Conservation genetics and biogeography of the Caribbean pine (Pinus caribaea var. bahamensis) in the Bahaman archipelago

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Conservation genetics and biogeography of the Caribbean pine (*Pinus caribaea* var. *bahamensis*) in the Bahaman archipelago

Thesis submitted by

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For the degree of

Doctor of Philosophy

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Declaration

I hereby confirm that this thesis is my own work and the material from other sources used in this work has been appropriately and fully acknowledged.

Michele Sanchez

London, September 2012

"All past and present organic beings constitute one grand natural system…" (Darwin 1859)

I would like to dedicate this work to my husband; whose support, encouragement and patience have been a constant throughout the years.

Abstract

The Bahaman archipelago contains large expanses of pine forests, where the endemic Caribbean pine *Pinus caribaea* var. *bahamensis* is the dominant species. This pine forest ecosystem is rich in species and also a valuable resource for the local economy. Small areas of old-growth forest still remain in the Turks and Caicos islands (TCI) and in some of the islands in the Bahamas; despite on-going severe infestation by pine tortoise scale insect *Toumeyella parvicornis* and high pine mortality in the former and intensive past commercial logging activities in the latter. For the first time integrated research on the genetics, morphology, ecology and biogeography of this variety was carried out throughout its whole distribution range. Past and present forest areas were mapped using historical physical maps and modern satellite imagery, showing forest loss due to urbanisation, pests and storm surges and expansions resulting mainly from dry-season human induced fires. Population genetic analysis using plastid and nuclear microsatellites revealed high ancient gene flow and recent genetic distance between populations of the Bahamas and the TCI; in addition to genetic structure within regions. Morphological differences were also observed and discussed. The variety showed high individual genetic and morphological variance and high plasticity. Despite the observation of good forest regeneration in normal circumstances, stochastic events did cause severe reductions in forest area and effective population size. A predominantly random and outcrossing breeding system was also inferred from the data, despite detection of some inbreeding in the smaller populations. Suggestions for the future conservation and management of the species included fire management and the creation or extension of *in-situ* conservation areas and *ex-situ* collections.

Acknowledgements

This research would not have been possible without the masterly guidance and support of my supervisors Dr. Martin Ingrouille and Dr. Mike Fay. I owe my deepest gratitude to my husband, colleague and field assistant Martin A. Hamilton for all his support throughout the years, sweat and hard work during fieldwork and encouragement during difficult times. Many thanks to my colleagues at Kew Gardens, whose valuable technical advice, time, flexibility and support contributed to the successful completion of this thesis, in particular Dr. Colin Clubbe, Robyn Cowan, Marcella Corcoran, Justin Moat, Susana Baena, Edith Kapinos, Dr. M. Dolores Lledó-Barrena, Sara Redstone, Noelia Alvarez and Kathleen Smith. I would also like to thank the valuable support from my parents, relatives, friends and PhD colleagues.

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Therefore, I offer my sincere gratitude to all of you who believed in me and my research and participated in some way to the completion of this work.

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Chapter 1 . Introduction

The Caribbean pine *Pinus caribaea* Morelet var. *bahamensis* (Griseb.) W.H.G.Barrett & Golfari is the only native pine tree in the Bahaman archipelago. It is important not only in the pine forest ecosystem, where it is the dominant species, but also for the local community. This species has high economic value locally and internationally. Commercial plantations are present in many countries and continents. The pine forests of The Commonwealth of the Bahamas (Bahamas) were intensively logged in the first half of the 20th century and as a result only a few areas of old-growth forest still remain. In the Turks and Caicos Islands (TCI), where *P. caribaea* var. *bahamensis* is the National Tree, forests are mostly old-growth. However, intensive forest decline has been observed in the past decade as a result of infestations by the exotic pine tortoise scale insect *Toumeyella parvicornis* Cockerell. *Pinus caribaea* is an IUCN red listed species under the category Lower Risk/least concern (Conifer Specialist Group 1998). At present, a new assessment is being undertaken for *P. caribaea* (pers.comm. Aljos Farjon). The assessment for *P. caribaea* var. *bahamensis* undertaken by Sanchez *et al*. (in press) found this variety to be in the category Vulnerable, due to recent and future threats.

Pinus caribaea var. *bahamensis* trees have withstood a harsh dry and warm climate, hurricanes, fires and logging in the past. Nonetheless, their ability to survive modern challenges of climate change, development and pest attacks is uncertain. Conservation measures and forest management are essential for preserving the species genetic diversity for the future and the survival of the biodiversity in the pine forest ecosystem. In this research, a survey of size and extent of these forests based on satellite imagery, historic maps and ground truthing served as basis for delimiting population area and analysis of forest changes through time. Past and modern patterns of gene flow and genetic structure of this variety throughout its whole distribution range were explored in detail for the first time through genetic analysis using nuclear and plastid microsatellites. Morphological and ecological data collected during fieldwork contributed to delineate regional and local differences or similarities among these trees, detect threats to the pine forest ecosystem and enhance knowledge of pine forest ecology and regeneration. Scientific knowledge gathered, analysed and discussed in this work was finally used as reference for suggestions for future targeted conservation efforts for the variety.

1.1. *Pinus caribaea* var. *bahamensis*

1.1.1. Taxonomy

In this thesis the classification adopted by Farjon and Styles (1997), in their monograph of *Pinus*, has been accepted, with the name for the studied variety being *Pinus caribaea* var. *bahamensis*. The name *P. caribaea* was first used in an obscure publication by Morelet (1851) citing a type specimen from the Isla de Pinos in Cuba (Little Jr. and Dorman 1954). Shaw (1914) in his revision of the genus *Pinus* combined *P. caribaea*, *P. elliottii* Engelmann, *P. cubensis* Sargent, *P. bahamensis* Grisebach, *P. heterophylla* Sudworth and *P. recurvata* Rowley under the name *P. caribaea*, including plants from the United States, Central America and the Caribbean. A thorough revision of *P. caribaea* as described by Shaw (1914) was undertaken by Little Jr. and Dorman in 1954, resurrecting *P. elliottii* as a distinct species comprising two varieties in the southeastern United States, *P. elliottii* var. *elliottii* and *P. elliottii* var. *densa*, and *P. caribaea* as a separate species in the Caribbean and Central America. Differences between these two species included geographical location, seed wings, cone size and number of needles per fascicle (Table 1.1). In 1962, *Pinus caribaea* was divided into three varieties, one of which is *P. caribaea* var. *bahamensis,* according to morphological characteristics and distribution range (Table 1.1) (Barrett and Golfari 1962). This remains the current accepted nomenclature (Farjon and Styles 1997). *Pinus bahamensis* is a synonym of *P. caribaea* var. *bahamensis*. Common names for this variety include Caribbean pine, Caicos pine, Bahamian pine and yellow pine.

Pinus caribaea is in the family Pinaceae Lindl., subgenus *Pinus* L. (Diploxylon or hard pines), according to Price *et al.* (1998). This subgenus is characterised by the presence of two fibrovascular bundles in the needles; while subgenus *Strobus* Lemmon (Haploxylon or soft pines) has only one fibrovascular bundle present. According to phylogenetic research by Gernandt *et al.* (2005), *P. caribaea* is in section *Trifoliae* Duhamel subsection *Australes* Loud., which includes pines from North America, Mexico, the West Indies and adjacent Central America. Main morphological features in this subsection are the presence of two to five needle-like leaves arranged in fascicles (condensed short shoots containing the needles), a persistent sheath (series of bud scales wrapping the needles) and two vascular bundles per leaf.

1.1.2. Morphology

According to Farjon and Styles (1997), *P. caribaea* var. *bahamensis* is a tree of medium size, with a distribution restricted to the Bahaman archipelago (Figure 1.1 and Table 1.1). Leaves are dark green and arranged in fascicles of two to three needles, varying from 13-26 cm long × 1.2-1.8mm wide. These fascicles are bundled in sheaths 15 to 20mm long (reduced to 10mm at maturity), with dark red-brown to grey-black imbricate scales. Stomata are present on all faces of the leaves, in eight to 11 lines on the convex abaxial face, and four to six lines on the adaxial face. They are monoecious (i.e. with male and female structures on the same tree). The female strobili usually develop on higher branches than the male strobili, facilitating wind driven seed dispersal. Seed cones vary in size from 4-12 cm long × 3-7 cm wide. Seeds are most frequently winged (articulate) and wind dispersed. Seedlings have an elongated stem with ascending green primary leaves.

Trees are monopodial, losing their lower branches as they mature. They rarely reach 24m in height. Young trees are conical with the presence of a developed leader branch and old trees have a rounder crown and usually lack the leader branch. Most tree height develops in the early years of growth until the tree reaches a diameter at breast height (dbh) of circa 10 cm. (Henry 1974). Observations and tree ring counts in the Bahamas pine forests have shown that most of the trees do not exceed 20 cm in dbh; and diameters of 15 cm and 25 cm corresponded, respectively, to trees of 33 and 78 years-old (March 1949). Trees rarely exceed 100 years old, but when they do a diameter beyond 30 cm has been observed (Miller, W. 1920, March 1949). A 112 year-old tree was recorded on Andros, Bahamas, during a survey by Henry in 1974. *Pinus caribaea* is short-lived when compared to other pine species, such as bristlecone pine *P. longaeva* D.K.Bailey, which can live for hundreds or even thousands of years (Connor and Lanner 1990).

Little study or observations of pine trees in the TCI have been carried out. Trees in the TCI are generally smaller than in the Bahamas, usually less than 15m tall, and this is probably an adaptation to the drier climate of the region (described in section 1.2.3 of this chapter). Morphological and ecological observations from this research are presented in Chapter 4, section 4.3.

The pine roots of *P. caribaea* var. *bahamensis* have also adapted to dry climate, low moisture content and shallow soils. Trees frequently form a flat sheet or plate of roots below the emerging limestone rock. Moisture is mainly obtained from rainfall which penetrates the porous rock or from the fresh water table through capillary movement. In some of the islands the water table is only 1.6m below the surface (Greaves 1978). Some authors suggest that adaptations to alkaline soils and/or drought resistance could be linked to the nature of their mycorrhizal associations (Greaves 1978). Mycorrhizal fungi create a network below the soil, improving hydraulic conductivity, transport of nutrients and water retention. They can also access the water contained in soil micropores during droughts, which cannot be accessed by the root system (Allen 2007). Fungi reported growing in association with *P. caribaea* and in alkaline soils are *Rhizopogon nigrescens* Coker & Couch and *Suillus cothurnatus* Singer; the former being present in the Bahamas soils (Redhead 1982). No data are available for the TCI.

1.1.3. Varieties

Other varieties of *P. caribaea* are *P. caribaea* var. *caribaea* and *P. caribaea* var. *hondurensis* (Sénécl.) W.H.G.Barrett & Golfari (Barrett and Golfari 1962). Both of them grow taller and have greater dbh than *P. caribaea* var. *bahamensis*. Other differences include number of needles, sheath size at maturity, colour and leaf disposition of primary leaves in seedlings, and geographic distribution, as seen in Table 1.1. *Pinus caribaea* var. *caribaea* is restricted to western Cuba, Pinar del Rio and Isla de la Juventud (Isla de Pinos); *P. caribaea* var. *hondurensis* is only present in Central America (Farjon and Styles 1997) (Figure 1.1). The evolutionary history of *P. caribaea* is briefly discussed in section 1.3.

Stands of *P. caribaea* var. *caribaea* in Cuba and adjacent islands occur in areas of low altitude, high rainfall and acidic soils. They often grow in association with *P. tropicalis* Morelet in Pinar del Rio and Isla de la Juventud (Greaves 1978). In Central America, *P. caribaea* var. *hondurensis* grow mostly in lowland coastal plains (littoral) from 50 to 500m altitude, but can adapt to higher altitudes inland (continent) (Greaves 1978). Annual precipitation experienced varies with altitude and distance from the coast (Greaves 1978, Farjon and Styles 1997, Dvorak *et al.* 2005). This variety grows in well-drained, sandy or gravelly acidic soils and possibly hybridizes with *P. oocarpa* Schiede and *P. tecunumanii* F.Schwerdtf. ex Eguiluz & J.P.Perry in the wild (Squillace and Perry Jr. 1992, Farjon *et al.* 1997, Farjon and Styles 1997). Interspecific crosses between *P. caribaea* var. *hondurensis* and *P. oocarpa, P. tecunumanii* and *P. elliottii* var. *elliottii have* been successful in the past, producing fertile hybrids (Nikles 1996, 2000). Natural hybridization between *P. elliottii* var. *elliottii* and *P. caribaea* var. *caribaea* have also been detected in plantations in Australia (Slee 1971). However, high segregation distortion present in the genetic maps of *P. elliottii* var. *elliottii* and *P. caribaea* var. *hondurensis* suggested some sort of pre- or post-zygotic mechanism to reduce success of inter-specific crosses (Shepherd, M. *et al.* 2003).

	P. caribaea			***P. elliottii***	
Varieties	***bahamensis***	***caribaea***	***hondurensis***	***densa***	***elliottii***
Distribution	Bahamas and the TCI	Cuba	Belize, Guatemala, Honduras, Mexico and Nicaragua	Florida	Florida, South Carolina, Mississippi, Louisiana
Altitude	0-30 m	45-355 m	50-850 m	1-20 m	1-110 m
No. of needles	(2) 3	3 (rarely 4)	3 (4 or 5)	2 (3)	2-3
Fascicle sheaths	15-20 mm (young) 10 mm maturity	15-20 mm	15-20 mm	10-15 mm	11-14 mm
Seeds	articulate (rarely adnate)	adnate	articulate (rarely adnate)	articulate	articulate
Seedlings	green, ascending primary leaves	green, ascending primary leaves	glaucous, spreading primary leaves	grass stage with a short stem	erect pencil-like stem
Cones (length)	4-12 cm	5-10 cm	6-14 cm	5-15 cm	9-14 cm
Tree height	8-20 m	10-25 m	10-45 m	(8)15- (26)30 m	15-30 m
Max. tree dbh	50 cm	70 cm	100 cm	50 cm	100 cm
Soil pH	7.5-8.5	4.5-6	4.3-6.5	3.6-8.4	3.6-8.4
Mean annual temperature	22-29 °C	22-28 °C	22-28 °C (littoral) 20-24 °C (continent)	23-27 °C	18-27 °C
Mean annual rainfall	750-1500 mm	1200-1700 mm	950-1300 mm	1000-1800 mm	1000-1800 mm
Drought duration	6 months (Bahamas) 8 months (TCI)	5-6 months	2-3 months (littoral) 6 months (continent)	6 months	4-6 months

Table 1.1. Range, morphological and ecological parameters among the three varieties of *Pinus caribaea* and varieties of *P. elliotii*. Data according to Little Jr. and Dorman (1952), Barrett and Golfari (1962), Sorensen (1963), Greaves (1978), Henderson (1984), Farjon and Styles (1997) and SERCC database (2012).

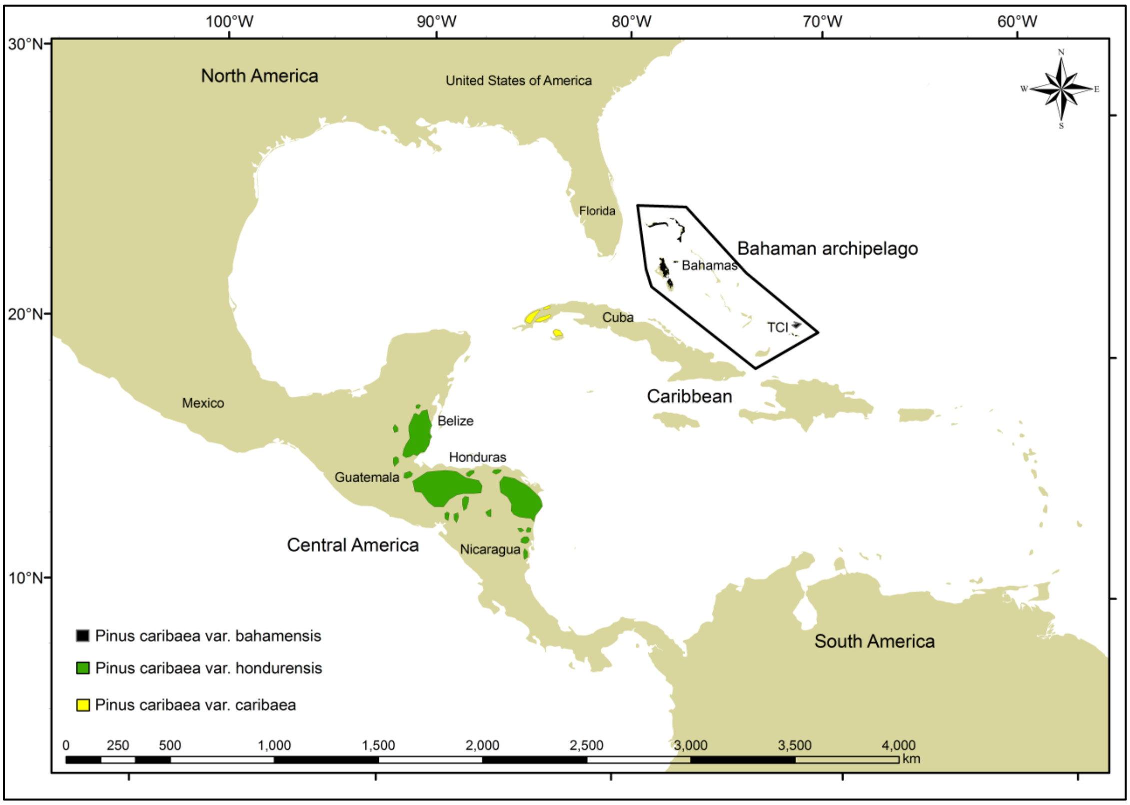

Figure 1.1. Distribution of natural populations of *Pinus caribaea* following Greaves (1978), Farjon *et al*.(1997) and this research.

1.1.4. Distribution range

Pinus caribaea var. *bahamensis* is endemic to the Bahamas and the TCI (Farjon and Styles 1997). Pine forests are located in the north and far south of the Bahaman archipelago, circa 600 km from each other. This variety is the only native pine tree present in the region; occurring on the islands of Abaco, Andros, Grand Bahama, New Providence (in the Bahamas), and Middle Caicos, North Caicos and Pine Cay (in the TCI) (Barrett and Golfari 1962) (Figure 1.2).

Recent research has found evidence of pine pollen resembling that of *P. caribaea* on the island of Abaco dating back to circa 8338 yr BP (years before present), during the Early Holocene (Slayton 2010). The oldest record for human presence in the northern Bahamas dates back to circa 1055-925 yr BP (Steadman *et al.* 2007), which leads to the conclusion that these are natural stands of pine forests and not human introductions.

Some isolated records for Berry Islands (Northrop and Northrop 1902) and Great Inagua (Farjon and Styles 1997) are mentioned in the literature, but no further evidence of pines in these islands has been confirmed (Miller, W. 1920, March 1949, Henry 1974, Greaves 1978).

Pine forests in the archipelago grow at low altitudes from sea level to 30 m. Latitudes vary from 21°45’N to 27°00’N and longitudes from 71°42’W to 78°50’W. The TCI is the most southern and eastern point in the distribution range for the variety, and most eastern for the species (Greaves 1978). The vegetation in the Bahamas islands shows a latitudinal gradient, with a decrease in rainfall from north to south and a change from tall and lush vegetation in the north to more compact and xeric vegetation in the south. The eastern side of many larger islands is also drier and more subject to salt spray than the western side, and there xeric vegetation develops (Whitaker and Smart 1997).

Pine forests in the northern Bahamas occur on the largest islands, covering an estimated area of 1782 to 2023 km² (March 1949, Greaves 1978). Recent changes in forest area and current forest distribution are explored in detail in Chapter 2, section 2.3.2.

1.2. The Bahaman archipelago

The Bahaman archipelago extends from circa 20°N to 27°N and 71°W to 80°W and has a tropical marine climate. It lies between Florida, USA (northwest), Cuba (west and southwest) and Haiti/Dominican Republic (south and southeast) and includes two countries, The Commonwealth of the Bahamas and the Turks and Caicos Islands. The former is an independent country and the latter is one of the 16 UK Overseas Territories. The archipelago covers an area of 260,000 km^2, but only a small percentage of its total area (5.5%) is currently emergent land (Figure 1.1) (Whitaker and Smart 1997). This low-lying carbonate land is scattered throughout the archipelago in the form of islands, cays and rocks, with more than 3000 of them in the Bahamas and circa 49 in the TCI (Buchan 2000, Hall and Close 2007). The Bahamas have a land area of 18,460 km^2 and 35 major islands, whereas the TCI have only 491 km^2 and six inhabited islands (Buchan 2000, The Commonwealth of the Bahamas 2005b, Clerveaux and Fisher 2006).

1.2.1. Population and development

It has been estimated that the first settlers reached the Bahamas from northern Cuba circa 800-900 AD, and the TCI from Hispaniola circa 1100 AD, following the driest period in the Caribbean from circa 550 BC to 950 AD (Berman and Gnivecki 1995, Marvel 2008). The oldest human fossil found in the archipelago, from Abaco, dates back to circa 1055-925 yr BP (957-1087 AD) (Steadman *et al.* 2007). The indigenous people, called Lucayans, inhabited several of the islands; and it is estimated that a population of 10,000 individuals lived in the TCI and over 100,000 in the whole archipelago (Marvel 2008). Their society was based on fishing, hunting-gathering and 'slash and burn' agriculture (Blick 2007). The Bahamas and the TCI were inhabited by the Lucayans until the early 16th century; when they were taken as slaves to work in Hispaniola and other islands or died from diseases brought by the Europeans (Doran Jr. 1958, Marvel 2008).

Most islands remained uninhabited until Europeans settlers arrived in 1718 AD, and new colonies were founded on the islands of New Providence, Eleuthera and Harbour Islands in the Bahamas. British loyalists from North America flocked into the Caribbean in the late 1700s, and many stayed in the Bahamas and Caicos islands. The population of New Providence grew quickly and by 1800 it had reached 3,000 people; however Abaco and Andros remained quite sparsely populated (Doran Jr. 1958). New crops such as cotton, citrus, pineapple and sisal were introduced; agriculture intensified and many slaves were brought into the new colonies to work the land (Mooney 1905, Doran Jr. 1958). Large plantations of cotton and sugar were established in the TCI at that time; e.g. Haulover Estate on Middle Caicos, Wade's Green plantation on North Caicos and Cheshire Hall Estate on Providenciales. However, productivity declined due to poor soils and pests and by 1813 most of these plantations has been abandoned and small subsistence agriculture took their place (Farnsworth 1996, Sadler 2008).

Logging, agriculture, non-seasonal fires and urbanisation could have affected pine forest size and distribution during this early colonisation time. Environmental pressures, e.g. logging, agriculture, hunting and introduction of invasive species, seem to have contributed to species composition and extinctions elsewhere in the past. During pre-historic times, the small population of San Salvador (circa 500-1000 people) was large enough to cause extinction of several marine taxa due to unsustainable and intensive use of the natural resources (Blick 2007). Intensive anthropogenic pressures, e.g. logging and grazing, during the 1800s have changed the species composition of St. Helena from scrub and forests to grasslands, reduced forest area and eventually led to species extinctions, e.g. *Trochetiopsis erythroxylon* (G.Forst) Marais, *Trochetiopsis* *melanoxylon* (R.Brown ex Aiton f.) Marais and *Commidendrum rotundifolium* (Roxb.) DC. (Cronk 1989).

The population of the Bahaman archipelago has grown significantly since the 18th century. The last population census in the Bahamas in the year 2000 counted a total of 303,611 inhabitants; almost 70% of them living on the small island of New Providence (The Commonwealth of the Bahamas 2008). The pine forests of this island have suffered greatly with this population increase, as it will be explored in Chapter 2, section 2.3.2. Grand Bahama, Abaco and Andros have, respectively, ≥ 46,000, 13,170 and 7,686 people (Dept. Statistics, The Commonwealth of the Bahamas 2008). Despite the fact that most of the communities on these islands are located on the coastal areas, an increase in population is usually followed by higher demand for land, food and water. These factors could affect and threaten existing forest areas. The recent increase in urbanisation and its effects on pine forest area in the Bahamas will be discussed in detail in Chapter 2, section 2.3.2.

The TCI have a smaller population, with circa 36,600 people in 2008; about 65% of them living in Providenciales, where no pines are present (TCI Census Atlas, The Turks and Caicos Islands 2001). Although urbanisation is not the major pressure on the TCI pine forests at the moment, almost all suitable land has already been developed and in the future development will reach rural parts of the islands. New roads, causeways, docks and houses pose a new threat to inland ecosystems like the pine forests. An increase in sea level could increase the salinity of the fresh water lenses and also shift urbanisation to inland areas, mainly in low-lying islands like those in the Bahaman archipelago; consequently threatening the pine forests. This will be discussed further in Chapter 2, section 2.4.

The increase in population numbers has created extra demand for fresh water, which is a scarce resource in the archipelago (Sealey 1985). In the TCI most of it comes from rainfall (collected in cisterns), desalination plants or importation (Ratter 1996); thus, it does not have an effect on the pine forests. In the Bahamas, however, most fresh water is obtained from the underground fresh water lenses through well fields located in the pine forests. Well fields on Andros supply fresh water for all the population of Andros and the majority of the population of New Providence. Other smaller well fields are present on New Providence, Grand Bahama and Abaco (Sealey 1985). Most of the water extraction occurs in pine forests, as these areas have deeper fresh water lenses. Water extraction in large volumes can reduce water availability for plants and increase salinity. An increase in salinity of the water lenses has already been observed on Andros and New Providence due to over-pumping (Whitaker and Smart 1997, Sealey-Sullivan *et al.* 2002). On Grand Bahama, many new coastal developments have canals linked to the sea for boat access. These canals can alter the hydraulic balance and cause salinization of adjacent areas (Whitaker and Smart 1997), as will be discussed in Chapter 2, section 2.4. Changes in local hydrology can become a real problem for forest ecosystems, mainly when predictions of an increase in water demand and decrease of the fresh water lenses due to sea level rise are taken into account. Therefore, a decrease in the amount of fresh water could also cause a decrease in the pine forests.

1.2.2. Geology, soils and hydrology

The Bahaman archipelago has been tectonically stable for the past 100,000 years, according to fossil reef and stalagmite records (Mylroie 2008). It is formed by several carbonate platforms, also known as banks. The main banks in the Bahamas are the Great Bahama bank (Andros, New Providence and the Exuma Cays), the Little Bahama bank (Grand Bahama and Abaco) and the Cay Sal bank. In the TCI, there are three banks, the Caicos bank, the Turks bank and the Mouchoir bank. The Cay Sal and the Mouchoir banks are the only mostly submerged and uninhabited banks (Figure 1.2) (Hearty and Neumann 2001). All the banks are shallow, less than 15 m deep, and isolated from each other and land by deep sea channels, which can reach a depth of more than 3,600m (Traverse and Ginsburg 1967). The Bahamas banks are separated from North America by the Florida Straits and from Cuba by the Old Bahama Channel. Other deep water channels separate Andros and New Providence/Exuma Cays (Tongue of the Ocean); the Little and Great Bahama banks (NW Providence Channel); Mayaguana and Great Inagua (Deep Channel); and the Caicos and Turks banks (Turks Island Passage) (Buchan 2000, Clerveaux and Fisher 2006, Hall and Close 2007, Reijmer *et al.* 2009). Therefore, it can be assumed that islands remained isolated even during low sea level events, given the stability of the archipelago and the depth of the sea channels.

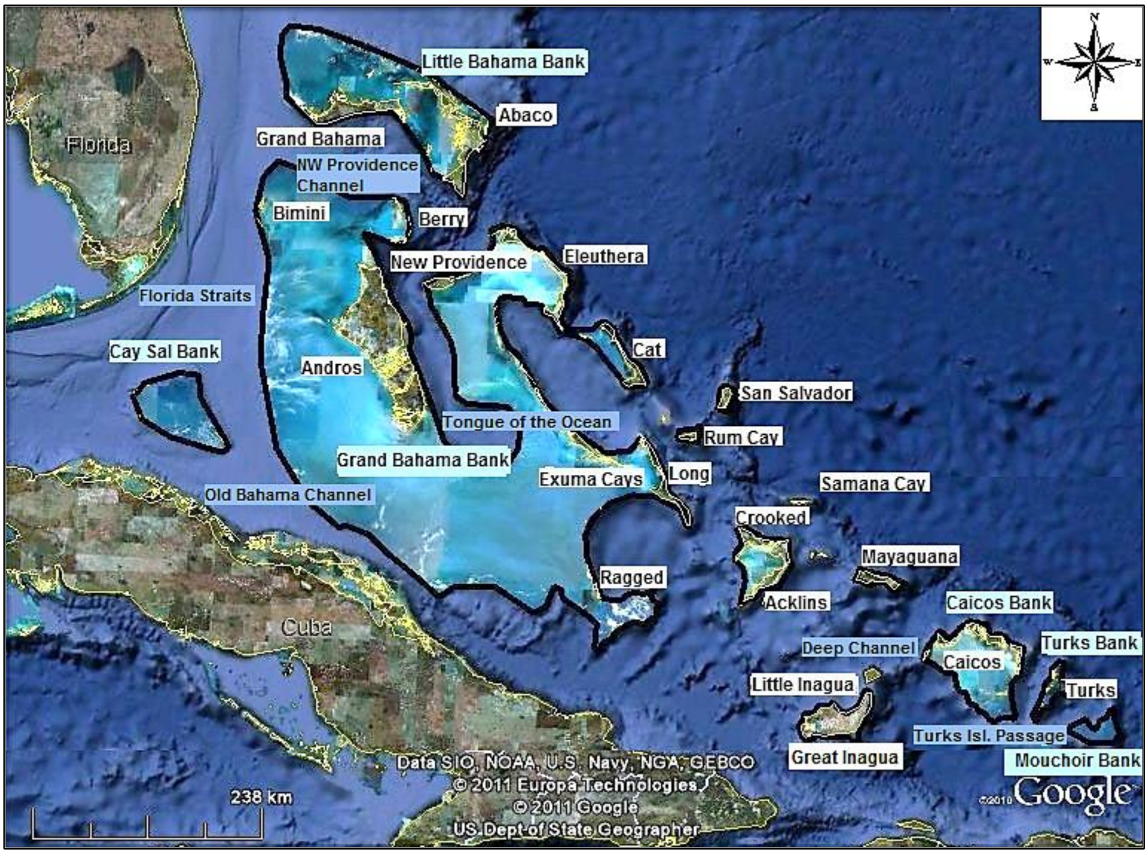

Figure 1.2. Map showing the Bahaman archipelago main islands, banks and deep water channels, following Hearty and Newmann (2001).

The vegetation on the islands changes according to soil type, proximity to the coast, salinity and species composition. The classification system for vegetation types in the Caribbean is complex and controversial. This work follows the most used and locally accepted terminology (Smith, I. K. and Vankat 1992, Areces-Mallea *et al.* 1999, Williams, S. 2009). The water salinity decreases linearly from the coast; and lusher, taller and less salt-resistant vegetation increases accordingly (Whitaker and Smart 1997). In the eastern parts of the islands the vegetation is a low and wind-swept scrub (dry evergreen scrub or limestone scrub), due to sandy soils and constant trade winds. Further inland, low hills run usually lengthwise along the islands; the soil is composed of coral sand and organic matter (loam), and limestone outcrops cover part of the land surface. The vegetation can grow taller and lusher, where it is protected from salt spray and the wind. Thus, the predominant vegetation changes to a dry evergreen forest (coppice or limestone thicket). The valleys below sea level are connected to the sea by brackish ponds or lakes; and in these areas halophytic plants thrive (salt marshes or salinas). Mangroves grow near the coast and by the sea. In larger and more protected islands, pine forests are found in valleys at sea level and low altitudes, where the land surface is almost completely covered with limestone rock (Nickrent *et al.* 1988, Smith, I. K. and Vankat 1992, Whitaker and Smart 1997).

The limestone has a honeycombed appearance caused by erosion, and is filled with loam-rich soil. A soil sample from New Providence pine forests was high in Ca (209.8 ppm), K (107.4 ppm) and NO_3 (278.3 ppm) (Mooney 1905). The soils are alkaline on all of the islands and do not retain much moisture (Greaves 1978). Pine Cay in the TCI is covered by aeolian sands, instead of limestone rock like all other pine islands. Most pine forest occurs in stony loam soil (3) and dry evergreen forest (mixed broadleaf forest) in black loam soil (2), as can be observed in the map below (Figure 1.3).

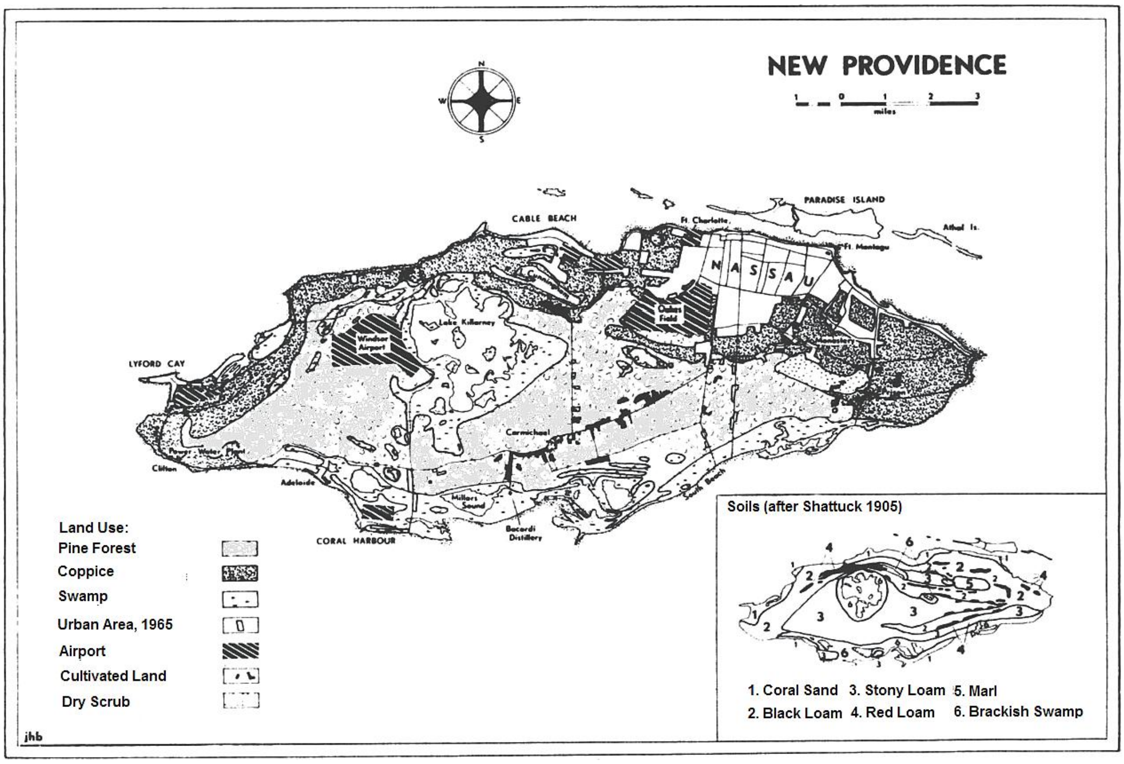

Figure 1.3. Map of vegetation and soil types on New Providence in 1961. Reproduction from Bounds (1981b)[1]. Soil types according to Mooney (1905). Pine forest areas are located mainly in stony loam and coppice in black loam.

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[1] Reproduction of Figure1- Land use of New Providence 1961 from the article *Land use on New Providence island. Bahamas 1960-1979,* Revista Geográfica, 94 pp.123-153 by J.H. Bounds in 1981 with permission from IPGH (Instituto Panamericano de Geografía e Historia).

Water can drain through fissures and cracks in the porous limestone rock into the freshwater aquifer (Sealey 1985). The presence of a shallow and wide root plate in trees of *P. caribaea* var. *bahamensis* can increase the absorption of water and nutrients; as minerals become available when water circulates and dissolves the porous rock (Kellogg 1951). The woody roots of these pine trees can also reach the water table through fissures in the rock or even dissolve the rock to a certain extent, due to acid exudates and CO_2. The freshwater aquifer (Lucayan limestone aquifer) lies on top of the saline groundwater (pre-Lucayan limestone and dolomites aquifer) and is mainly calcitic (alkaline). Its thickness varies from 43.0 m in the northern islands to 10.5 m in the southern parts of the archipelago; depending on rock permeability, rainfall and evapotranspiration. In a couple of hours after a storm circa 60 to 100% of the rainfall can pass through the ground surface and phreatic zone and reach the water table (Whitaker and Smart 1997). Northern islands in the archipelago have deeper fresh water lens than southern ones and higher hydraulic conductivity. This conductivity is important in the retention of the freshwater lens and avoidance of its mixing with salt water during high tides. In general, northern islands have higher rainfall, less evapotranspiration and deeper freshwater aquifers then southern ones (Whitaker and Smart 1997), thus higher availability of fresh water. Proctor (1955) observed that growth and establishment of vegetation in the TCI could be limited by low rainfall, porosity of the limestone and sea influence. Morphological differences between pine trees in the Bahamas and the TCI may be related to a plastic response to water availability. This possibility will be explored in Chapter 4, section 4.4.

It seems that climate plays a big role in the regulation and availability of water in these islands. Therefore, sea level rise and increase in global temperatures and water demand due to development can have a huge impact on the flora, fauna and inhabitants of this archipelago.

1.2.3. Climate

The climate in the Bahaman archipelago is tropical marine with two main seasons, a wet summer (May to October) and a drier winter (November to April). Mean average temperature is 25 °C, and annual temperature varies from 22 °C to 29 °C (The Commonwealth of the Bahamas 2005b). The wet season corresponds to the hurricane season. The northern part of the archipelago, where the largest islands are located, has higher rainfall and cooler winter temperatures, due to winter cold fronts from the USA and larger land mass (Whitaker and Smart 1997, Reijmer *et al.* 2009). The average annual precipitation varies from 1500 mm (Grand Bahama) to 1000 mm (South Andros) in the Bahamas and from only 750-800mm in the northwest part of the TCI and circa 550 mm in South Caicos and Grand Turk (Barrett and Golfari 1962, Greaves 1978, Sears and Sullivan 1978, Farjon and Styles 1997, Mylroie 2008). The TCI and other small islands are drier, as clouds tend to dissipate or be displaced by wind before rain occurs (Whitaker and Smart 1997).

Easterly and south-easterly moderate trade winds prevail during summer. Cold fronts change the wind pattern to south or south-west and then north-west to north, affecting the Bahamas archipelago and the Greater Antilles (Stone 1953, Ricklefs and Bermingham 2008). Hurricanes do not follow these patterns and they can have highly variable wind direction (Reijmer *et al.* 2009). Wind patterns have a huge influence in migration and gene flow in wind-dispersed trees, such as the Caribbean pine. A migration of *P. caribaea* var. *hondurensis* in Central America following trade wind direction (north-easterly to easterly) in the Yucatán peninsula was suggested by Dvorak *et al.* (2005). They also suggested an influence of hurricanes in seed dispersal.

The Bahaman archipelago lies within the North Atlantic hurricane belt in the Atlantic basin. It is often hit by hurricanes during the hurricane season, which lasts from June to November. A recent study by Vecchi and Knutson (2010) counted six hurricanes per year on average for the Atlantic Basin from 1878-2008, based on the ‘best track’ dataset from the National Hurricane Centre (NHC) Atlantic Basin hurricane database (HURDAT). They also observed that an average of 1.8 hurricanes per year make landfall in the USA . Hurricanes usually approach the archipelago from the south and east. In the first half of the 20th century alone, more than 80 hurricanes had adverse effects on the Bahamian islands (Buchan 2000). Hurricanes affecting pine islands in the archipelago since 1920 are listed in Table 1.2 below. The pine forests of the northern Bahamas were hit by 15 out of the 17 hurricanes recorded for that period, but only four of those were recorded for the TCI. In the past 12 years, seven major hurricanes hit pine islands in the archipelago, and severe damage was observed for some of the pine forests.

Hurricane	Year	Category	Wind Speed (km/h)	Pine Islands damaged	Major damage reported
#6	1926	4	212	Andros, New Providence	wind, storm surge
#4	1928	3	203	Grand Bahama, TCI	wind, floods
#2	1929	4	222	Andros, New Providence	storm surge, floods < 5m
#4	1932	5	254	Abaco, TCI	wind, floods
#5	1941	3	173	Andros (North), New Providence	wind
#4	1947	5	271	Abaco	wind, floods
Betsy	1965	4	225	Abaco, Andros, New Providence	wind, floods, storm surge < 2m
Inez	1966	2	166	New Providence, Grand Bahama	tornado in Nassau, wind, floods
David	1979	1	145	Andros, New Providence	wind, floods
Andrew	1992	4	240	Andros (North), New Providence	storm surge, floods
Floyd	1999	4	249	Abaco, Grand Bahama, New Providence	floods < 5m in Abaco, storm surge
Michelle	2001	2	164	Abaco, Grand Bahama, New Providence	floods < 2.5 m in Nassau
Frances	2004	4	231	Grand Bahama	wind, floods < 3m
Jeanne	2004	3	195	Abaco, Grand Bahama	wind, floods < 1.5m
Wilma	2005	2	178	Grand Bahama (Southwest)	wind, storm surge < 4m, floods
Hanna	2008	1	139	TCI (Caicos Islands)	wind, floods
Ike	2008	4	215	TCI (Caicos Islands)	wind, storm surge, floods

Table 1.2. List of hurricanes in the northern Bahamas and the TCI since 1920 causing severe damage on islands with pine forests. Parameters are for the islands listed and include hurricane category based on Saffir-Simpson scale (1- to 5), average wind speed and damage reported. Data compiled from Henry (1974), NOOA Miami Regional Library archive, UNYSIS® hurricane database and hurricane city database.

Hurricanes can have diverse impacts on forests, depending on their intensity and duration. Trees can be uprooted and snapped by strong winds. They can also be affected by floods during intense rainfall or sea-water intrusion inland (storm surges). Hurricanes have a huge impact on the local fauna, flora and the ecosystem as a whole (Tanner *et al.* 1991). Discussion about specific hurricane damage observed in the archipelago and effects of flood and storm surge on the pine forest ecosystem will follow in Chapter 2, section 2.4.

Recent prediction models on climate change for the North Atlantic mid region show that hurricane frequency will tend to decrease during the 21st century due to increased static stability and reduced vertical circulation; however, wind speed and destructive power of these hurricanes will tend to increase considerably with an increase in ocean temperature and water vapour (Geng and Sugi 2003, Bengtsson *et al.* 2007). An increase in hurricane intensity mainly over the Gulf of Mexico and the Caribbean has already been reported by Elsner and Jagger (2010) for the period from 1943 to 2008. Changes to the Bahamas climate during the 20th century, probably from high pressure over North America, have been reported by Chenoweth (1998). Such changes included winter drought, higher frequency of summer tropical cyclones and lower temperatures.

Predicted changes to the global climate could have devastating effects in low-lying islands; e.g. Bahamas and TCI. The fast response of natural systems to contemporary climate change has also been demonstrated by Parmesan and Yohe (2003). Their study reviewed over 1,500 species around the globe, showing changes in species phenology and plant-insect interactions, shifts in species range towards the poles and extinctions of restricted range alpine and polar species correlated to climate change. Modern reduction in forest area related to sea level rise has been observed in the Florida Keys (1931 to 1991), as a result of low survival rates of forest species due to stress, increased salinity and rise in groundwater (Ross *et al.* 2009). Predictions for the Texas region, USA, show that by the year 2080 floods caused by storm surge during high impact hurricanes could be as high as 1.8m (Mousavi *et al.* 2011). Changes in climate and hurricane intensity could have devastating effects on the pine forests of the Bahaman archipelago and its regeneration.

1.2.4. Palaeoclimate and vegetation

Sea level in the Bahaman archipelago fluctuated during the Pleistocene (1.8 million yr BP to circa 11,700 yr BP) and the Holocene (after 11,700 until present) varying from 5-95 m below modern sea level during glacial periods and 5-18 m above during interglacial periods (Pregill and Olson 1981, Tushingham and Peltier 1991, Lundberg and Ford 1994, Potter and Lambeck 2003, Mylroie 2008). These fluctuations are usually estimated from studies of the fauna (i.e. benthonic, planktonic and corals) and speleothems (e.g. stalactites and stalagmites), and can differ in different areas of the globe. The approximation of global sea level presented by Shackleton (1987) show values lower than 100 m below present during the last glacial with a significant rise in the interglacial (Figure 1.4). Tushingham and Peltier (1991) observed that towards the end of the Pleistocene, circa 16,000 yr BP, sea level was as low as 95 m below current in the Bahamas/Florida area; causing exposure of large parts of land and the reduction of distance between land masses. This proximity of land masses can be seen in Figure 1.5 (after Dutch 2006), which simulates a sea level 100 m below present. It seems that under these conditions, the Little Bahama Bank (Grand Bahama and Abaco) is one land mass, and New Providence is also linked to other islands in the Great Bahama Bank; however, Andros remains isolated. The TCI land mass is considerably extended and islands could have been linked together. Colonisation by new species or expansion of occurring species in the archipelago could have been facilitated by these conditions. A recent study by Jardón-Barbolla *et al.*(2011) estimated the expansion of *P. caribaea* var. *bahamensis* in the Bahamas circa 97,900 yr BP. A drop in sea level around that time can be observed in Figure 1.4 (after Shackleton 1987), which could have driven *Pinus* dispersal and colonisation of new exposed land. New land was built-up by carbonate deposits during sea level highstands, becoming exposed for plant colonisation during periods of low sea level. Reid (2010) has shown that circa 95% of the carbonate deposits found on New Providence Island were built-up during sea level highstands circa 130,000 yr BP; and the same occurred in the Caicos Islands. An older expansion time, circa 331,100 yr BP, was estimated for *P. caribaea* var. *hondurensis* in northern Central America (Jardón-Barbolla *et al.* 2011); also coinciding with a glacial period and probably low sea level. These cycles of lower and higher sea levels, due to melting of the ice sheets, happened at intervals of circa 20,000 to 40,000 years; and contributed to land expansion and exposure (Eberli 2000).

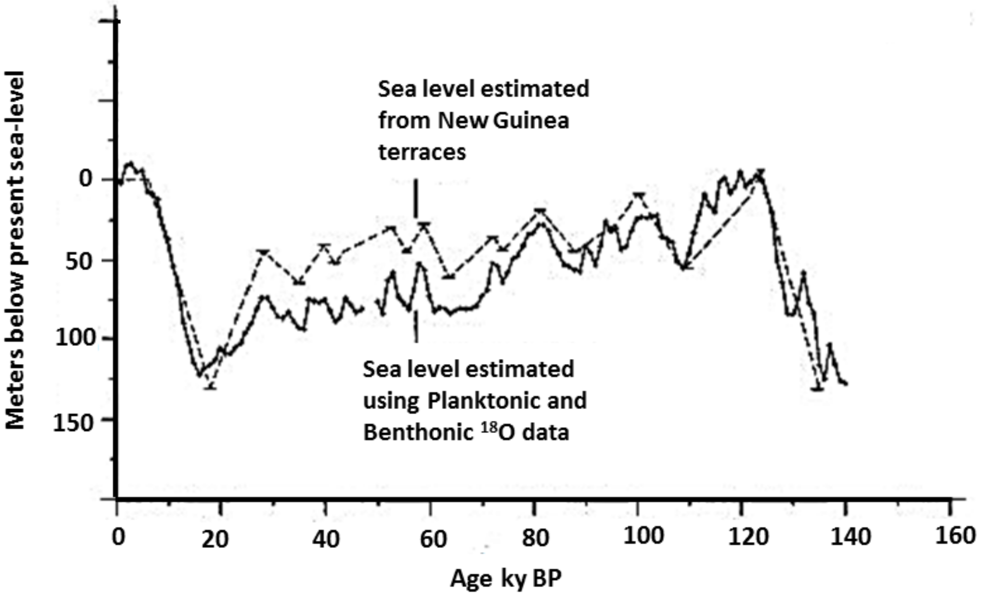

Figure 1.4. Sea level changes in the past 160,000 years (Shackleton 1987)[2]. Graph shows a steep increase in sea level after the glacial periods, circa 130,000 yr BP and after 18,000 yr BP.

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[2] Reproduction of Figure 5 from *Quaternary Science Reviews*, 6 (3-4), N. J. Shackleton, Oxygen isotopes, ice volume and sea level, pp. 183-190, Copyright (1987), with permission from Elsevier.

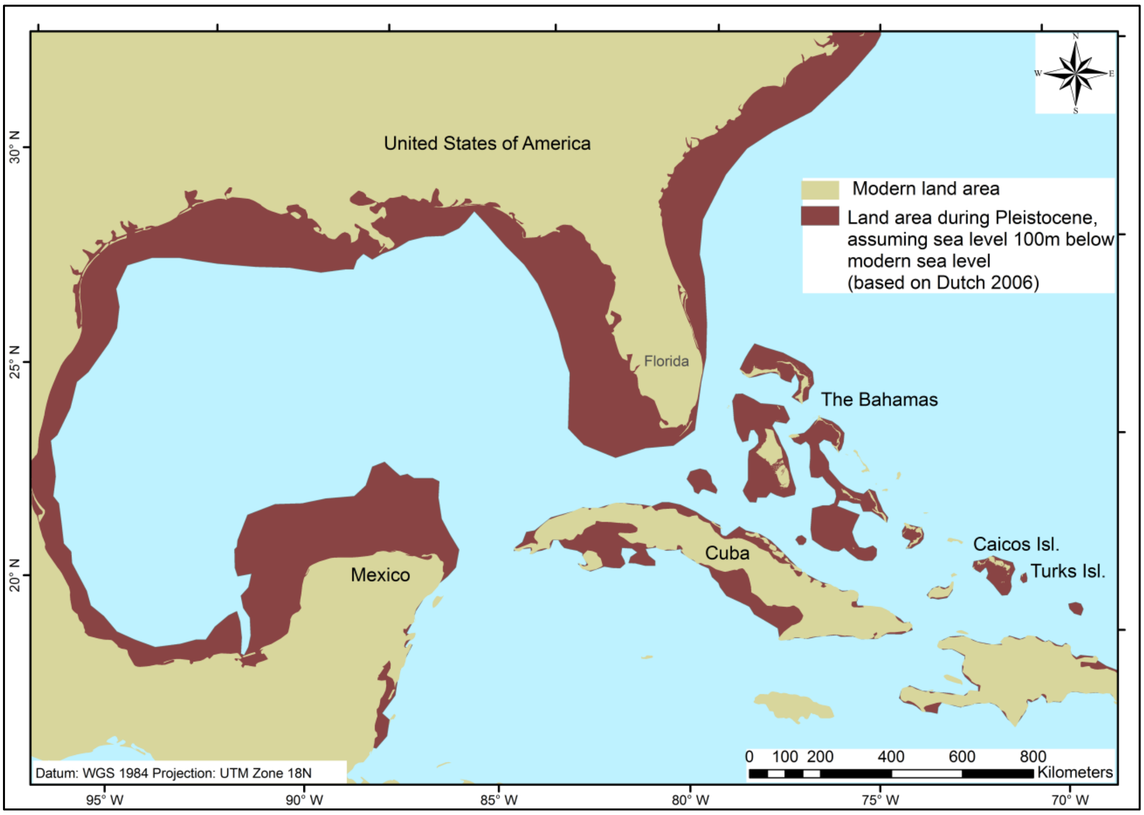


Figure 1.5. Emergent land during the Pleistocene in the Gulf of Mexico and the Caribbean Sea. Model assumes a sea level 100 m below the modern sea level, based on simulation by Dutch (2006). Increase in land mass of the Bahamas, southern USA and the Caicos Islands reduced distance between these areas.

These changes in sea level, expanding and contracting emergent land mass, in addition to changes in temperature and rainfall have driven extinctions, speciation, and morphological and ecological adaptations (Pregill and Olson 1981, Hewitt 2000). Savannahs, deserts and scrub woodlands thrived during the colder and drier conditions during the last glacial maximum (23,000 yr BP to 18,000 yr BP) and land bridges, due to low sea level, facilitated dispersal of species to more favourable conditions. *Pinus* populations migrated south during this period, as ice sheets covered most of the northern areas of North America, becoming abundant along the southeast coast. According to MacDonald *et al.* (1998), when the climate became milder and ice started melting during the interglacial, *Pinus* populations started migrating towards the north and west (circa 10,000 yr BP). However, the southern refuge remained and by circa 6,000 yr BP two distinct distributions of *Pinus* could be observed in North America, one in the southeast and another in the north and west. Observations of fossil pollen records by Williams *et al.* (2004) and Willard (2007) confirm the *Pinus* expansion in the Great Lakes and in southern USA around 13,000 and 11,000 yr BP. They also observed a decrease in *Picea*, Cyperaceae, *Carya* and *Quercus* pollen, as the vegetation changed from cool mixed forests to temperate deciduous forest and warm mixed forests around 15,000 and 5,000 yr BP. These were not only changes in distribution range and species composition, but also number of populations, density and genetic structure.

Pregill and Olson (1981) observed habitat loss and extinction of several species of reptiles and birds in the Great Bahama Bank at the end of the Pleistocene and start of the Holocene. Sea level rise and the change from xeric to wet conditions reduced open dry savannah, which was the habitat for many of these species. Records of pine wood and cones dating back to the Pleistocene have also been found in Cuba and Central America (Iturralde-Vinent *et al.* 2000, Bush *et al.* 2009). However, the earliest record of *Pinus* pollen in the Bahaman archipelago, found on the island of Abaco, dates back 8,338 yr BP during the Holocene (Slayton 2010). Slayton has also noticed a shift in vegetation on that island around 6,961 to 6,431 yr BP. Wetlands increased, probably due to a rise in sea level (Figure 1.4) and salinization of the fresh water table in low-lying areas. Lusher vegetation dominates from 6,306 to 3,252 yr BP, with high incidence of pines with many hardwoods and shrubs in the understory. From 3,252 to 2,062 yr BP, the climate becomes drier and pinelands become more open with an understory of mainly grasses. A marked increase in pine is observed after this period, coinciding with human presence and an increase in fire frequency around 822 yr BP. The same was observed by Kjellmark's analysis of fossil pollen in North Andros (Kjellmark 1996). Drier climate in association with increased fire frequency after human arrival on this island had contributed to the expansion of pine forests and changes in species composition. Therefore, glacial and interglacial periods, climate change and human disturbance seemed to have influenced changes in vegetation types, new colonisations, extinctions and the expansion and reduction of pine forests. Small rises in sea level can have a great effect on low-lying islands, as will be discussed in Chapter 2, section 2.3.3.

1.3. Phylogenetics and ancient migration routes

Phylogenetic studies of Pinaceae based on plastid DNA show a Cretaceous origin in the Northern Hemisphere (Laurasia) for the crown-group Pinaceae, circa 87 to 72 million yr BP, with major diversification of the genus *Pinus* during the Miocene (23 to 5.3 million yr BP) (Gernandt *et al.* 2008, Lin *et al.* 2010). This diversification and range expansion coincides with cooler temperatures and creation of new mountainous habitats (LePage 2003). The lack of species diversity and low morphological and genetic divergence between species of the genus *Pinus* has been reported as the result of recent radiation of extant sections and high extinction rates (Farjon 2003, Willyard *et al.* 2007). The highest species diversity is found in the New World (Gernandt *et al.* 2005), with 70 species in North and Central America (Price *et al.* 1998). Not one of the 11 genera and 225 species of Pinaceae has been recorded in the Southern Hemisphere (Farjon 2003).

A phylogenetic study by Gernandt *et al* (2005) proposed a subdivision of subgenus *Pinus* into distinct lineages from the Old and New World: section *Pinus* in Eurasia/Mediterranean (subsections *Pinus* and *Pinaster*) and section *Trifoliae* Duhamel in North America, Mexico, the Caribbean and Central America (subsections *Australes*, *Ponderosae* and *Contortae*). *Pinus caribaea* is in subsection *Australes* with other species including *P. elliottii*, *P. cubensis*, *P. echinata* Mill., *P. occidentalis* Sw., *P. palustris* Mill., *P. pungens* Lamb., *P. rigida* Mill., *P. serotina* Michx. and *P. taeda* L.

The evolutionary history and ancient migration patterns of *P. caribaea* are still controversial. Relatedness between *P. caribaea* and *P. elliottii* var. *densa* from Florida has been implied by several authors, and links between North America, Central America and the Caribbean have been suggested. Chemical analysis of turpentine carried out by Mirov *et al.* (1965) revealed similarities between *P. caribaea* and other pines from Mexico and western North America. It has also shown a closer relationship between *P. elliottii* var. *densa* and *P. caribaea* than *P. elliottii* var. *elliottii*. A theory of pine migration from western North America to Mexico and Central America, and then further into the Caribbean and southern Florida was proposed, suggesting a possible migration during hurricane events. Study of plastid markers by Nelson and Nance (1994) also confirmed high similarities between *P. caribaea* var. *bahamensis* (from Andros island, Bahamas) and *P. elliottii* var. *densa* (from Florida, USA). Research by Dvorak *et al.* (2000) using random amplified polymorphic DNA (RAPD) suggested a common ancestor for subsections *Oocarpae* and *Australes* with the Mesoamerican ancestor of *P. caribaea* migrating southwards from Mexico to Central America and later into the Caribbean and Florida. The phylogenetic tree showed *P. caribaea* var. *hondurensis* in an intermediate position between the Central America and Florida/Caribbean clades. *Pinus caribaea* var. *bahamensis* and *P. elliottii* var. *elliottii* had a sister relationship (68% bootstrap support) and were both in the Florida/Caribbean clade which also included *P. caribaea* var. *caribaea* and *P. elliottii* var. *elliottii* (84% bootstrap support). A more recent study using plastid microsatellites supported this theory and suggested that *P. caribaea* var. *bahamensis* and *P. caribaea* var. *caribaea* originated from independent colonisation events to the Caribbean from a common Central American ancestor (Jardón-Barbolla *et al.* 2011). Zheng and Ennos (1999) used nuclear isozymes to infer that isolation of these varieties could have led to genetic drift and differentiation.

An alternative migration pattern has been discussed by other authors. An ancient migration route from southeastern North America to the Caribbean and further into Central America was inferred by Farjon (1996), based on a common ancestor for *P. caribaea* and *P. occidentalis* (from Hispaniola). Adams and Jackson (1997) supported this theory, reporting the existence of a Caribbean clade in subsection *Australes* (including *P. elliottii* and *P. caribaea*). They infer that the lack of diversification in this clade would have been the result of recent divergence and that similarities between Caribbean pines and *P. serotina* suggest a common North American ancestor and a migration route from North America to the Caribbean. More recent research based on comparative mapping of *P. elliottii* and *P. caribaea* also showed these species to be allopatric and supported a recent divergence time and common ancestor (Shepherd, M. and Williams 2008).

Many other species show links between the Antilles and Central America, North America and South America through long-distance migration and successive extinctions and re-colonization events (Delgadillo 2000, Santiago-Valentin and Olmstead 2004). Many similarities in the flora of south-east North America (Florida) and the Bahaman archipelago can still be observed. Common taxa present in pine forests of both areas include *Angadenia berteroi* (A.DC.) Miers, *Croton cascarilla* (L.) L., *Metopium toxiferum* (L.) Krug & Urb., *Zamia pumila* L., *Byrsonima lucida* (Mill.) DC., *Sabal palmetto* (Walter) Lodd. ex Schult. & Schult.f., *Tetrazygia bicolor* (Mill.) Cogn., *Bletia* *purpurea* (Lam.) DC. and many more (Correll and Correll 1982 , Austin 1998). Low endemism in the Bahaman flora and fauna (Ricklefs and Bermingham 2008) could have been the result of extinction events during the glacial and inter-glacial periods, associated to changes in sea level and climate (this chapter, sections 1.2 and 1.5). Further inferences on migration routes within the Bahaman archipelago will follow in Chapter 3 section 3.4.

1.4. Pine forest ecology

Pines have managed to successfully colonise and adapt to new environments. Wind dispersed seed, adaptation and resilience to prolonged dry seasons, resistance to fire and good regeneration have contributed to the existence and survival of pine forests in the Caribbean (Farjon 1996, Dvorak *et al.* 2005).

1.4.1. The pine forest ecosystem

Pine forests are open, savannah like, monospecific pine stands, with an undergrowth of herbs and shrubs (Farjon *et al.* 1997). *Pinus caribaea* var. *bahamensis* is the dominant taxon in the pine forests of the Bahaman archipelago (Figure 1.6). These pines grow mainly on honeycombed limestone substrate with pockets of soil, giving rise to the common name of ‘pine rockland’ for this kind of ecosystem in the Bahamas and Florida (Austin 1998). In the TCI it is locally known as ‘pine yards’ (Earle-Mundil 2010). Pine Cay, TCI, has the only pine forest growing on a sandy substrate. Pine forests are usually located in the intermediate area between wetlands and uplands, in the central part of the islands, where ridges provide some protection from the salt spray and there is enough fresh water for tree growth (Areces-Mallea *et al.* 1999). In low areas with high salinity, mangroves prevail; however, sometimes small blocks of stunted pine trees can be found growing on little raised ‘islands’ of soil among this kind of vegetation. In the TCI, the vegetation is limited by salinity, low rainfall and rock porosity (Proctor 1955). Pine forests cover smaller areas than in the Bahamas and trees are often stunted in growth (Figure 1.6).

The density of pine stands can vary from 28,000 to 49,000 trees/km^2 (2.8 to 4.9 trees/100 m^2) in some areas of the Bahamas. Density, diversity and height of the undergrowth depend mostly on soil, fire frequency and water availability (Myers, R. *et al.* 2004). Species composition varies slightly according to soil water retention. Some authors have even divided these forests into wet and dry barrens (Correll and Correll 1982) or *P. caribaea* var. *bahamensis* woodlands on limestone and *P. caribaea* var. *bahamensis* saturated woodlands (Areces-Mallea *et al.* 1999). Palms are often present in the undergrowth, with *Coccothrinax* spp. being more abundant in the Bahamas and *Sabal palmetto* in the TCI (Sealey-Sullivan *et al.* 2002). Many hardwood shrubs also grow in the understory, e.g. *Tabebuia bahamensis* (Northr.) Britton, *Metopium toxiferum, Tetrazigia bicolor, Sideroxylon salicifolium* (L.) Lam., *Acacia choriophylla* Benth., *Coccoloba diversifolia* Jacq., *Swietenia mahogani* L.; however, their size is usually limited by the frequent fires (Kjellmark 1996, Green 2011). Other climbers and shrubs include *Angadenia berteroi*, *Cassytha filiformis* L., *Erithalis fruticosa* L., *Ernodea serratifolia* Correll, *and Randia aculeata* L. (Green 2011). Orchids found in these forests include *Bletia purpurea*, *Eulophia alta* (L.) Fawc. & Rendle and *Calopogon tuberosus* var. *simpsonii* (Small) Magrath in the Bahamas and *Encyclia rufa* (Lindl.) Britton & Millsp. in the TCI, among many others (Correll and Correll 1982). Ferns like *Pteridium caudatum* (L.) Maxon are abundant in the Bahamas pine forests, sometimes covering large open areas and growing-up to almost 2m tall (Correll 1979). Endemic plants like *Stenandrium carolinae* Leonard & Proctor in the TCI (Earle-Mundil 2010) and *Lantana ovatifolia* Britton on Grand Bahama (Sanders 1987) are also present in these ecosystems. Density and size of the undergrowth have a direct effect on pine regeneration, as it will be explained in the next section.

Figure 1.6. Pine forests in the Bahamas and in the TCI. Monospecific stands of *P. caribaea* var. *bahamensis* and undergrowth with palms, shrubs and herbs on Abaco, Bahamas (left) and on Pine Cay, TCI (right).

This ecosystem is also home to several rare and threatened animals, which depend on this habitat for their survival, including the Andros boa *Epicrates striatus fowleri* (Durden and Knapp 2005), the Atala butterfly *Eumaeus atala atala* (Smith, E. M. 2002) and the IUCN red listed species Andros Island rock iguana *Cyclura cychlura* subsp. *cychlura* (Knapp, C. R. and Buckner 2004, Knapp, C. R. and Owens 2005), Bahamas parrot *Amazona leucocephala* (Stahala 2005, O'Brien *et al.* 2006, BirdLife International 2008a), Kirtland's warbler *Dendroica kirtlandii* (Radabaugh 1974, BirdLife International 2008b) and the Turks and Caicos rock iguana *Cyclura carinata* (Iverson 1978, Gerber 2004).

1.4.2. Forest regeneration and fire

The forests of *P. caribaea* are fire climax communities. Mature pine trees have a thick and fire-resistant bark, enabling them to withstand natural fires. The arrangement of the branches in open crowns and the protection of buds by bundles of needles are also adaptations to fire. Cones can protect seeds from burning and germination from the soil seed bank can occur and even increase after fires of low intensity (Habrouk *et al.* 1999, De las Heras *et al.* 2006).

The natural fire regime is similar to that observed in pine rockland habitat in Florida. They are frequent (every three to seven years) during the rainy season, triggered by lightning strikes and usually of low intensity (Sealey-Sullivan *et al.* 2002). These fires are fuelled by the pine needles and the undergrowth vegetation. They can travel fast through the exposed limestone, soil and vegetation, helping to remove competition to the pine seedlings. Space, extra light and nutrients available after the fire provide the essentials for forest regeneration, improving seed germination and establishment (Shirley 1945, Sealey-Sullivan *et al.* 2002, Prasad *et al.* 2007-ongoing).

When fires are suppressed or have not occurred for many years, hardwood trees and shrubs become dominant, pines do not regenerate and the ecosystem starts changing from pine forest to dry evergreen forest (Sealey-Sullivan *et al.* 2002, Myers, R. *et al.* 2004). Fire suppression can also increase fuel load due to an increase in the number of flammable shrubs, resulting in high intensity fires which can potentially kill mature pine trees and reduce diversity (O'Brien *et al.* 2010). Regeneration after fire will depend on seed source and environmental conditions for seed establishment. If mortality of mature trees is high, regeneration could be impaired by lack of seed source (Ross *et al.* 2009).

This is of high concern in the TCI, where most of the mature trees have died due to an exotic scale insect infestation. Too frequent fires can also kill seedlings and young trees and reduce pine regeneration (O'Brien *et al.* 2008).

The effects of fires in regeneration and change in vegetation will be discussed further in Chapter 2, and the importance of fire in forest management in Chapter 5.

1.4.3. Seed and pollen dispersal

Pinus pollen and seeds are wind dispersed. During pollen dispersal, mature pollen is released from the male strobili in a semi-dry state of < 10% water content. Its small size (60-67μm), surface ornamentation and low weight contribute to excellent aerial buoyancy and the ability to travel long distances (Doyle, J. H. *et al.* 2002, Fernando *et al.* 2005). Pine pollen can travel up to 1 km transported by turbulence; from 1 to 1000 km due to hurricanes, weather fronts and storms, cyclones and monsoons (Williams, C. G. 2010). However, circa 99% of total pollen falls close to the source (Williams, C. G. 2008). Distances of 78 to 140 m from the source have been reported for different pine species (De Lucas *et al.* 2008). Pollen of *P. caribaea* var. *bahamensis* usually disperses from January to March for several weeks and in vast quantities (Farjon and Styles 1997). Once the pollen reaches the female cone, it is captured by the pollination drop exuded by the micropyle located between the micropylar arms of the female cone (Fernando *et al.* 2005). It takes almost a year for fertilisation of the ovule to happen, and another year for the seeds to develop and mature (Owens *et al.* 2003). Second year cones ripen in late summer to early autumn during the rainy season. They can change rapidly from green to brown in the final stages of maturity; but they only open in dry conditions to release the seeds, usually in August/September (Henry 1974, Greaves 1978). Thus, there is enough moisture available for seed germination and no standing water in the soil, which can kill the seeds. The majority of pines can be self-fertilised. Nonetheless, cross-fertilisation is more common and produces higher number of viable seeds per cone (Laner 1998).

Seeds of *P. caribaea* are small, light and wind dispersed. Seeds disperse shorter distances than pollen. Studies on *P. halepensis* Mill. and *P. pinaster* Aiton, both wind-dispersed species, reveal seed dispersal distances of 15 to 60 m from the source (Nathan *et al.* 2000, González-Martínez *et al.* 2006). However, long-distance dispersal by hurricanes has been suggested previously. It has been observed in *P. caribaea* var.

hondurensis that cones could withstand flooding and still keep seed viability for germination after water receded (Dvorak *et al.* 2005). This could have occurred in the Bahaman archipelago; as deep channels separating the Little and Great Bahama Banks and Andros and New Providence are, respectively, circa 50 km and 30 km wide (Figure 1.2).

Patterns of pollen flow and seed dispersal play an important role in the genetic structure and diversity of populations; this will be discussed in Chapter 3, section 3.4.

1.5. Human action

Pine survival and forest regeneration depend on many natural factors, as outlined before; however, they can also be altered and hindered by human action. Past and present threats to this habitat include logging, human-ignited uncontrolled fires, invasive species, loss of habitat and insect pests. Threats to this ecosystem and some of its implications to forest loss, vegetation changes and loss of species richness are discussed below.

1.5.1. Logging

The Caribbean pine is an excellent tree for timber due to its straight trunk, lack of lower branches, close-grained wood and resistance to termites (March 1949, Kellogg 1951, Snyder *et al.* 2007). These pine trees have been used locally on a small scale since the first island inhabitants and settlers came to these islands. It has been reported that settlers used to extract wood from Grand Bahama in the 17th century (March 1949). There are no reports of any commercial exploitation of pine forests in the TCI, but there has been small local use of charcoal and timber for construction (Faizool 1998). However, large scale commercial exploitation, mostly for exportation, occurred in the Bahamas in the 20th century; with extraction of turpentine and also felling of trees for timber and pulpwood. Most pine forests in the Bahamas belong to the Bahamas government, being on state land or Crown land (The Commonwealth of the Bahamas 2005a). All commercial logging was undertaken on Crown land through concessions that the Bahamas government granted to The Bahaman Timber Company, The Bahamas Cuban Company, Abaco Lumber Company, Owens of Illinois Lumber Company and Owens-Illinois of the Bahamas Ltd. (Miller, W. 1920, Henry 1974, Radabaugh 1974). The licenses issued before 1966 stated that only trees with a dbh > 0.15m and height >

0.9m could be harvested. After 1967, a minimum tree size was not observed; however, five mature trees should be left per acre (equivalent to 1235 trees per km^2) (Henry 1974). Thus, young growth and seed bearers (mature trees capable of producing seed) would be a source for natural regeneration (March 1949, Kellogg 1951, Radabaugh 1974). An estimated area of exploitable pine forest in the Northern Bahamas of 1,457 km^2 was reported by Kellogg (1951).

Island	**Logging activity**	**Years**	**Area logged (km^2)**	**Wood volume (m^3)**
Abaco	timber	1905-1944	1,388	489,138
New Providence	timber	1923-1929	N/A	1,974
Grand Bahama	timber	1944-1956	535	221,665
Andros	timber	1948-1967	162	173,574
Grand Bahama	pulpwood	1957-1959	516	128,920
Abaco	pulpwood	1959-1967	634	2,689,329
Andros (North Andros)	pulpwood	1966-1969	183	596,012
Andros (Mangrove Cay)	pulpwood	1970-1974	N/A	N/A
Total		**1905-1969(74?)**	**3,418**	**4,300,612**

Table 1.3. Commercial logging activities in the Bahamas pine forests, based on data reported by Henry (1974).

All the islands containing pine forests in the Bahamas were exploited; however, some areas on the islands were not exploited. Type of exploitation, intensity and duration varied among islands, as shown in Table 1.3. On some of the islands, such as Abaco, part of the forest was felled more than once; explaining the high values observed for area logged and volume of wood extracted. Logging activities varied according to quality and size of the trees and lasted for 69 years. Operations started on Abaco in 1905 and this island was the most heavily logged of all, due to large tree stands and ease of access, followed by Andros and Grand Bahama (March 1949, Henry 1974). New Providence forest area is small in comparison with the other forests in the Bahamas. Forest exploitation only started in 1923 and by 1926 the pine forests were almost all clear cut (Miller, W. 1920, Kellogg 1951). Henry (1974) reported that the major damage to the pine forest on New Providence was caused by charcoal burners and timber removal for domestic use, instead of commercial timber production. In 1969, a large amount of pine seed (185kg) was collected from felled trees in North Andros to be sold to research stations abroad, other commercial companies and also to be stored for future use (Henry 1974, Greaves 1978).

Commercial logging activities ended in the Bahamas in 1974. Greaves (1978) reported that only circa 121 km^2 of forests remained unexploited in all the northern Bahamas; Little Abaco with 17 km^2 and South Andros with 104 km^2. Nowadays, the forests are only harvested by small local furniture businesses with legal permits (Myers, R. *et al.* 2004). Different levels of regeneration, from good to non-existent, have been reported and observed after logging activities ended (Henry 1974, Radabaugh 1974, Snyder *et al.* 2007). The effects of logging on forest regeneration and loss of gene diversity will be discussed in the following chapters.

1.5.2. Human ignited uncontrolled fires

Human ignited fires and pine forests share a long history in the archipelago. Slayton (2010) and Kjellmark (1996) showed a correlation between increase in fire frequency and human settlements dating back to circa 1000 yr AD, probably related to slash and burn agricultural practice. Adaptations of *Pinus caribaea* to fire allow it to survive natural fires and take advantage of these events for regeneration, as previously mentioned in section 1.4.2. However, non-seasonal and frequent fires (< three years) can have negative effects on pine survival and the pine forest ecosystem. Frequent annual fires can kill seedlings, young trees and shrubs (Myers, R. *et al.* 2004). These frequent fires favour the development of grasses and ferns, e.g. *Pteridium caudatum*, suppressing pine and shrub regeneration (Sealey-Sullivan *et al.* 2002). They also increase fuel load and fire intensity, which can damage the canopy and enhance tree death (O'Brien *et al.* 2010). A study in the pine forests of Abaco have shown that pine regeneration is low to non-existent in frequently burned areas, even when the fire intensity is low (O'Brien *et al.* 2008). Another detrimental effect of such fires is promoting the spread of invasive species, which will be discussed in the next section.

Most fires in the Bahamas are human ignited and occur during the dry season, mainly from April to June (Myers, R. *et al.* 2004). Miller’s study (2007) in an area of pine forest on Abaco showed that most fires were primarily human ignited and occurred every one to four years. From 2000 to 2008, an average of 141 fires per year was observed in the Bahamas using MODIS active fire remote sensing technology (Robbins *et al.* 2010). It was also reported that the main ignition sources in rural areas were uncontrolled fires for slash and burn agriculture or pasture improvement and intentional fires for hunting animals (mainly wild boar). In urban areas fire sources were campfires and intentional fires. Such events are not so common in the TCI, but they do occur occasionally, e.g. the fire in the pine forest of North Caicos in 2009. The effects of this devastating fire on the pine forest will be discussed in Chapter 2, section 2.4.

1.5.3. Agriculture and invasive species

Agriculture in the Bahamas and the TCI is on a small scale, and agricultural land covers only circa 1.5% of the Bahamas. Produce is sold mainly on the local markets, although Abaco and Grand Bahama produce and export citrus and vegetables (The Commonwealth of the Bahamas 1999). The main environmental problems associated with agriculture in the archipelago are the spread of invasive species and uncontrolled fires derived from slash and burn agricultural practices. Slash and burn agriculture has been used in the Bahamas since the first Lucayan Indian settlements (Blick 2007). This practice is common in areas with poor soil and a thin soil top layer. Trees are stripped, cut down and burned in order to clear and fertilize the soil before cultivation. This practice increases nutrients in the soil (N, P, K, Mg, Ca, S and micronutrients) in the first three to four months after the burn due to extra organic material and ashes; after that, the levels in organic matter and nutrients start to drop until they are similar to or below the initial state at the end of one year. After three to four years the soil is practically infertile and eroded, and crops become weak and more susceptible to pest attacks (Cochrane and Sánchez 1982, Sandford *et al.* 2001). Besides soil depletion, this kind of agriculture can reduce the number of species in the soil seed bank by > 50% in tropical forests (Ewel *et al.* 1981). It also favours the spread of invasive plants and increases the risk of uncontrolled fires.

A report for the Caribbean region carried out in 2003 for The Nature Conservancy (Kairo *et al.* 2003) listed 327 exotic plants growing in the region, with 179 of these species encroaching natural habitats or becoming invasive. The main invasive species observed in the archipelago are *Casuarina equisetifolia* L., *Leucaena leucocephala* (Lam.) de Wit, *Melaleuca quinquenervia* (Cav.) S.T.Blake, *Schinus terebinthifolia* Raddi and *Pennisetum purpureum* Schumach. (The Commonwealth of the Bahamas 1999, Sealey-Sullivan *et al.* 2002, Myers, R. *et al.* 2004, Hardman 2009). These species are all exotics in the Bahaman archipelago. They are fast growing, have an efficient reproductive system, and can spread quickly through disturbed and open areas. Thus, they can alter ecosystems, replacing native vegetation and reducing diversity (Sealey 2006). Many of them start in urban areas or agricultural fields. They benefit from fires, which clear vegetation, increase soil fertility and open new areas for colonisation (Davis *et al.* 2000, Hardman 2009). Effects of invasive species on pine plantations of *P. caribaea* have been observed in Trinidad, where elephant grass or Napier grass (*P. purpureum*) hinders pine regeneration and contributes to intense fires. Lack of light due to grass cover and hot fires fed by higher quantities of fuel (grass) kill seedlings and damage mature trees, favouring further invasions (Robbins *et al.* 2010). *Melaleuca quinquenervia* increases fire intensity and drives fires to the canopy, due to its fire resistant bark and highly flammable leaves rich in volatile oils (O'Brien *et al.* 2010).

Another factor of concern is that best land for agriculture in the archipelago is located inside pine forests. These areas are inland, which make them less subject to salt spray; they are mainly in rural and uninhabited or low populated areas, have good water resource and are flat. A road was started in South Andros by the Bahamas Agricultural and Investment Corporation in 2010 to provide access to pine forest area for use as agricultural land. This road cuts through circa 4.8 km of old-growth dry evergreen forest (coppice) before reaching one of the few old-growth pine forests in all of the Bahamas. A rapid assessment of the area conducted in 2010 for The Nature Conservancy has shown that apart from the destruction of ecosystem balance, loss of biodiversity and fragmentation in these unique forests, there can be alterations to fire frequency and introduction of invasive species (Lloyd and Slater 2010). Fortunately, recent expansion of the West Side National Park on Andros encompassing the pine forests of Mangrove Cay and South Andros has stopped threats and protected this important area of old-growth forests (The Bahamas National Trust 2012). Nonetheless, the increases in population numbers will only enhance food demand and the need for agricultural land, putting more pressure on forest ecosystems across the archipelago.

1.5.4. Insect pests

Exotic or alien insect species have become a worldwide problem due to the movement of plants, soil and forestry products between countries (Liebhold 1995, Pimentel *et al.* 2005). Most of these species thrive and multiply due to lack of natural enemies, lack of built-up resistance by the native vegetation, fast adaptation to new environments, efficient predation of local food sources and habitat disturbance (Liebhold 1995, Davis *et al.* 2000). The proximity of the Bahaman archipelago to the southern USA and other Caribbean islands, similarities in climate, extensive importation of goods and lack of control and quarantine facilitate the movement of pests in the region. More than 900 exotic insect species and circa 11,500 native insect species have been observed in

Florida (Pimentel *et al.* 2005). Many insect pests have been recorded in the Caribbean region in the past, and many more are still being found nowadays; many of these spreading from agricultural crops and ornamental plants into the forest ecosystem. The Caribbean Plant Protection Commission has recommended regulations for 419 pests in the Caribbean region up to 2003 (Kairo *et al.* 2003).

Reduced growth and reproduction, tree death, loss of biodiversity and vegetation shifts are some of the consequences of pest attacks. More than 360 exotic species of insects are present in the forests of the USA, costing the country more than 13.5 billion US dollars per year (Pimentel *et al.* 2005). An example of rapid outbreak of pine shoot beetle *Tomicus piniperda* L. happened in the USA, and in only two years this pest had spread through six U.S. states into Canada (Liebhold 1995). In the 1990s, an attack of spruce trees (*Picea* spp.) by spruce beetle (*Dendroctonus rufipennis* Kirby) in Alaska caused great tree mortality and a shift from spruce forest to grasslands in the area. Warm weather, drought-stressed trees and a change in the life cycle of the insect from semivoltism (one generation every other year) to multivoltism (more than a generation a year) were the main causes for this insect outbreak and the severe damage it caused to the forests (Logan *et al.* 2003, Berg *et al.* 2006).

Similar conditions could have led to the outbreak of pine tortoise scale insect (*Toumeyella parvicornis* Cockerell) in the TCI. This exotic pest was probably introduced to the TCI on live, cut Christmas trees imported from the USA by local residents. Since the insect was first recorded in 2005, it has spread to all three islands containing pine forests and caused high levels of tree mortality and serious risks for the ecosystem survival (Hamilton 2007). Reduced photosynthesis, weakening and die back of the trees happen when the insects feed on their sap and sooty mould (ascomycete fungus) grows on excreted honey dew covering the leaf surfaces (Figure 1.7). This insect species is known from Mexico, USA and Canada, where it has one or two life cycles a year, but it could have evolved to having more than two generations per year in a warmer country (Malumphy *et al.* 2012). This factor, in conjunction with abundant food source (pine trees), drought-stressed trees that are less able to recover from infestations and maybe a lack or low number of predators are probably the causes of the success of this invasive pest and the severe threat to the pine forests in the TCI. Further considerations and discussion will follow in the next chapters.

Other insect pests have been observed in the pine forests of the archipelago in the past and during this work . Kellog (1951) reported pine tree death due to southern pine bark beetles (*Dendroctonus* spp. and *Ips* spp.) in an area of 1.2 km^2 on New Providence, Bahamas. He suggested that drought could have increased tree susceptibility to attack. Similar infestations have also been observed throughout Central America in *P. caribaea* forests (Billings *et al.* 2004) and in mixed forests in South Carolina, USA, after Hurricane Hugo (Gardner *et al.* 1992). Another study by Christiansen *et al.* (1987) showed that pine tree resistance to pine bark beetles was related to carbon balance of the trees. Loss of canopy or reduction in photosynthetic capacity, often associated with extended drought, damage by fire or lightning, and competition for light and nutrients, could reduce the defence of trees and increase damage caused by insects.

The introduction, spread and consequences of invasive pests poses a real threat in small, low-lying islands with warm climates and subjected to hurricanes and droughts; like those found in the Bahaman archipelago.

Figure 1.7. Tree mortality and infestation by pine tortoise scale insect in the TCI. Dead pine trees on the left, and on the right infested young sapling covered in sooty mould.

1.6. Plantations and other uses

The Caribbean pine is an important economic species in many countries across the world. This pine has been used widely in plantations in tropical and subtropical lowlands, because it grows in poor soils, withstands warm temperatures and low rainfall, is fire resistant, grows fast and its wood is of good quality (Kemp 1973). Most plantations were established in the 1960s, 1970s and 1980s. In the Caribbean region, provenance trials were carried out in Costa Rica, Trinidad, Jamaica, Puerto Rico and Venezuela (Cruz 1988, Liegel 1991). Experimental research and commercial plantations are also present in South Africa, Nigeria, Zimbabwe, Venezuela, Brazil, Argentina, Colombia, India, the Philippines, Fiji and Australia (Barnes *et al.* 1977, Le Maitre 1998, Moura and Dvorak 2001). Several genetic improvement programme for *P. caribaea* were developed in Australia, South Africa and Zimbabwe (Nikles 1996, Hodge and Dvorak 2001, Evans 2003).

Plantations with exotic pines were originally introduced into disturbed, unproductive land to restore forest cover and boost economic growth (Kemp 1973). Nonetheless, they have caused ecosystem disruption and loss of biodiversity in many areas, mainly where they replaced native vegetation. However, in highly disturbed areas they can provide habitat for native species (Brockerhoff *et al.* 2008). A study on forest regeneration in Sri Lanka has demonstrated that *P. caribaea* trees can be used as a pioneer species in reforestation. They provide protection for late-successional native trees, and once native forest is established pines can then be removed (Ashton *et al.* 1997). Regeneration of grasslands in anthropogenic sites in Uganda has also been improved and speeded up in areas with pine plantations in comparison with open areas (Zanne and Chapman 2001).

Other uses of *P. caribaea* include the removal of oleoresins for the production of gum resin and turpentine (Jantan and Ahmad 1999) and the extraction of tannins from the pine bark to be used in many different products in the pharmaceutical, leather and wood industries (Almeida 2006). New research includes the use of pine leaves to produce natural insecticides against *Aedes aegypti* L. mosquitoes, which transmits the dengue virus (Kanis *et al.* 2009).

1.7. Legislation and conservation areas

Legislation protecting the flora and fauna and regulating conservation areas varies in the Bahamas and the TCI. In the TCI, the first law created to protect the local flora was the Plant Protection Ordinance in 1921. It regulated the importation of diseased or invasive plants. In 1975 the National Parks Ordinance recognised the conservation of natural areas designating national parks, nature reserves, sanctuaries and areas of historical interest, but the first protected areas were only established in 1992. At the same time the Turks and Caicos National Trust (TCNT) was created and given the responsibility to manage several of the national parks and reserves; including the Conch Bar Cave National Park on Middle Caicos. This National Park is the only protected area containing pine forests in the TCI. A new Ordinance in 1989 gave power to the

Governor to declare new conservation areas and require environmental impact assessments (EIA) for new developments in protected areas (Garland-Campbell 2008).

In the Bahamas, the legislation has protected forests against illegal logging and fires through its Penal Code since the beginning of the 20th century (Henry 1974). A Plant Protection Act, similar to the one in the TCI, has been in place since 1916. In 1964, the Agriculture and Fisheries Act gave power to the Minister of Agriculture and Fisheries to declare protected areas. In 1959, the responsibility to manage and oversee protected areas was transferred to the Bahamas National Trust (BNT) (The Commonwealth of the Bahamas 2005a). The Bahamas Environment, Science and Technology Commission (BEST) was instituted in 1994, and some of its responsibilities included the coordination of policies and programmes for protection of the environment and to develop National Action Plans. The first National Biodiversity and Action Plan was published in 1999 (The Commonwealth of the Bahamas 1999) and a more recent National Environmental Management Action Plan in 2005 (The Commonwealth of the Bahamas 2005a). A National Forest Policy was adopted in 1998, following the Physical Landscape of the Bahamas Act in 1997, which declared the Caribbean pine a protected species. It was not until 2010, that the Bahamas government instituted the Forest Unit and the National Forest Plan through the Forestry Act. This act regulates the declaration of forest reserves, protected forests and conservation forests, issuing of licenses, permits and leases, offences such as illegal logging and fire, and other issues (The Commonwealth of the BahamasThe Commonwealth of the Bahamas 2010).

The Bahamas have five National Parks containing large tracts of pine forests. The Lucayan National Park, established in 1970, and the Rand Nature Centre, established in 1992, are both on Grand Bahama and have an area of, respectively, 0.16 km^2 and 0.4 km^2. The Abaco National Park was created in 1994 and covers an area of 82 km^2. Blue Holes National Park, part of the Central Andros National Parks, was established in 2002 and covers an area of 134.5 km^2. The West Side National Park on Andros, created in 2002 and recently expanded, is the largest of all with an area of 5260.9 km^2, including the west side of Central Andros, Mangrove Cay and South Andros (The Bahamas National Trust 2012). More details about these protected areas and pine forest management will follow in Chapter 5.

1.8. Thesis outline

The distribution and size of the pine forests in the Bahaman archipelago is currently unknown and so is the genetic structure and variability of the populations of *P. caribaea* var. *bahamensis*. Morphological adaptations of the species throughout the archipelago to different ecological factors are also not well studied. In order to understand the effects of natural disasters and anthropogenic action in forest regeneration and the genetics of the species, information about the historical forest distribution and its changes through time will be needed. Better knowledge of the species genetics, morphological adaptations, size, distribution and response to external pressures can have a large impact on the future conservation of these pine forests and the species in the Bahaman archipelago.

In Chapter Two past and current distribution and size of the pine forests in the archipelago are estimated through the analysis of historical maps and satellite imagery. Changes in forest area derived from stochastic events and urbanisation are reviewed and future threats identified. Genetic structure and variability of the species across its distribution range, using plastid and nuclear microsatellite regions, is the focus of Chapter Three. Morphological and ecological data collected from pine forests in all seven islands where the species occur are compared in Chapter Four. Threats, variations and adaptations of the species will also be identified at a regional and local level. This is followed by a general discussion and suggestions for the conservation and management of these pine forests in Chapter Five, based on observations and results obtained.

Chapter 2 . Biogeography

2.1. Introduction

The range occupied by a species and population sizes are usually not fixed through time in natural systems. Population decline or expansion depends on biotic and abiotic factors, such as habitat, climate, genetic pool, mating system and other natural and human pressures. The study of the biogeography of a species, which encompasses its distribution in space through time, is important in detecting and quantifying these spatial changes, identifying the driving forces for such changes and in enhancing knowledge of species biology and ecology. Therefore, biogeography is not only based on the comparison of historic and modern geographical distribution data, but also comprises patterns of variation, interactions of these organisms with the physical and biotic environment and the effects of natural and human disturbances on their distribution (Lomolino *et al.* 1953/1998). Geographical Information System (GIS) and remote sensing have advanced the science of biogeography, enabling not only the compilation and manipulation of huge quantities of data, but also comparisons and simulations of a wide range of ecological and geographical parameters, such as vegetation, soils and land uses (Lomolino *et al.* 1953/1998).

Remote sensing allows the direct observation, analysis and measurements of variables, the production of maps and the detection of changes in time and space. It can also provide the support needed for decision making in species conservation and management. It has been widely used in the past decades, becoming an important tool in the fields of conservation biology and ecology (Horning *et al.* 2010). The comparison of recent and historical imagery allows the mapping and measurement of changes through time in distribution area and size for a species or individual populations. GIS is a computer system used to visualise, store and analyse geographical data (Maguire 1995). It allows the visualisation of remote sensing imagery, digitized physical maps and many other geo-referenced data layers, e.g. roads, collection points and vegetation boundaries, the graphical manipulation and spatial analysis of the data and the production of maps (Horning *et al.* 2010). These systems also provide a good range of options for maps, layouts and outputs and are widely used in cartography (Buckley *et al.* 2005).

GIS, remote sensing and ecological data are important tools in biogeography. They assist in the mapping of species distribution, biodiversity and land cover, in the identification of environmental and anthropogenic pressures, and in the management of conservation areas (Foody 2008). Du Puy and Moat (1996) created a GIS of Madagascar to visualise, overlay and analyse maps of primary vegetation, geology and protected areas in the region, in order to evaluate and guide conservation of the local flora. Another study by Améztegui *et al.* (2010) estimated expansion patterns for *P. uncinata* Ram. in northeast Spain during the second half of the 20th century, correlating this expansion with topographical and socio-economic variables, using sets of aerial photography from 1956 and 2006. Forest fragmentation in western Kenya in the past 90 years has been observed through GIS analysis of older aerial photography, topographical maps and more recent satellite imagery (Mitchell *et al.* 2006). GIS has also been used to detect areas of suitable habitat for future introduction of *Triunia robusta* (C.T.White) Foreman in south-east Queensland, Australia, based on modern and historical species distribution (Powell, M. *et al.* 2005).

Very few maps of land use and land cover are publicly available for the Bahamas and almost none for the TCI. Some previous studies and maps for the Bahamas included land use on New Providence in 1961 and 1979 by Bounds (1981b), pine forest area, logging activities and forest regeneration in most of the Bahamas in the late 1960s and early 1970s by Henry (1974) and vegetation and land use maps of the early 1970s by the Directorate of Overseas Surveys (D.O.S) and the Bahamas Department of Lands and Surveys. More recent maps for the Bahamas were developed for the Bahamian Archipelago Landcover Mapping Project by The Nature Conservancy (TNC), the US Forest Service International Institute of Tropical Forestry (IITF), the EROS Data Centre (EDC) and the United States Geological Service (USGS). Land cover and land use maps were produced using LANDSAT Thematic Mapper (TM) satellite images from 2000-2001 and image analysis (Sealey-Sullivan *et al.* 2002). Restricted access to these latter maps; older imagery and different methodology from this work were the main points for deciding not to include them in this research.

Updated maps of the pine forest distribution in the archipelago are important for future conservation work and studies of changes in pine forest distribution, pine population sizes and adaptations to environmental and human pressures. Green (2011) identified the need for estimates of population size and area in order to conduct more accurate conservation assessment and effective management of the Caribbean pine in the Turks and Caicos Islands.

This chapter will focus on mapping the pine forests of the Bahaman archipelago, estimating the past and modern distribution and area and detecting some trends in forest expansion or decline related to external pressures, such as natural disasters, development, economic activities and pests.

2.2. Material and Methods

2.2.1. Bahamas

Detailed information on methodology used to map and estimate pine forest area in the northern Bahamas follows and a simplified workflow is shown in Figure 2.8.

2.2.1.1. Satellite imagery and Geographic Information System (GIS)

A GIS project for the northern Bahamas was created by the GIS Unit at the Royal Botanic Gardens, Kew (Kew) using ESRI® ArcMap™ v.9.2 (©1999-2006 ESRI Inc.) (Figure 2.1). The raster images used were Orthorectified Landsat Thematic Mapper (TM) Mosaics by GeoCover™. Source Landsat imagery dated from 1990 (+/- 3 years), had seven TM bands and a mixed resolution of 28.5m and 30.0m. Landsat Mosaics (5° latitude × 6° longitude) were in MrSID™ compressed format, 24-bit colour, with three spectral bands (Band 7, mid-infrared light, 2.08 to 2.35µm, displayed as red; Band 4, near-infrared light, 0.76 to 0.90µm, displayed as green; and Band 2, visible green light, 0.52 to 0.60µm, displayed as blue) (Tucker *et al.* 2004). Images with bands 7,4,2 RGB have good definition between land and water and distinctiveness among vegetation types (Horning *et al.* 2010). Water is shown in blue and vegetation in green (Figure 2.2, bottom). Images were path oriented, with datum World Geodetic System 1984 (WGS84) and projection Universal Transverse Mercator (UTM) and then orthorectified using nearest neighbour. LOCAL (Locally Optimized Continuously Adjusted Look-up-tables) stretch was applied for contrast enhancement. Absolute positional accuracy was <50m root-mean-square error. The GIS was used for data collecting, handling and processing and for the production of thematic maps.

On-screen digitizing, also called heads-up digitizing, was the method used to create polygons containing pine forest vegetation. Although feature recognition algorithms can be used to identify different features in an image, e.g. vegetation types and water bodies, visual interpretation by a user is usually more accurate and reliable. On the other hand, this can be a time consuming process when dealing with large areas (Horning *et al.*

2010). This research used only visual interpretation of imagery, due to small area covered and easy identification of target vegetation, i.e. pine forests. Identification of vegetation was based on satellite imagery from Google™ Earth v.5. Satellite imagery was either ©GeoEye (IKONOS) or ©Digital Globe (QuickBird), with resolution, respectively, 3.28m and 0.65m, and positional accuracy of <25m. The dates of these images varied from island to island, as follows: Abaco images 2003-2008, Andros 2001-2008, Grand Bahama 2002-2010 and New Providence 2004-2007. The benefits of fine spatial resolution imagery, such as IKONOS and QuickBird, in ecological studies and in the identification of vegetation types have been highlighted in the past (Aplin 2005).

Visual interpretation of colour, texture and shades produced by different kinds of vegetation was used to spot and delimit pine forests. In the pine forest habitat, pine trees are much taller than the undergrowth, casting more shade on the ground. These characteristics in association with the presence of a round canopy and dark green colour make it possible to distinguish pine forest from other broadleaf vegetation, e.g. coppice or scrub. Salt marshes or wetlands are also easily differentiated from pine forest, as they are usually flooded, pinkish or brown in colour and with lower vegetation (Figure 2.2). Google™ Earth imagery was more recent and enabled visualization of individual trees, having better resolution than the Landsat imagery used in the GIS (described above), thus the choice for their use in the identification of forest type and drawing of pine forest boundaries and coastlines. Forest boundaries were manually drawn from 500m eye altitude images, which provided the clearest identification of different vegetation types. Areas of pine forests <0.001 km² were not digitized, as they were smaller than the map scale. Some areas lacked good resolution imagery or had high cloud coverage, e.g. parts of Andros and Grand Bahama (Figure 2.3). In this case, forest boundaries were drawn at an eye altitude of 2.5 km in Google™ Earth or directly in the GIS project through observation of colour and texture of imagery and comparisons with previously identified forest areas during ground truthing. Island coastlines were all digitized at 300m eye altitude in Google™ Earth, and boundaries were drawn at high water marks visible on the imagery, including tidal areas with vegetation present (wetlands, mangroves, salt marshes) and continuous land. Major roads on each island and access roads in pine forests were also digitized. Lakes and ponds were digitized directly in the GIS.

Forest and coastline polygons and roads polylines were saved as .kml files (Keyhole Markup Language) and imported into the GIS using ESRI® ArcView GIS v.3.3 (©1992-2002 ESRI Inc.). The ArcView 3.x extension (kml2shp.avx), a converter for points, lines and polygons, was used to convert .kml files into shapefiles (.shp). The application of another extension (3d_to_2d.avx) was necessary to convert 3d shapefiles to 2d shapefiles, as Google™ Earth shapefiles contain additional height values (Z). Projection datum WGS84 for each new shapefile was applied in ESRI® ArcCatalogue v.9.3 (©1999-2008 ESRI Inc.). Final shapefiles were then added as layer themes to an ESRI® ArcMap™ v.9.2 (©1999-2006 ESRI Inc.) project and exported with their final projection of UTM 18N. Several other layers were added to the GIS project, e.g. ground truthing points, DNA and data collecting points and genetic data (Figure 2.1).

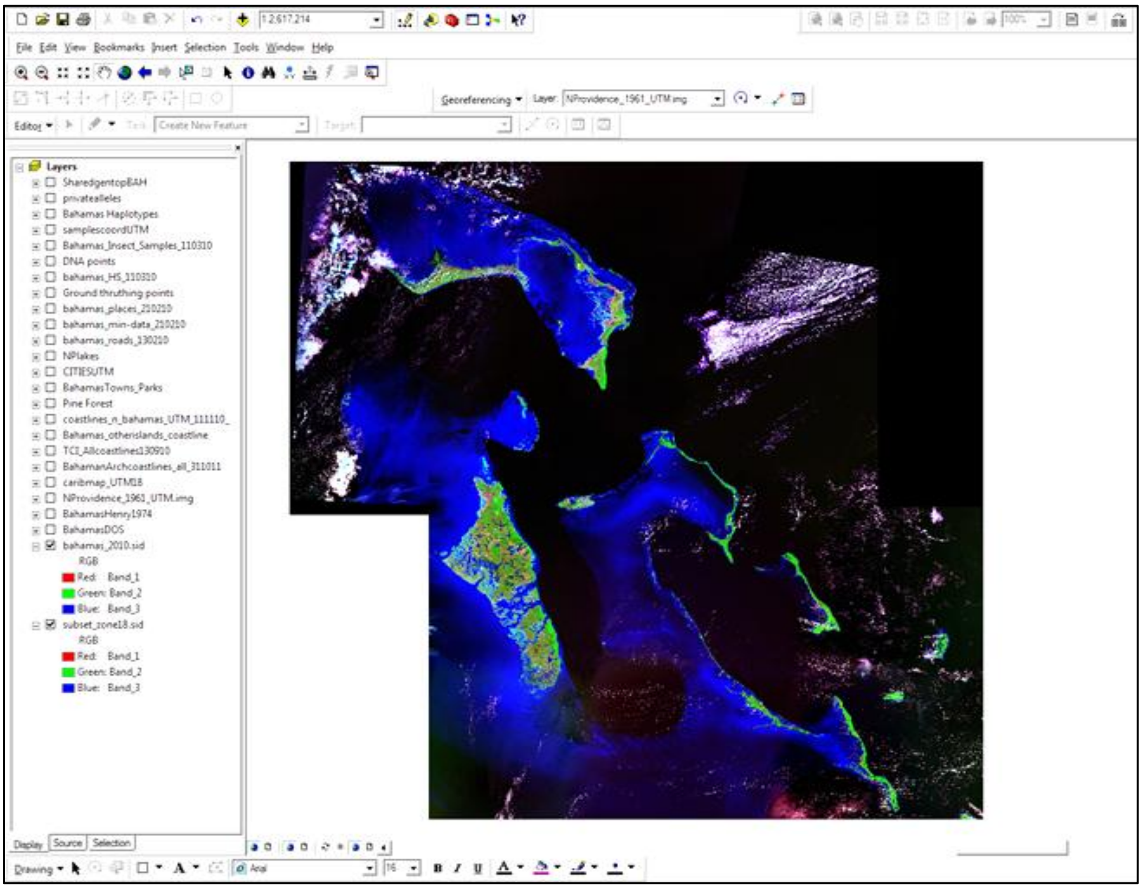


Figure 2.1. GIS of the northern Bahamas showing raster images and layers in ArcMap™ v.9.3

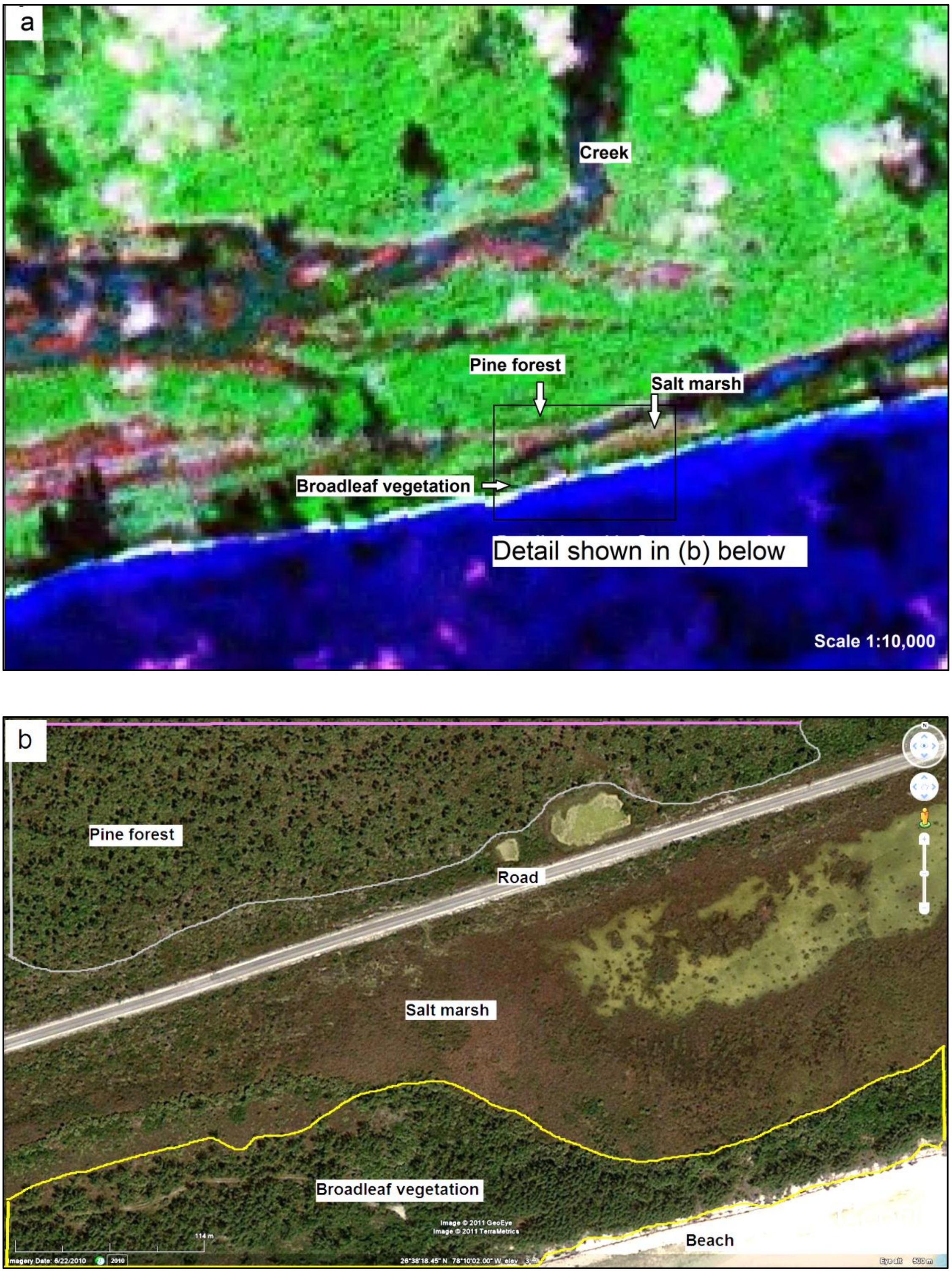


Figure 2.2. Comparison of Landsat Thematic Mapper mosaic image (a) and Google™ Earth v.5 satellite imagery (b), showing vegetation types and features. Images from Grand Bahama Island. Google™ Earth image (b) is a detailed view at 500m eye altitude of highlighted area in (a). Individual trees and different vegetation types can be easily identified in the higher resolution imagery.

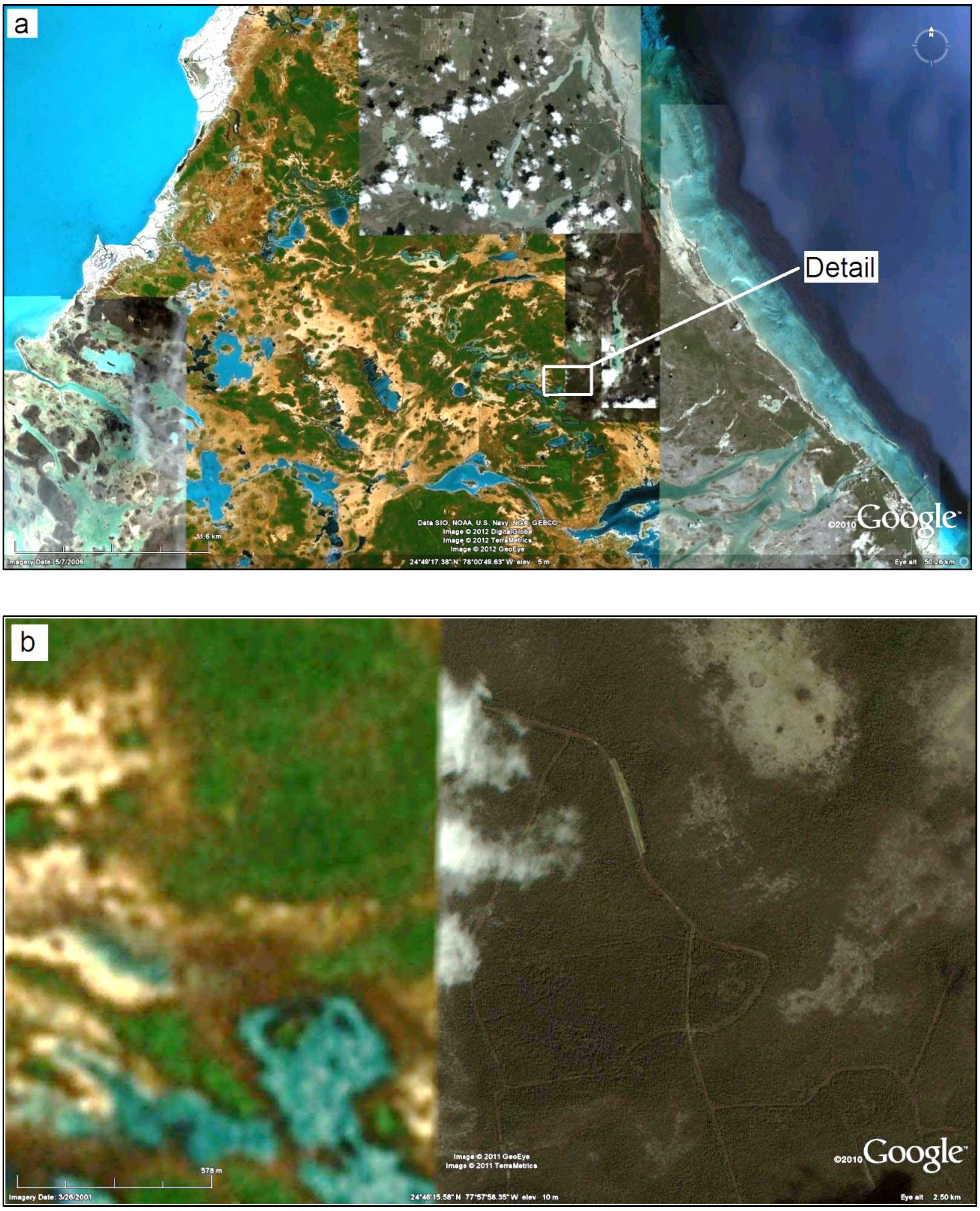


Figure 2.3. Mosaic of images (tiles) for northern Andros viewed in Google™ Earth v.5. Area highlighted in (a) is shown in detail in (b). Low image resolution and presence of clouds across tiles and regions can lower the accuracy of drawn forest boundaries.

2.2.1.2. Historical maps

A total of 20 physical maps of land cover and land use for the islands of New Providence, Abaco, Andros and Grand Bahama containing pine forest boundaries were digitized and used to estimate pine forest area in the Bahamas during the 1960s and 1970s. Maps of land cover and forest regeneration for most parts of the islands were obtained from D.O.S. maps containing Henry’s (1974) survey of the Bahamas pine forests, logging activities and its subsequent regeneration. Additional maps from the D.O.S and the Department of Lands and Surveys, Nassau, Bahamas, provided information for the rest of the areas not previously covered. The map of land use in New Providence in 1961 produced by Bounds (1981b) was also used for mapping vegetation changes in this island. Map sources and dates are provided below and coverage of these maps is shown in Figure 2.4 (Bounds 1981 not represented in the image):

Abaco- Abaco forest types and regeneration maps A, B and C (1973), 1:50000, D.O.S. Bahamas 3092R, 3092S and 3092T (Directorate of Overseas Surveys 1973a, b, c). Prepared by D.O.S. in 1973 and printed by Ordnance Survey in the same year, © Bahamas Government 1973. Base map derived from Bahamas 1:25000 series D.O.S. 358 (1963); with a major revision from 1967 air photography by Owen & White Ltd. Forest types and regeneration information supplied by P.W.T. Henry in 1971 based on 1958 aerial photography, 1:14,000, and 1969 field observations (74/733344 S, 74/733345 S and 74/733346 S). Detail of Little Abaco present in Map A was scanned and digitized separately.

Andros- Andros forest types maps A, B, C and D (1974), 1:50000, D.O.S. Bahamas 3092V, 3092W, 3092X and 3092Y (Directorate of Overseas Surveys 1974a, b, c, d). Prepared by D.O.S. in 1973 and printed by Ordnance Survey in 1974, © Bahamas Government 1974. Base map derived from Bahamas 1:25000 BLS series 315 (1970). Forest types information supplied by P.W.T. Henry in 1971, based on 1958 aerial photography, 1:14,000, and 1969 field observations (74/740248 S, 74/740249 S, 74/740250 S and 74/740251 S).

Grand Bahama- a) Grand Bahama forest types and regeneration sheets 1 and 2 (1973), 1:50000, D.O.S. 3092Q (Directorate of Overseas Surveys 1973d, e). Prepared by D.O.S. and printed by Ordnance Survey in 1973. Base map derived from Bahamas 1:25000 series, D.O.S 358 (1963), © Bahamas Government 1973. Information on forest types and regeneration supplied by P.W.T. Henry in 1971 (74/733347 S, sheets 1 and 2). **b)**

Grand Bahama and Abaco (1963), sheets 9 to 15, 1:25000, UTM 17N, and D.O.S. 358, Series E811 (Directorate of Overseas Surveys 1963a, b, c, d, e, f, g). Prepared and published by D.O.S. in 1963, © The Crown 1963. Maps were based on aerial photography from 1958 and 1959, and field survey from 1962.

New Providence- a) Land use of New Providence in 1961 by Bounds (1981b, a), based on aerial photography from the 1950s and earlier and personal observations and ground truthing. **b)** New Providence maps sheets 1 and 2 (1975), 1:25,000, UTM 18N, BLS Series 316, Ed.1(Department of Lands and Surveys 1975a, b). Prepared and published by the Department of Lands and Surveys, Nassau, Bahamas and printed by Ordnance Survey, © The Commonwealth of the Bahamas 1975. Maps based on 1974 aerial photography and field observations by the Department of Lands and Surveys in the same year.

All maps were either photographed with a Leaf digital camera (56 megapixels) or scanned in a HP Designjet T1120 SD (300×300 dpi); except the 1961 map of New Providence, which was scanned in an HP Photosmart CN245. Raster images were then imported into ArcMap™ v.9.2. Spatial references (WGS84/UTM 17N or WGS84/UTM 18N) were added to the raster images in ArcCatalogue v.9.3, following the projections of the original maps. Images were then geo-referenced in ArcMap™ 9.2, matching distinctive features visible in the satellite imagery of the area against the same features or points in the GIS coastline and road layers. Geo-referenced images were saved as Imagine files (.img), added to ArcMap and re-projected into WGS84/ UTM 18N, if not already in this projection.

Maps could then be visualised digitally and polygons drawn over pine forest boundaries drawn on the map, using on-screen digitizing. Transition areas containing a mix of broadleaf and pines were not considered as pine forest in this research. New layers containing historical pine forest for each island were created. The year recorded on each historical map for field observations or survey was used as the year representing pine forest status in that area, instead of date of map publication. This seems to be a more accurate representation, due to the fact that many maps were published several years after ground truthing (field observations), and changes to these forests could have occurred in the meantime. Thus, historical forest status was represented for New Providence in 1961 and 1974, for western Grand Bahama in 1962, and for eastern Grand Bahama, Abaco and Andros in 1971. Final layers were added to the GIS project.

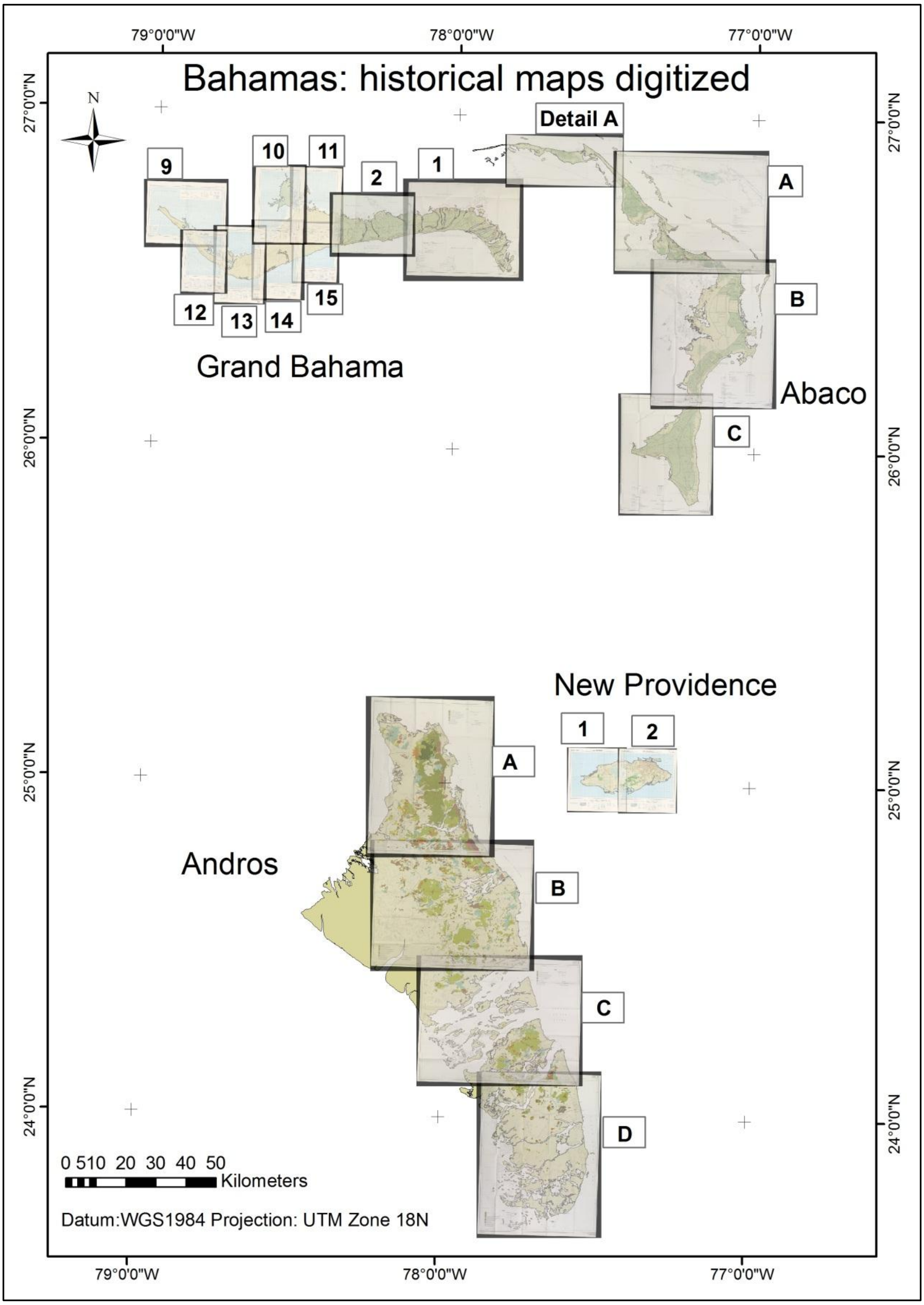


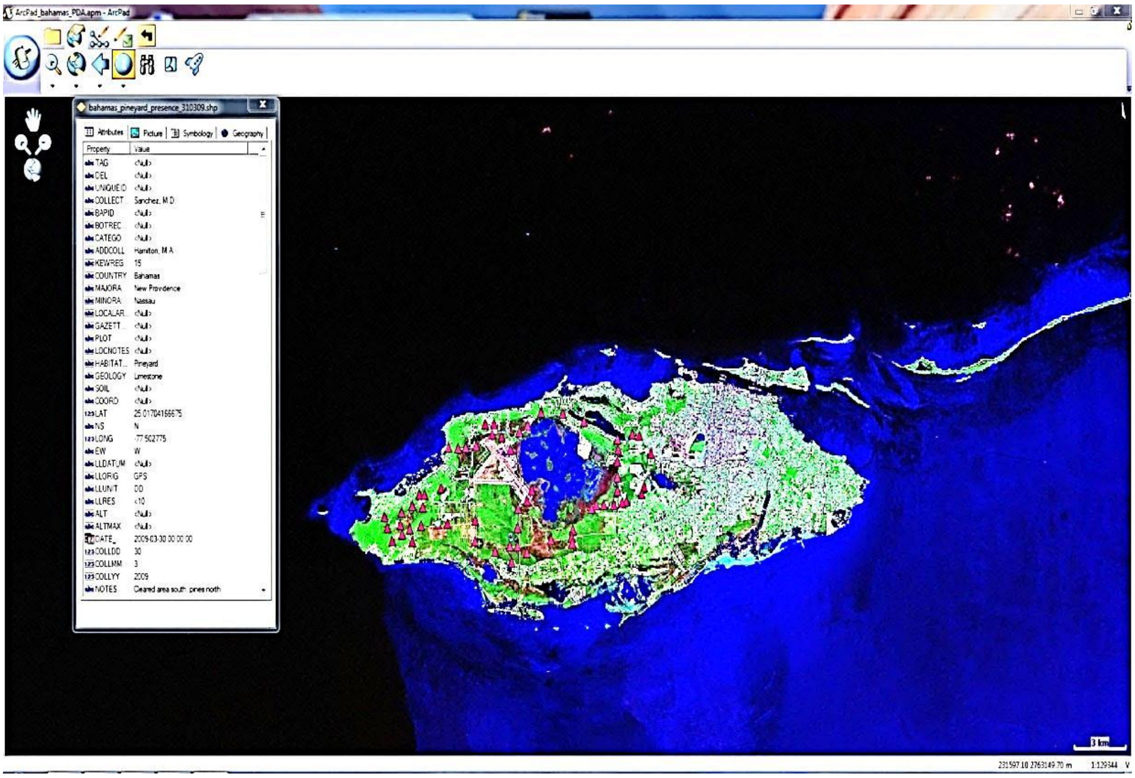
Figure 2.4. Historical maps for the northern Bahamas processed and imported into the GIS for estimation of historical pine forest. Letters and numbers correspond to original labelling of map sheets by the D.O.S. or Department of Lands and Surveys. Map numbers and references are found in the main text (section 2.2.1.2)

2.2.1.3. Ground truthing

Ground truthing was carried out on the islands of Abaco, Andros, Grand Bahama and New Providence (northern Bahamas). In 2008 and 2009, all islands were visited during a two-week period in February and/or March. In February 2010, the field visit was restricted to the islands of Andros and Grand Bahama. The island of Andros required a different sampling strategy, due to its large size and difficulty of access. In the first year, fieldwork was done in North Andros; followed by Mangrove Cay and South Andros in the successive years. A total of 885 points with associated GPS coordinates were recorded for presence or absence of pine forests during ground truthing. The number of points varied among islands; 565 for Grand Bahama, 153 for Andros, 106 for Abaco and 62 for New Providence. Most points were recorded while hiking or driving; however, in areas with restricted access, visual assessments were made from boats or airplanes. Presence or absence of pine forest was also recorded for the DNA sampling plots and used for ground truthing. All ground truthing points are shown in Figure 2.6.

Data collecting targeted the recording of as many points as possible throughout the islands, including forested, non-forested and transition areas. Field data was recorded on handheld computers with built-in GPS (Fujitsu-Siemens N560) running ArcPad™ v.7 (©2002-2006 ESRI Inc.) or Asus® Eee PC™ Netbook connected to BlüeNEXT BN-901S Bluetooth© GPS with an accuracy of +-10m, running ArcPad™ v.8 (©1995 - 2009 ESRI). Data from the updated GIS was transferred to the handheld computers before fieldwork, e.g. raster images, pine forest boundaries, roads, coastlines and previous data points. Ground truthing points, including habitat information along with other data and GPS coordinates, were recorded in the field using pre-defined tables (Figure 2.5). Main table fields included geographical location (e.g. village, island), GPS coordinates, habitat (pine forest, other vegetation type or anthropogenic) and notes. Data was then used to refine pine forest boundaries.

All data collected was exported to a database created using Brahms v.6.04 (©1985-2010 Department of Plant Sciences, University of Oxford), where it could then be ‘cleaned’, manipulated and exported to other software.



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BAPID	<Null>
BOTREC	<Null>
CATEGO	<Null>
ADDCOLL	Hamilton, M.A
KEWREG	15
COUNTRY	Bahamas
MAJORA	New Providence
MINORA	Nassau
LOCALAR...	<Null>
GAZETT...	<Null>
PLOT	<Null>
LOCNOTES	<Null>
HABITAT...	Pineyard
GEOLOGY	Limestone
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COORD	<Null>
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EW	W
LLDATUM	<Null>
LLORIG	GPS
LLUNIT	DD
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ALT	<Null>
ALTMAX	<Null>
DATE_	2009-03-30 00:00:00
COLLDD	30
COLLMM	3
COLLYY	2009
NOTES	Cleared area south: pines north

Figure 2.5. Screen shot of ArcPad™ project used for data collection in the field; showing ground truth points (pink triangles) on New Providence and attributes table (left) for a selected point as an example.

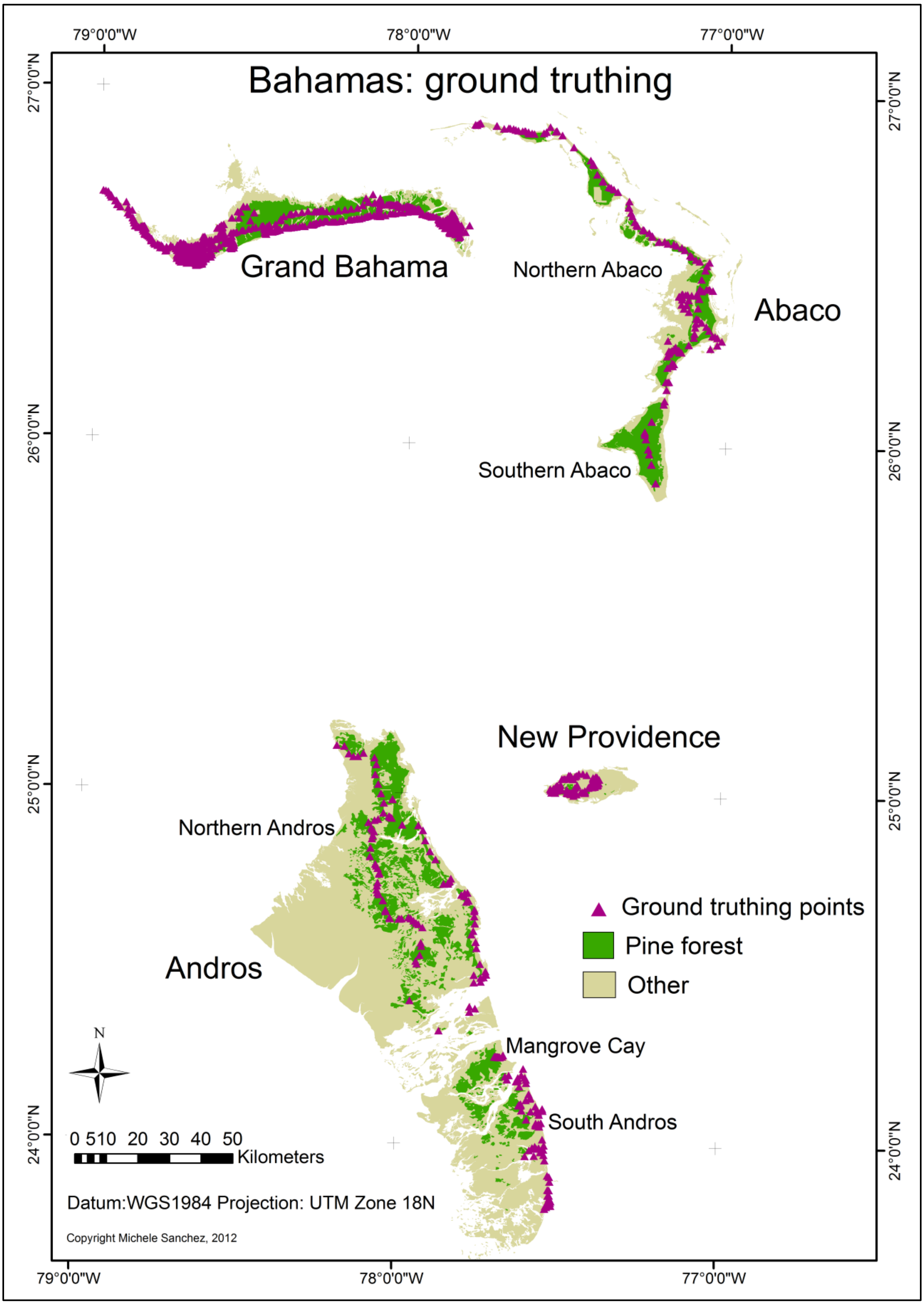


Figure 2.6. Ground truthing in the pine forests of the Bahamas. Ground truthing points were used to refine maps of modern pine forest. Feature ‘Other’ corresponds to other non-mapped features, e.g. vegetation types and urban areas.

2.2.1.4. Map accuracy

Positional and thematic map accuracies were tested for the maps of recent pine forest area (2008-2009) in the Bahamas. A total of 89 thematic accuracy points, (26 for Andros, 22 for Grand Bahama, 23 for Abaco and 18 for New Providence) were obtained during fieldwork in these islands (Figure 2.7). These points were geo-referenced and contained information about the presence or absence of pine forest on the ground. They were plotted in the GIS map and used to compare data on the ground against existing pine forest theme and ‘other’ theme (not pine forest) on the map. A confusion matrix could then be created, showing concordance or discordance in data from the ground (ref. data) and map (Table 2.1). This matrix was used to calculate thematic map accuracy for the theme pine forest, using the binomial test of thematic map accuracy in Rossiter (2004). This test is calculated as follows: p(overall map accuracy)=$\sum n_i$(all positive matches)⁄n(number of points) and s (standard error)= $\sqrt{}$p·(1-p)⁄n; and probability of theme being accurate at 95% confidence interval is p+- (s·Z-α), considering a two tailed normal score Type I error α. Producer’s thematic accuracy was calculated as $\sum n_{for}$(positive matches for forest)⁄n(total number of forest ground truth points); and user’s thematic accuracy was $\sum n_{for}$(positive matches for forest)⁄n(total number of forest map points) after Foody (2002) (Table 2.1).

In order to calculate positional accuracy, a set of two visible/recognizable points in the imagery for Grand Bahama and New Providence and four points on Andros and Abaco were generated in Google™ Earth, transferred to the GIS and then to ArcPad™. These points were compared to points at the same location obtained during fieldwork. The distances between the coordinates for a generated point for a feature (reference point) and the real position of the same feature on the ground (ground truth point) were used to calculate the positional accuracy of the map. Coordinates for the x-axis and y-axis in meters where obtained in ArcMap™ v.9.3 for all points, and represented as $x_{reference}$, x_{ground}, $y_{reference}$ and y_{ground}. Calculations of root mean square error (RMSE) for both axis and overall followed the National Standard for Spatial Data Accuracy (NSSDA) (Greenwalt and Shultz 1968, Subcommittee for Base Cartographic Data 1998). Accuracy for both axes were calculated as $RMSE_x=\sqrt{}\ [\sum\ (x_{reference}-x_{ground})^2/n]$ and $RMSE_y=\sqrt{}[\sum\ (y_{reference}-y_{ground})^2/n]$, where n means total number of points compared. Overall map positional accuracy ($RMSE_r$) was calculated by the formula $RMSE_r=$

$2.4477 \cdot 0.5 \cdot (RMSE_x + RMSE_y)$, which considers errors in the x-axis and y-axis to be independent and normally distributed.

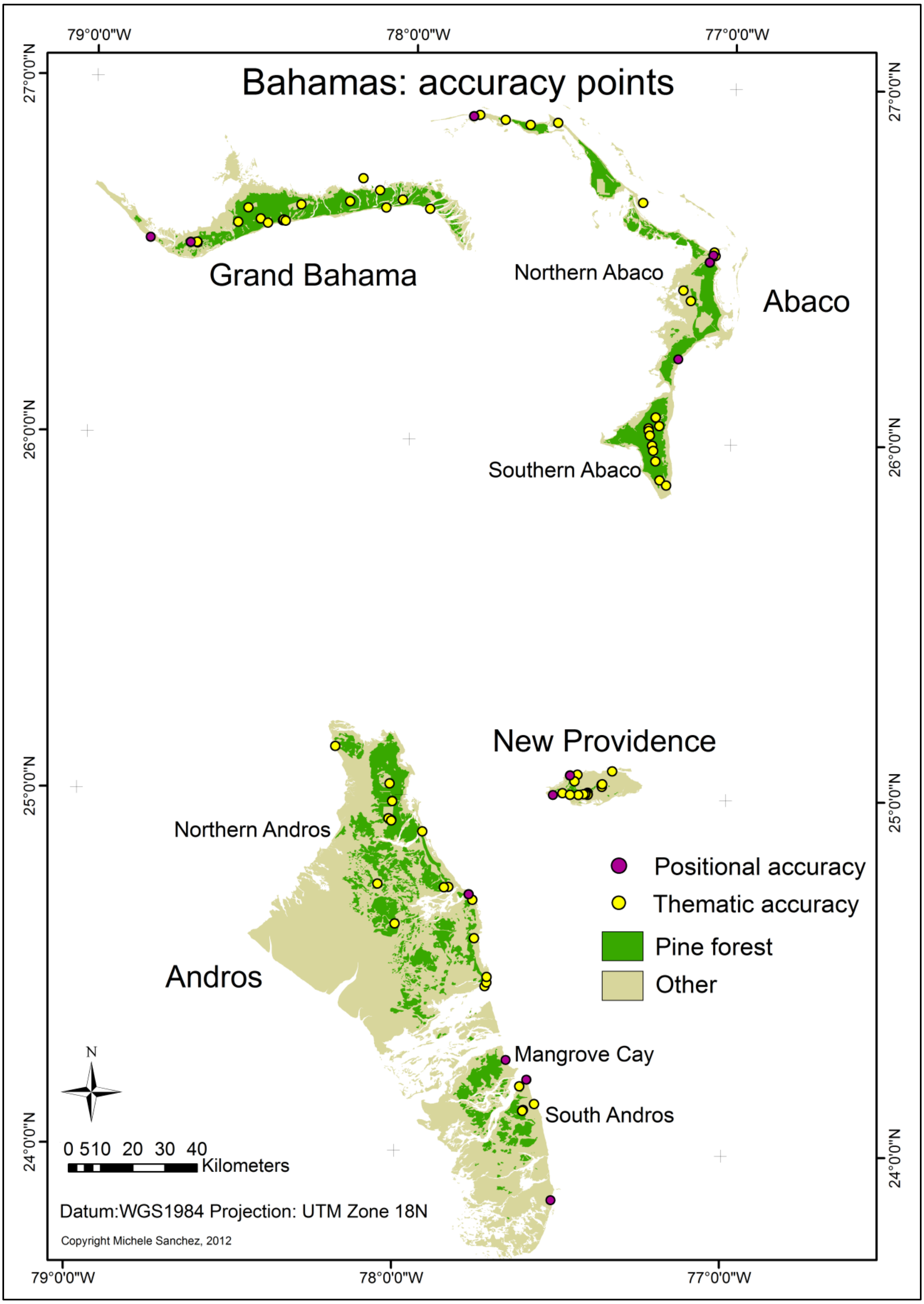


Figure 2.7. Positional and thematic accuracy points in the Bahamas. Points collected in 2008-2010, used to test the thematic and positional accuracy of the maps of modern pine forest produced.

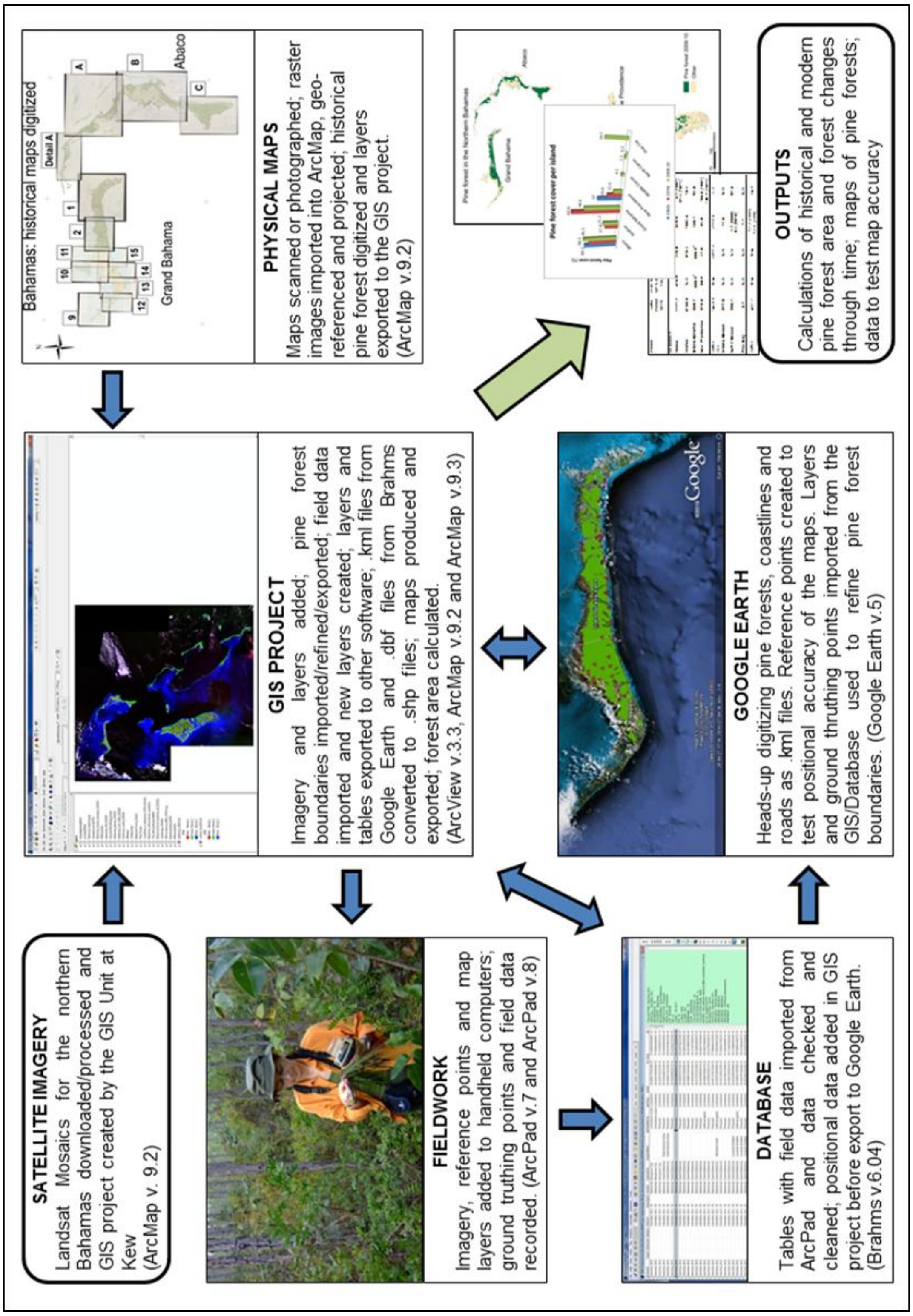
2.2.1.4. Producing maps and calculating areas

Tables (.dbf) containing the field data were added to a Brahms v.6.04 database following fieldwork. These tables could then be imported into the GIS or exported as .kml files and loaded into Google™ Earth v.5 after positional data was added. Ground truthing points were used to guide adjustments in existing pine forest boundaries in Google™ Earth. After completion, .kml files containing refined pine forest boundaries were transferred to the GIS as layer themes, following transformations explained in section 2.2.1.1. Themes, points, lines and boundaries, in the GIS were frequently compared to the images from Google™ Earth in order to increase accuracy.

After final refinement of boundaries in 2010, areas of disturbance (e.g. farms, houses, clearings etc.) > 0.1 km² were excluded from the pine forest area. Water bodies and other forest types, when clearly identifiable, were also excluded. These areas were compared in Google™ Earth and the GIS images, and then clipped in ArcMap™ v.9.2. When areas were not visible in the GIS they were first digitized in Google™ Earth and then transferred to the GIS before clipping. Maps containing final modern pine forest area could then be produced in ArcMap™ v.9.3. The area of modern pine forest for each island was calculated using statistical analysis available in this software.

Pine forest layers digitised from historical maps were used to produce maps of past pine forest area for each island, and statistical analysis was used to calculate the historical pine forest area for each island. Historical pine forest in the Bahamas was calculated based on the following assumptions. No data were available for the island of Andros and the eastern part of Grand Bahama in the 1960s; and the western part of Grand Bahama in the 1970s. It is known from Henry's report (1974) on logging activities in the Bahamas that logging on Grand Bahama happened from 1944 to 1959, with most activities concentrated in the eastern part of the island; and on Andros from 1966-74 (Table 1.2 in Chapter 1). The same report also mentioned tree death following storm surge by Hurricane Betsy in 1965 for some areas previously logged on Andros, which could have changed total forest area. However, it is likely that forest area for western Grand Bahama remained more or less the same from 1962 to 1971, as good regeneration was observed for all areas by the end of the 1960s (Henry 1974). Therefore, no estimation of historical pine forest in the 1960s was calculated; and estimations for the 1970s in the Bahamas assumed that forest area for western Grand

Bahama during the 1960s and 1970s remained the same.



SATELLITE IMAGERY
Landsat Mosaics for the northern Bahamas downloaded/processed and GIS project created by the GIS Unit at Kew (ArcMap v. 9.2)

FIELDWORK
Imagery, reference points and map layers added to handheld computers; ground truthing points and field data recorded. (ArcPad v.7 and ArcPad v.8)

DATABASE
Tables with field data imported from ArcPad and data checked and cleaned; positional data added in GIS project before export to Google Earth. (Brahms v.6.04)

GIS PROJECT
Imagery and layers added; pine forest boundaries imported/refined/exported; field data imported and new layers created; layers and tables exported to other software; .kml files from Google Earth and .dbf files from Brahms converted to .shp files; maps produced and exported; forest area calculated. (ArcView v.3.3, ArcMap v.9.2 and ArcMap v.9.3)

GOOGLE EARTH
Heads-up digitizing pine forests, coastlines and roads as .kml files. Reference points created to test positional accuracy of the maps. Layers and ground thruthing points imported from the GIS/Database used to refine pine forest boundaries. (Google Earth v.5)

PHYSICAL MAPS
Maps scanned or photographed; raster images imported into ArcMap, geo-referenced and projected; historical pine forest digitized and layers exported to the GIS project. (ArcMap v.9.2)

OUTPUTS
Calculations of historical and modern pine forest area and forest changes through time; maps of pine forests; data to test map accuracy

Figure 2.8. Workflow showing the mapping and ground truthing processes in the Bahamas.

Changes in pine forest through time could be visualised when modern and historical pine forest layers were overlaid. Layers for unchanged forest area were created using the extract and clip option in the analysis tools menu of ArcToolbox in ArcMap v.9.3, as these areas were present in both layers of modern and historical forest. Areas of expansion of forest corresponded to those only present in the modern maps, whereas areas of loss of forest referred to the ones only present in the historical maps.

The percentage of pine cover per island was calculated dividing the sum of pine forest area on each island*100 by total island area, using reference values in table 2.3.

2.2.2. TCI

2.2.2.1. Acquiring GIS and maps of the pine forests of the TCI

Development of a GIS project for the TCI and ground truthing of pine forest area were carried out by the GIS unit at Kew and Kew's UK Overseas Territories (UKOTs) Conservation Programme coordinator Martin Hamilton, as part of the TCI Pine Recovery Project. The GIS was developed using ArcGIS 9 (ESRI® ArcMap™ 9.2 © 1999-2006 ESRI Inc.). Imagery consisted of aerial photography at 0.25m (date uncertain, circa 2001, NAD 1927 UTM Zone 19N projection) of the islands of Pine Cay, Middle Caicos and North Caicos, and an additional Quickbird image of Middle Caicos from 2008 (2.4 m resolution multispectral and 0.6 m panchromatic). Images were re-projected to WGS 1984 UTM Zone 19N and added to a final GIS project (Figure 2.9). Ground truthing was undertaken in 2008 by Susana Baena of the GIS Unit resulting in the production of maps containing pine forest area for each of the three islands (Salamanca *et al.* 2010). Access and use of shapefiles of forest area, coastlines for the three main islands and area of pine forest on North Caicos burnt in 2009 were kindly granted by the GIS unit and the UKOTs Programme.

Additional coastlines for the rest of the TCI were digitized following methodology used for the northern Bahamas and described in section 2.2.1.1. A map of modern pine forest in TCI, showing changes from 2008 to 2010 was produced in ArcMap™ versions 9.2 and 9.3. No historical data were available to calculate area of forest in the 1960s and 1970s.

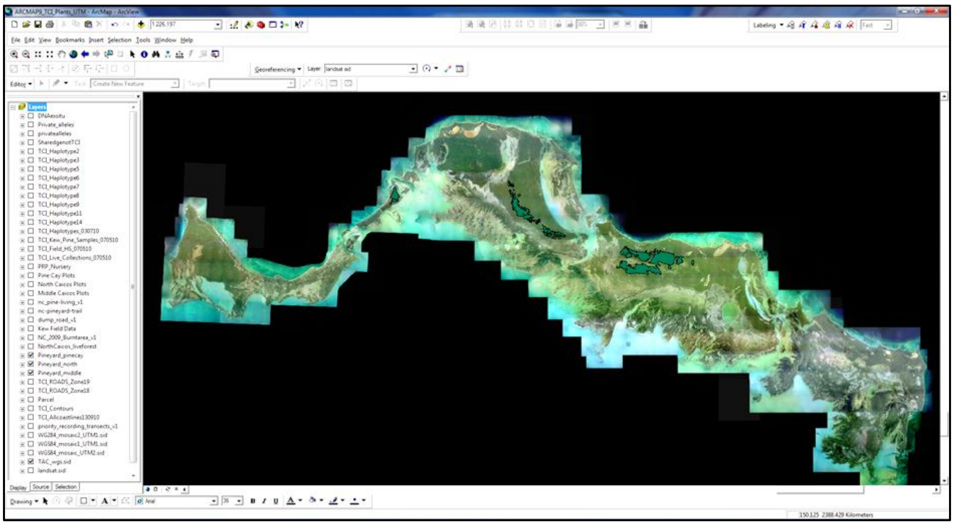


Figure 2.9. Screenshot of GIS project of the Turks and Caicos Islands. Figure shows a mosaic of aerial photography and shapefiles.

2.2.3. Simulation of sea level rise

A map of the Bahaman archipelago showing a simulation of inundation events resulting from sea level rise was produced in ArcMap™ v. 9.3, using raster images obtained from the Centers for the Remote Sensing of Ice Sheets (CReSIS). These images were created using the Global Land One-km Base Elevation (GLOBE) digital elevation model (DEM) and GIS raster analysis. Inundation zones were delimited according to elevation and distance to the coast. The spatial resolution for GLOBE images was circa 1 km at the Equator (30 arc seconds of latitude and longitude). GIS raster analysis first identified cells contiguous to the ocean in the DEM, secondly reassigned as oceans those cells with the same or lower elevations than the sea level rise, and then repeated the process with adjacent cells.

2.3. Results

2.3.1. Map accuracy and errors

Map scale for the pine forest theme varied among maps. Modern pine forest was mapped at a scale of approximately 1:18,000 and the map scale in the historical maps varied from 1:25,000 to 1:50,000. Map scale for 1960s pine forest on New Providence is unknown.

Positional and thematic accuracy of historical maps is unknown. Modern maps of pine forest had an overall thematic accuracy for the theme pine forest of 89.9%, according to calculations based on Foody (2002); and between 83.6% and 96.2% (95% confidence interval), following the binomial test of Rossiter (2004) (Table 2.1). Producer's accuracy was higher than user's accuracy, but both of them were >88%. Defining vegetation types and boundaries was more difficult and subjective in areas with poor imagery. In addition, most of these areas were also inaccessible, and lacked ground truthing points. Small areas of forest observable on the ground could have been ignored or missed in the map due to the mapping scale.

	Forest (ref.data)	Other (ref.data)	Total
Forest (map)	46	6	**52**
Other(map)	3	34	**37**
Total	**49**	**40**	**89**

Producer's thematic accuracy	93.9%
User's thematic accuracy	88.5%
Overall thematic map accuracy	89.9%
Binomial test of thematic accuracy	> 83.6% < 96.2%
Positional accuracy x (RMSEx)	7m
Positional accuracy y (RMSEy)	6m
Positional accuracy map (RMSEr)	16m

Table 2.1. Confusion matrix, thematic and positional accuracy values for maps of modern pine forest in the Bahamas. Thematic accuracy calculations, including confusion matrix (above) based on Foody (2002) and binomial test of Rossiter (2004). Positional accuracy calculations according to NSSDA standards (Subcommittee for Base Cartographic Data 1998). Confidence level used for all tests was 95%. RMSE means root mean square error.

Island	Estimated forest area before ground truthing	Estimated forest area after ground truthing	Overestimation (%)
Abaco	607.4	542.6	10.7
Andros	1121.2	1094.5	2.4
Grand Bahama	516.9	456.1	11.8
New Providence	48.8	24.8	49.2
Total	**2294.3**	**2118.0**	**7.7**

Table 2.2. Estimates of modern pine forest area before and after ground truthing in the northern Bahamas. Forest area is given in km^2.

Positional accuracy of the modern forest maps was circa 16 m; meaning that an error up to 16 m can be present in the real position of an object in the map and on the ground (Table 2.1). Differences between reference and ground points varied from 0.07 m to 17 m on the x-axis and 0.9 m to 14 m on the y-axis. These axes refer to longitude (x) and latitude (y) on a map. The highest error values were observed in western Grand Bahama and South Andros. The western Grand Bahama image was originally in a different UTM Zone (17N) than the rest of the images for the other islands (18N). Another source of error accountable for this is the GPS accuracy. The GPS used in the field had an accuracy of <10 m, according to manufacturer's manual. The overall accuracy for the x-axis and y-axis was, respectively, circa 7 m and 6 m. Therefore, worst overall accuracy of the map would be 27 m, i.e. maximum difference of 17 m between the reference and ground point plus 10m GPS accuracy. However, the accuracy for the majority of the points would be within the overall accuracy of the axes, i.e. ≤7 m.

All ground truthing points and DNA points (Figure 2.6) were used to refine boundaries of the modern pine forest theme. An overestimation of modern pine forest area for all islands can be observed when comparing area estimations before and after ground truthing (Table 2.2). The largest estimation error found was for the island of New Providence, where the forest area was reduced by 49% after final map refinements. Estimation errors for Grand Bahama and Abaco had similar values, and Andros showed the smallest value of all with only 2.4% area overestimation.

2.3.2. Pine forests through time

After the modern and historical pine forest themes for the Bahamas were digitized and refined, calculations of forest area were performed for comparison of changes through time. No precise value for total historical pine forest area in the 1960s or 1970s could be obtained, due to lack of historical data for the TCI and part of the Bahamas. An estimated value for historical pine forest in the Bahamas was calculated, as previously explained in section 2.2.1.4.

Total modern pine forest area for *P. caribaea* var. *bahamensis* is 2131 km^2 (2008-2010), with most of it being located in the Bahamas (2118 km^2), and only a small part in the TCI (13 km^2). Pine forest cover, i.e. calculated pine forest area per country*100/total island area, is 28.4% for the Bahamas and 2.7% for the TCI (Table 2.3).

2.3.2.1. Bahamas

Modern pine forest distribution in the Bahamas is shown in Figure 2.11, and pine forest area per island, total forest area per country and changes in forest area through time are shown in Table 2.3.

Total pine forest area increased by 5.3% from 1971/1974 to 2008/2010 (34 to 38 years) in the Bahamas; and decreased by 24.8% during 3 years (2008-2010) in the TCI (Table 2.3). The highest changes in pine cover per island occurred on Grand Bahama and on New Providence (Figure 2.10). New Providence is the smallest island with pine trees in the Bahamas, and contains the smallest forest area in that country. Meanwhile, Andros has the largest area of pine forest per island, twice or more that on any other island in the archipelago, being also the largest island in the whole archipelago (Figure 2.11, Table 2.3). Pine forests are present on the islands of Abaco, Andros, Grand Bahama, New Providence, Middle Caicos, North Caicos and Pine Cay. Analysis of the satellite imagery has failed to detect pine presence in the other islands of the Bahamas and the TCI, including the Berry Islands and Great Inagua.

The biogeography of *Pinus caribaea* var. *bahamensis* is next explored and detailed for each island where it occurs.

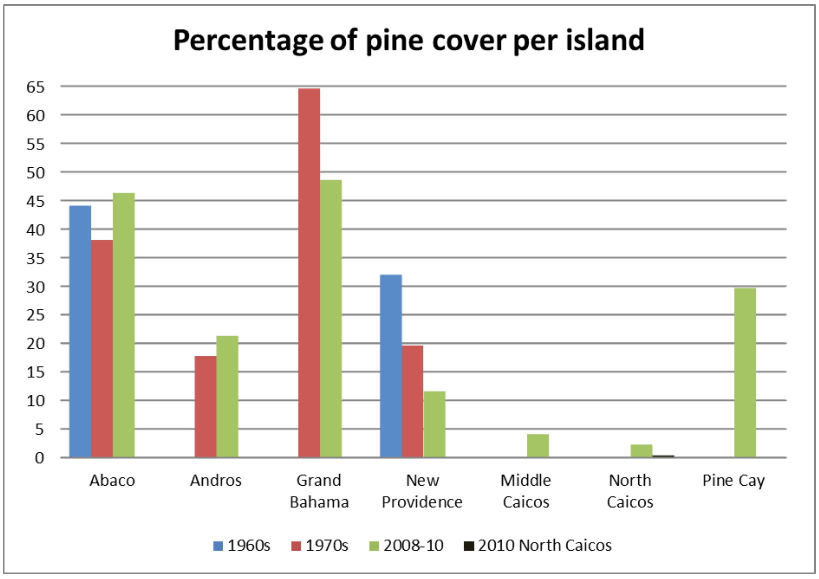


Figure 2.10. Changes in pine forest cover per island through time in the Bahaman archipelago. Pine forest cover percentage refers to percentage of pine forest area relative to total island area for each island. Major forest losses were observed on Grand Bahama, New Providence and North Caicos.

Island	Total island area	Pine forest area in the 1960s	Pine forest area in the 1970s	Pine forest area in 2008-2010	Loss or gain of pine forest since the 1960s/1970s†
Bahamas					
Abaco	1171.7	516.2	446.9	542.6	5.1 (1967) 21.4 (1971)
Andros	5136.6	N/A	916.4	1094.5	19.4
Grand Bahama	938.7	606.2•	606.2•	456.1	-24.8
New Providence	212.9	68.2	41.8	24.8	-63.6 (1961) -40.7 (1974)
Total	**7459.9**	**N/A**	**2011.3**	**2118.0**	**5.3**
TCI					
Middle Caicos	277.9	N/A	N/A	11.5	N/A
North Caicos	206.1	N/A	N/A	4.7 (2008) 0.4 (2010)	-91.5
Pine Cay	3.7	N/A	N/A	1.1	N/A
Total	**487.7**	**N/A**	**N/A**	**17.3 (2008)** **13.0 (2010)**	**-24.8**

Table 2.3. Changes in pine forest area in the Bahaman archipelago. Total island area (only shown for islands with pine forests) and pine forest area in km². •Values for western Grand Bahama refer to forest status after D.O.S. maps in 1962 (1963a, b, c, d, e, f, g) and eastern Grand Bahama in 1971(1973d, e). † Percentage of pine forest loss (-) or gain based on forest status after D.O.S. and Department of Lands and Surveys maps (1973a, b, c, 1974a, b, c, d, 1975a, b); since 1962 or 1971 for Grand Bahama, 1971 on Andros, or according to year in brackets. Forest area for TCI after Salamanca *et al.* (2010).

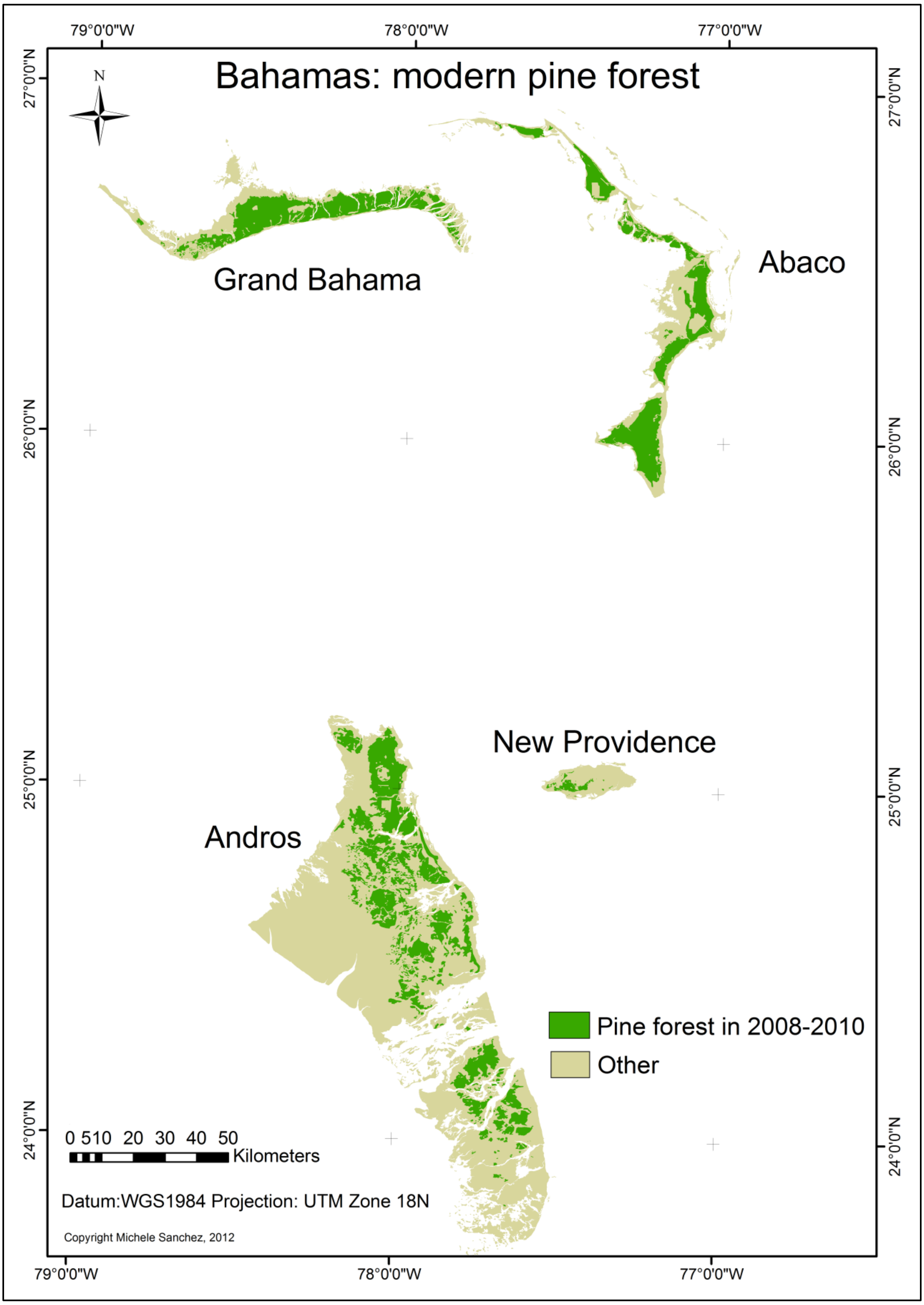


Figure 2.11. Modern pine forest area in the Bahamas. Pine forest area digitized from satellite imagery in Google™ Earth and ESRI® ArcMap™ v.9.2 and 9.3; and features refined through ground truthing in 2008-2010. Feature 'Other' corresponds to other non-mapped features, e.g. vegetation types and urban areas.

a) Abaco

Abaco Island comprises Little Abaco and Great Abaco, in addition to numerous cays. In this thesis, the island was represented in two parts: southern Abaco, corresponding to the southernmost part of Great Abaco, and northern Abaco, which included the rest of Great Abaco and Little Abaco.

Total pine forest cover on this island increased circa 8% in the past 42 years (1967-2009), and is at its highest at the moment (Figure 2.10), based on the comparison of historical and modern forest maps. Circa 46.3% of the island is covered in pine forests, as seen in Figure 2.11.

Abaco pine forests decreased from 516.2 km^2 to 446.9 km^2 between 1967 and 1971. This period was just after the second cycle of logging on the island (1959-1967) (Tables 2.3 and 1.2). Henry (1974) reported many dead trees and regeneration varying from non-existent to low in the pine forests in southern Abaco, south of Crossing Rock, and smaller areas in northern Abaco in 1971. These areas can be observed in Figures 2.13 and 2.17. Dead forest covered 69.3km^2 and areas with low regeneration were 194.5 km^2. Many of these areas have recovered since then and forest has been re-established, as seen in Figures 2.15 and 2.19.

Modern pine forest on Abaco, 542.6 km^2, shows an increase in area of 5.1% compared to the 1967 forest and 21.4% relative to 1971 forest (Table 2.3). Nevertheless, stretches of land along the coast, where most towns are located, have lost pine forest. The Marsh Harbour area has shown visible loss of forest since 1971 (Figure 2.15). Another place where forest has been lost is in the agricultural plot southwest of Treasure Cay airport in northern Abaco. A large extent of land between Spring City and Lake City in northern Abaco has been allocated to agricultural plots (Figure 2.20). This can already be seen in the 1960s and 1970s maps (Figures 2.12 and 2.13). An expansion of pine forest in this area since then can be observed in Figure 2.15. The most western land is mainly covered by salt marshes (Figure 2.20).

The only area of old-growth forest still present on the island is situated in Little Abaco, with an area of 22.52 km^2. The Abaco National Park is located in southern Abaco in the district of South Abaco, covering an area of 82 km^2 (The Bahamas National Trust 2012).

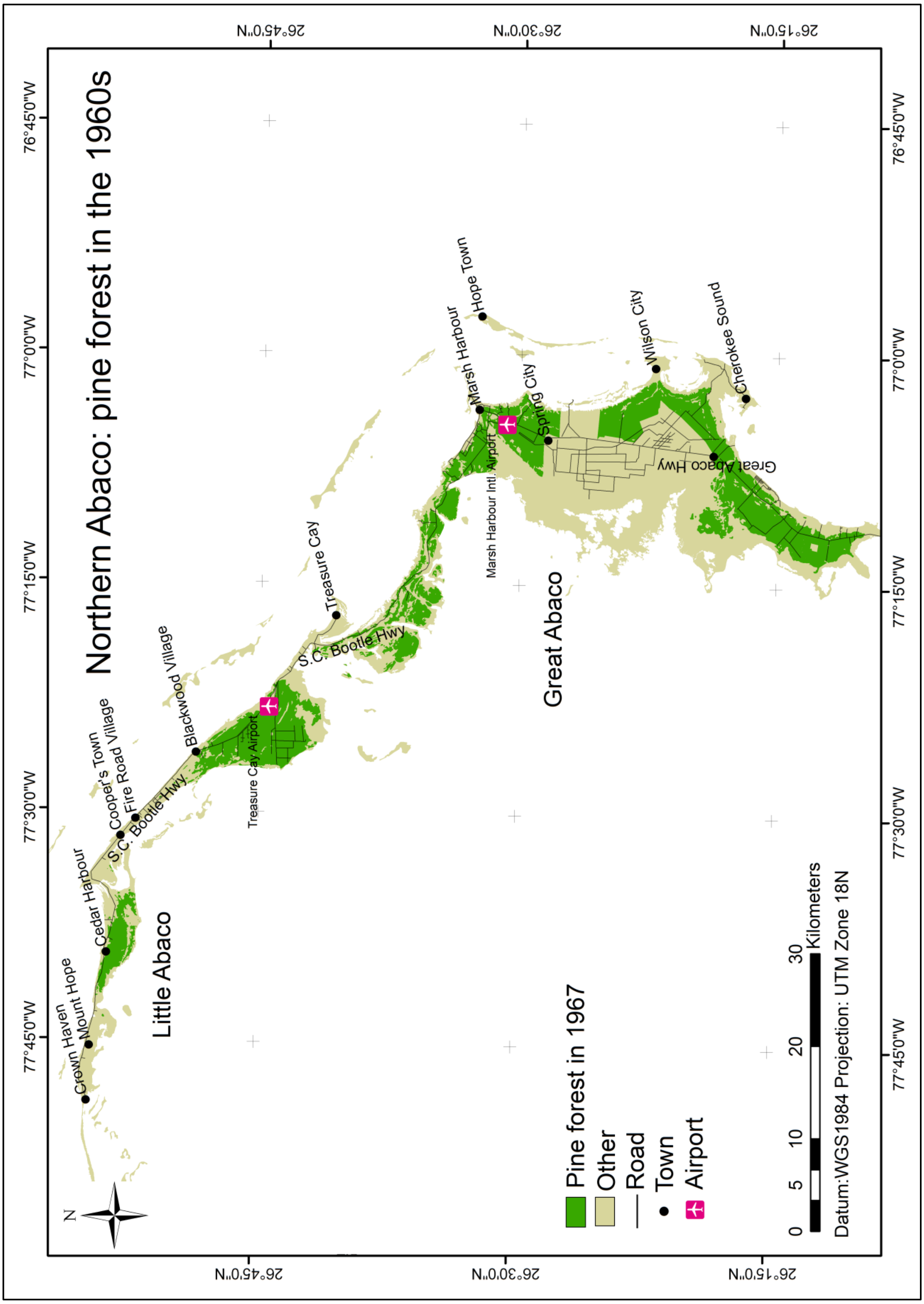


Figure 2.12. Pine forest area in northern Abaco in the 1960s. Pine forest area digitized from historical maps in ESRI® ArcMap™ v.9.2 and 9.3. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

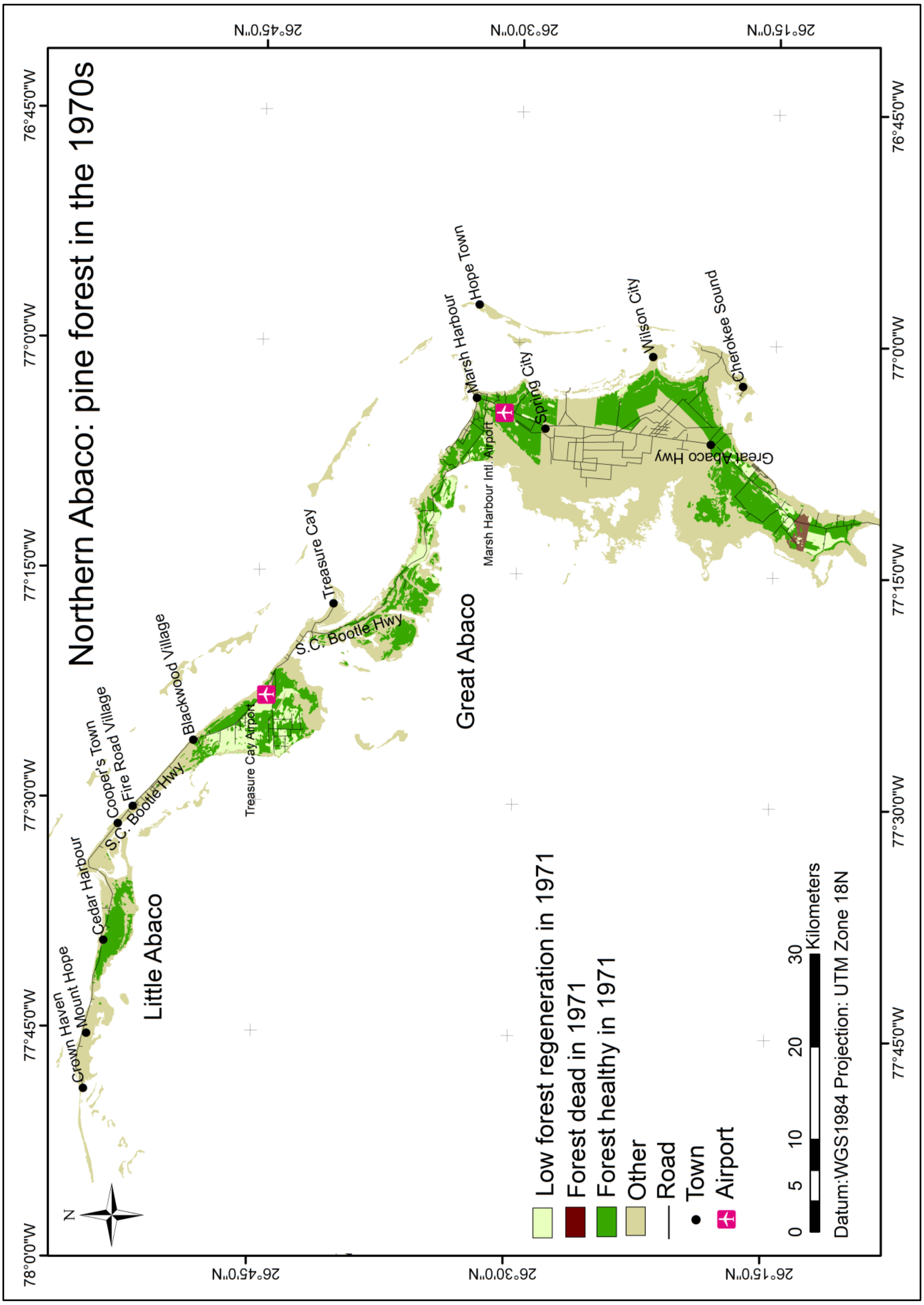


Figure 2.13. Pine forest area in northern Abaco in the 1970s. Pine forest area digitized from historical maps in ESRI® ArcMap™ v.9.2 and 9.3. Data on forest regeneration after logging as in Henry (1974). 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

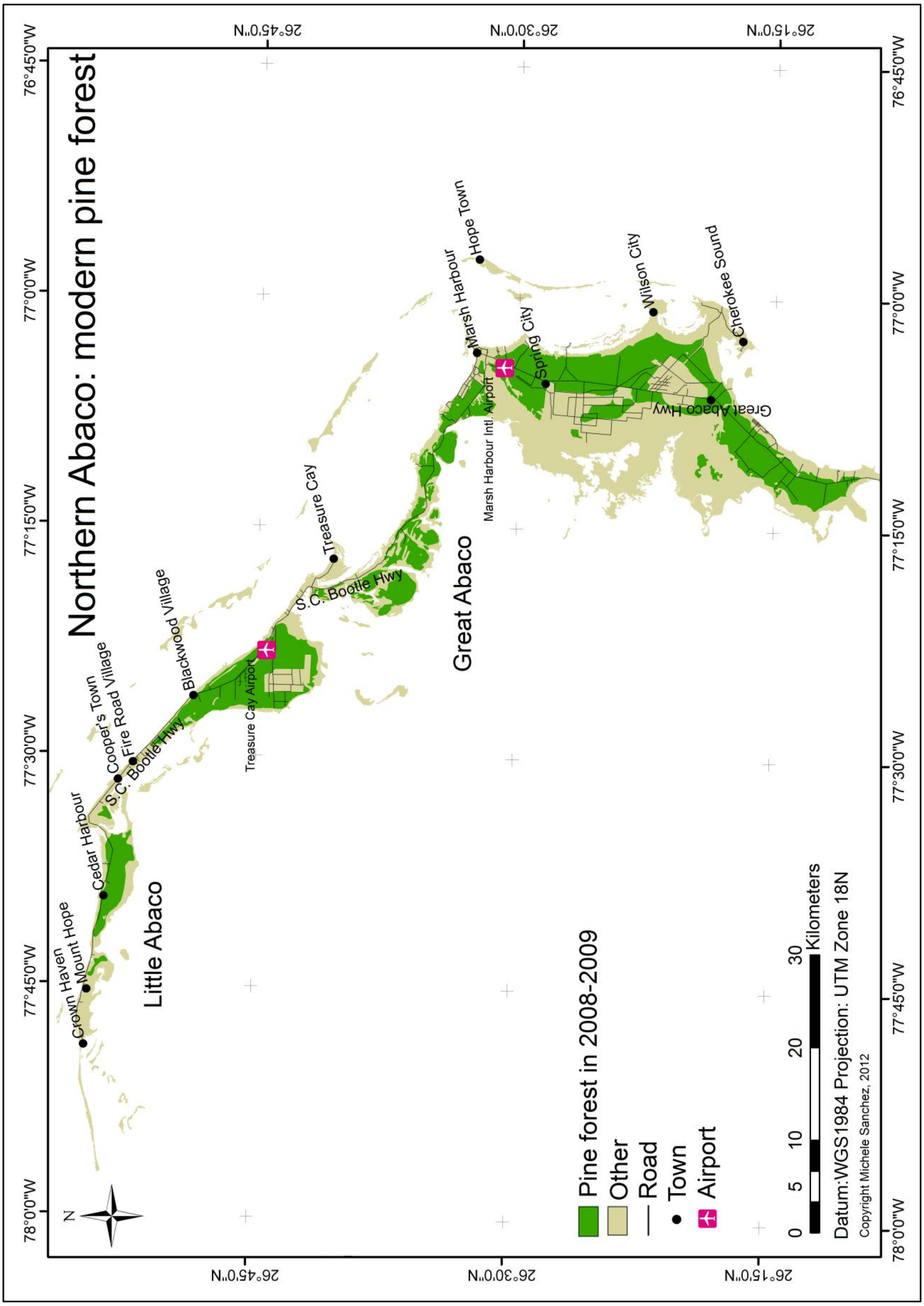


Figure 2.14. Modern pine forest area in northern Abaco. Pine forest area digitized from satellite imagery in Google™ Earth and ESRI® ArcMap™ v.9.2 and 9.3; and features refined through ground truthing in 2008-2009. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

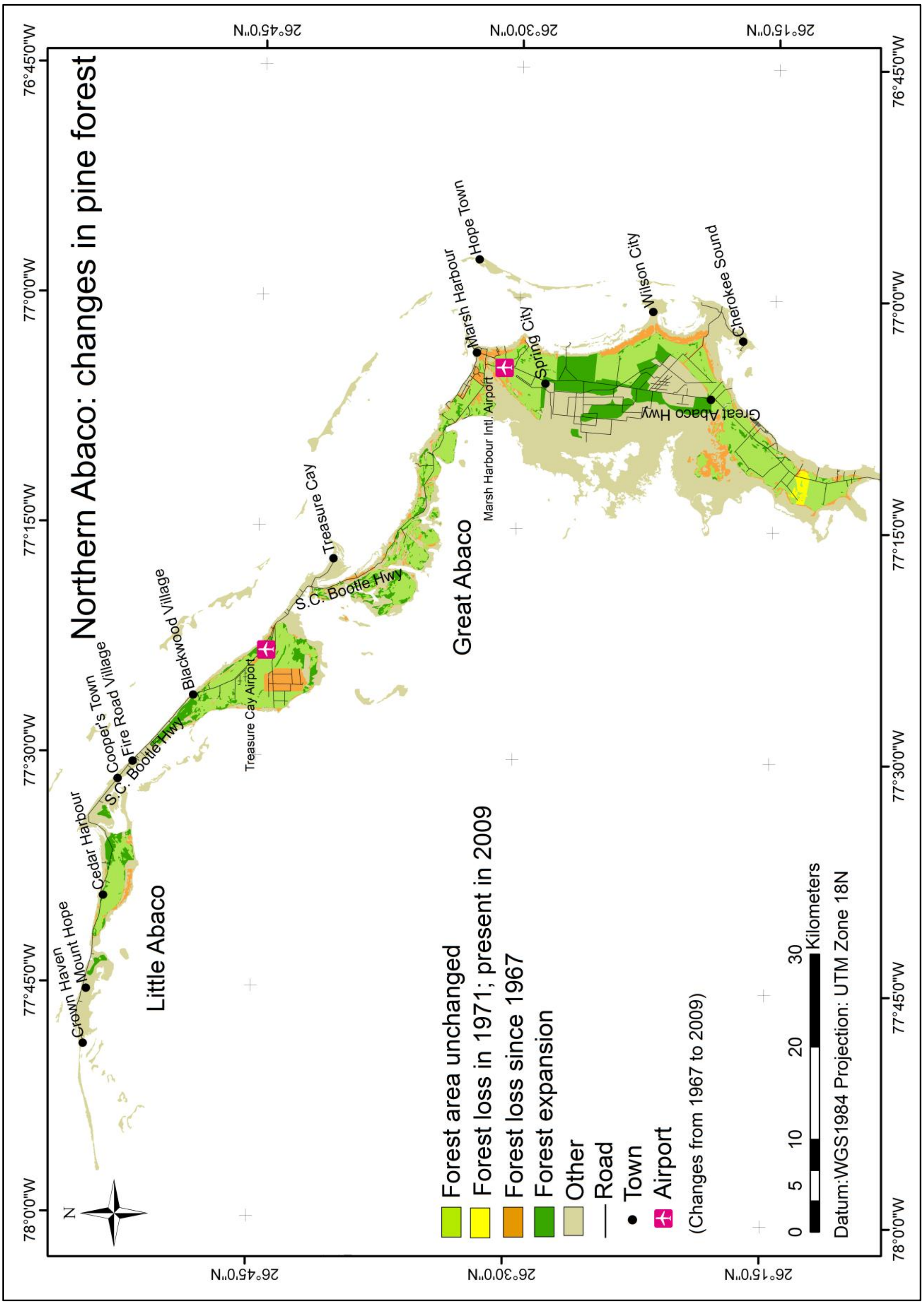


Figure 2.15. Changes through time to the pine forests of northern Abaco. Unchanged forest area corresponds to areas of pine forest present during 1967, 1971 and 2008-9 surveys; forest loss to areas not present anymore in 2008-9 survey; and forest expansion to new areas not present or mapped in 1967 and 1971 surveys. ‘Other’ means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

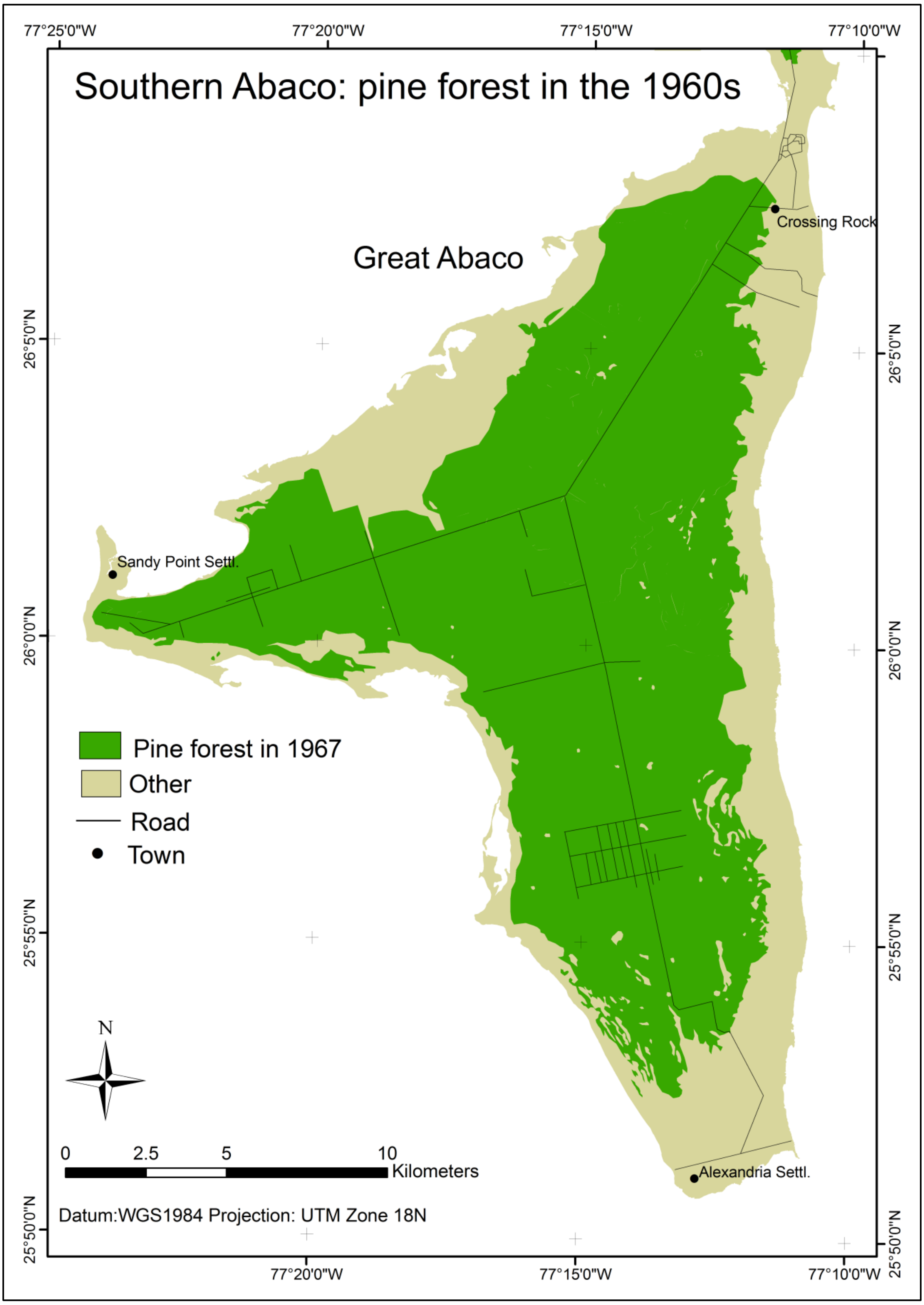


Figure 2.16. Pine forest area in southern Abaco in the 1960s. Pine forest area digitized from historical maps in ESRI® ArcMap™ v.9.2 and 9.3. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

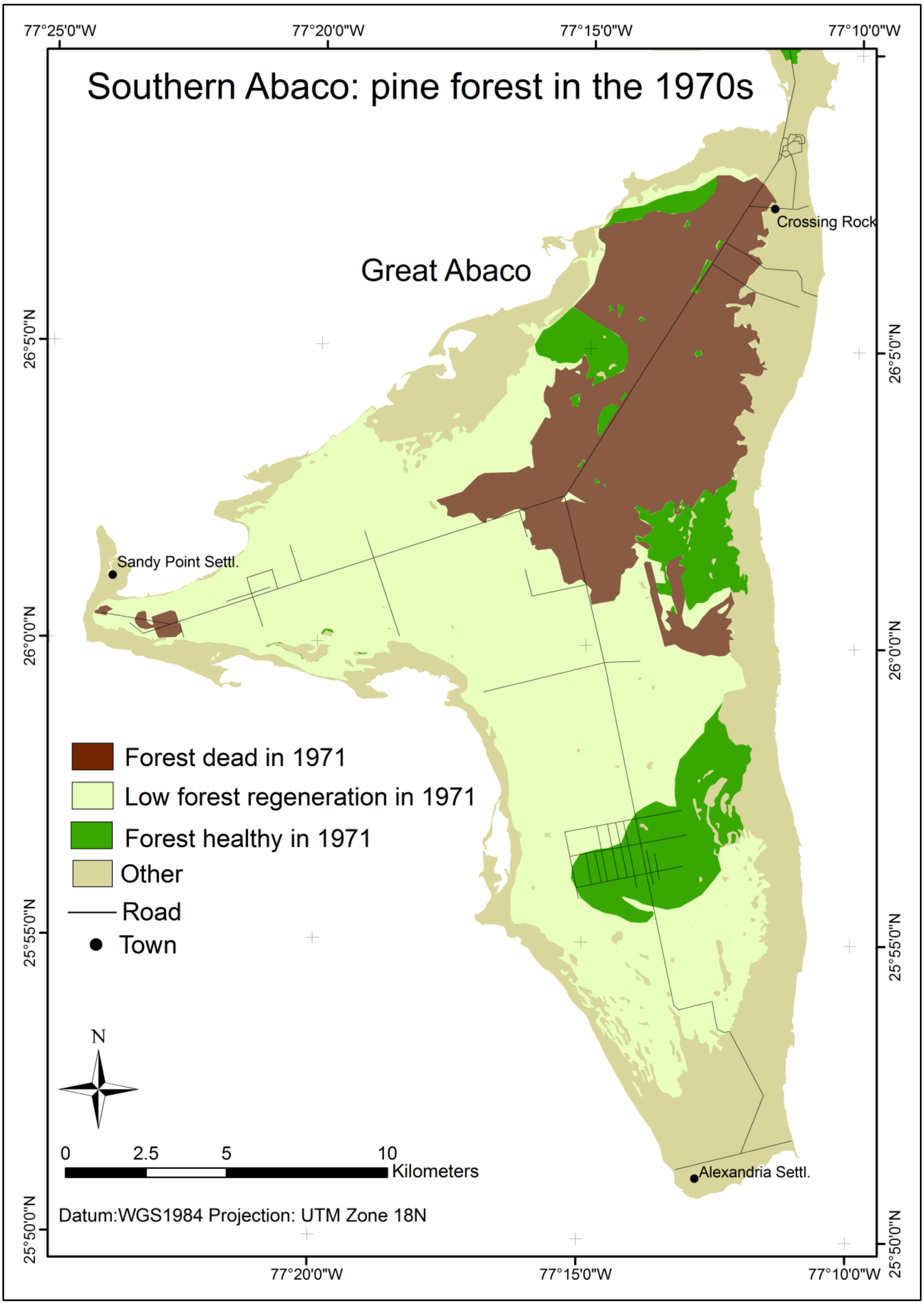


Figure 2.17. Pine forest area in southern Abaco in the 1970s. Pine forest area digitized from historical maps in ESRI® ArcMap™ v.9.2 and 9.3. Data of forest regeneration after logging as in Henry (1974). ‘Other’ means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

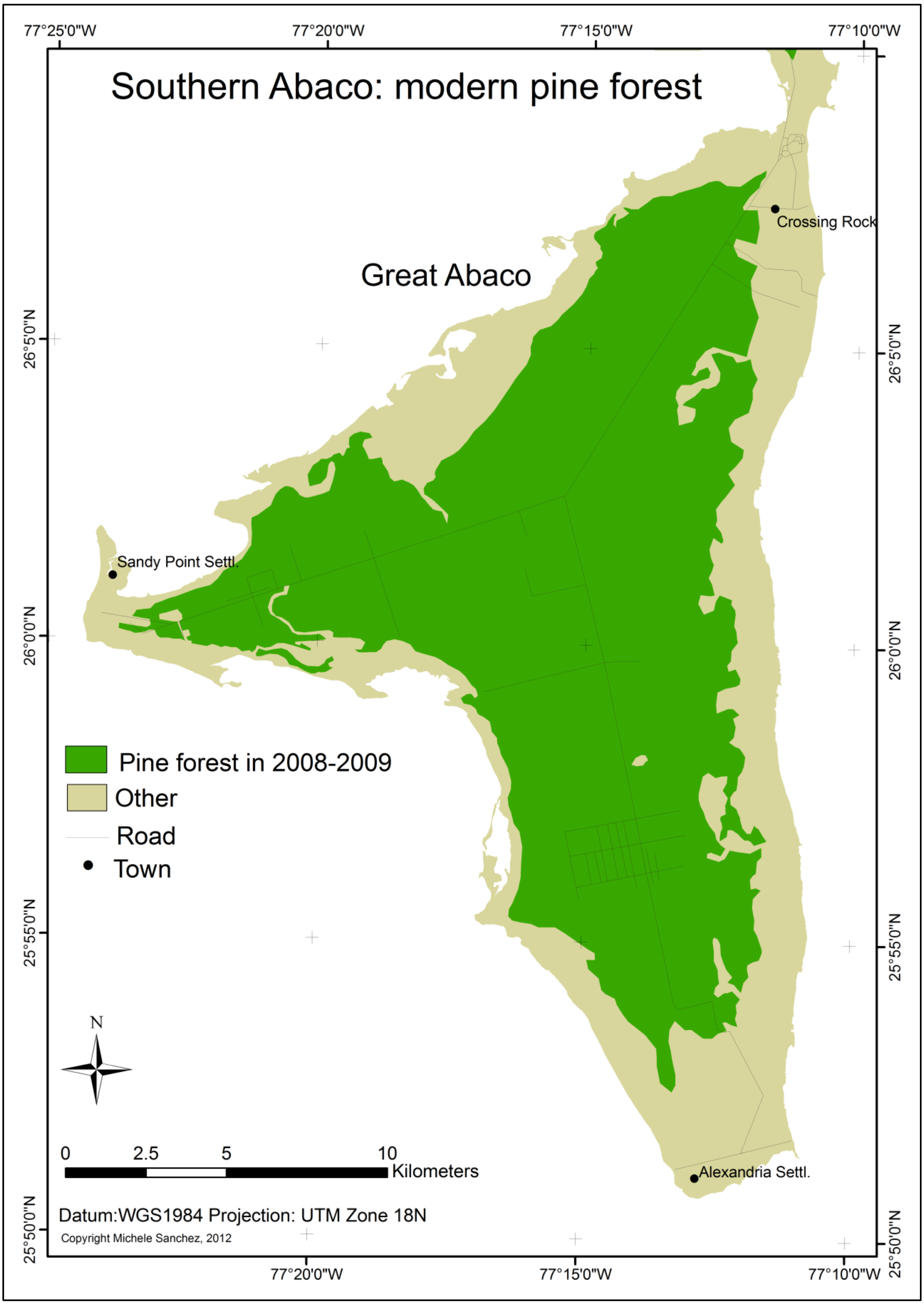


Figure 2.18. Modern pine forest area in southern Abaco. Pine forest area digitized from satellite imagery in Google™ Earth and ESRI® ArcMap™ v.9.2 and 9.3; and features refined through ground truthing in 2008-2009. ‘Other’ means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

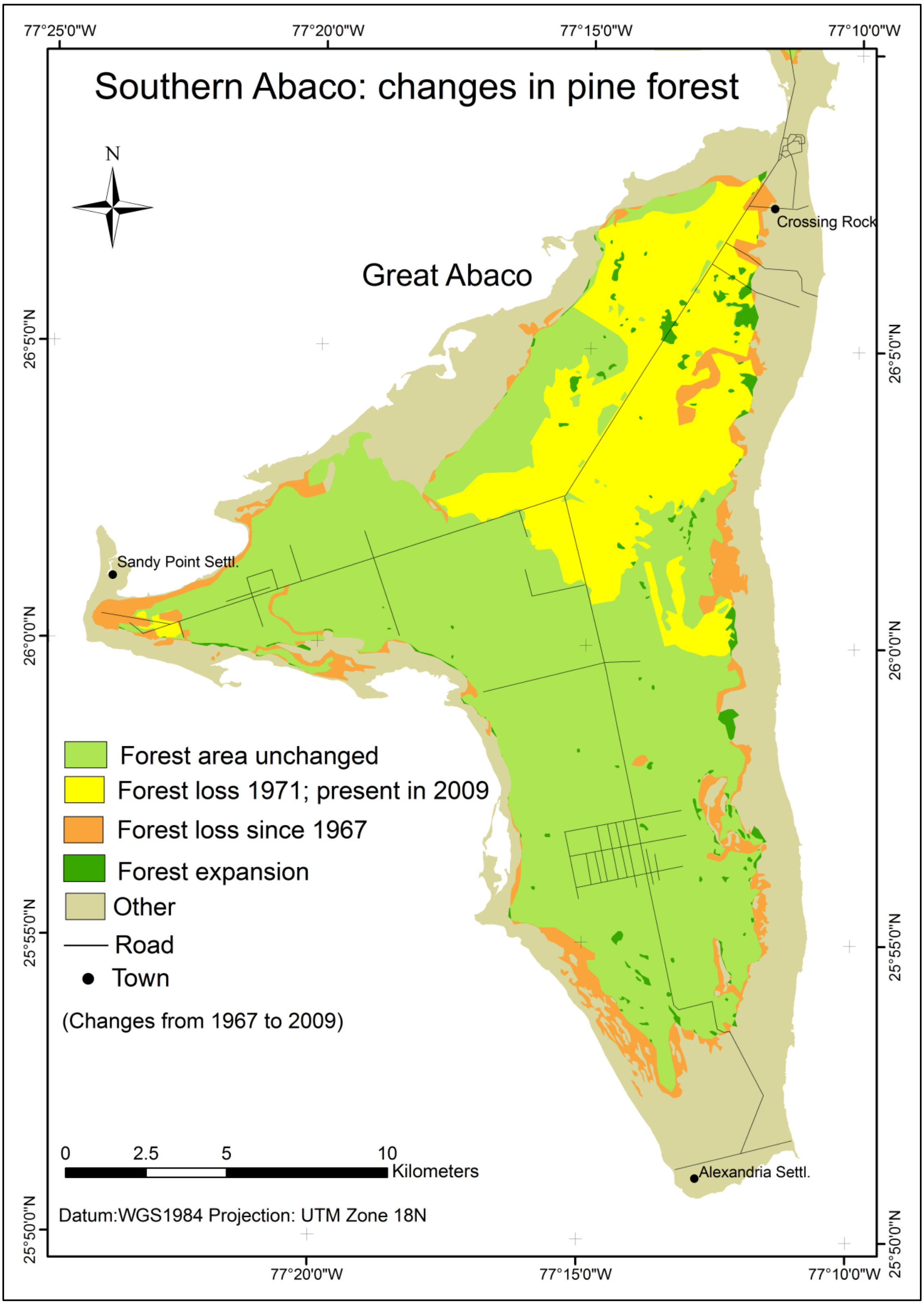


Figure 2.19. Changes through time to the pine forests of southern Abaco. Unchanged forest area corresponds to areas of pine forest present during 1967, 1971 and 2008-9 surveys; forest loss to areas not present anymore in 2008-9 survey; and forest expansion to new areas not present or mapped in 1967 and 1971 surveys. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

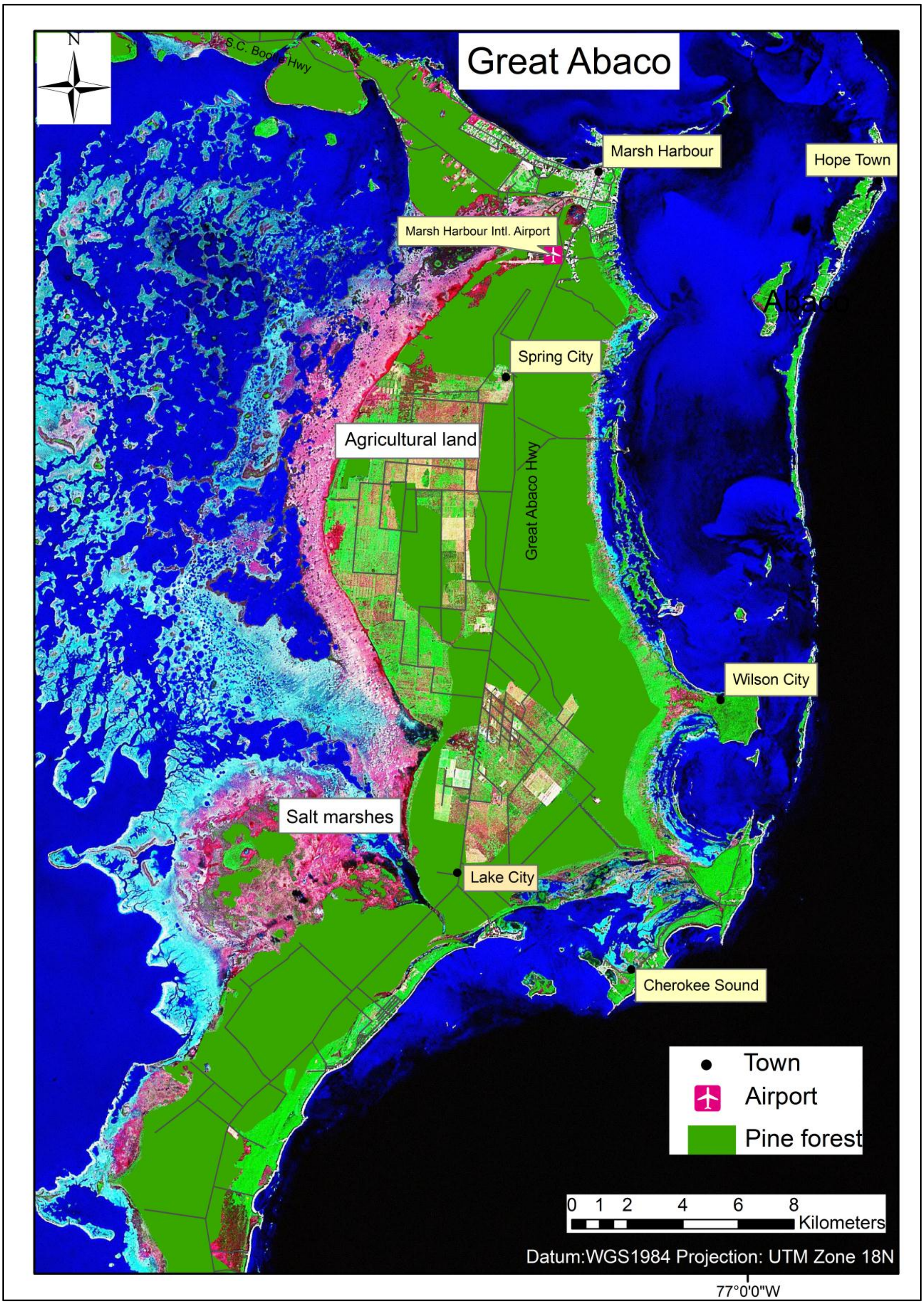


Figure 2.20. Satellite image of Great Abaco, showing location of modern pine forest, salt marshes and agricultural land. Pine forest is shown in dark green, salt marshes in pink and agricultural land as a mosaic of brown, green and light brown.

b) Andros

Andros Island is divided in four main districts: North Andros, Central Andros, Mangrove Cay and South Andros. In this thesis North and Central Andros districts were grouped together as northern Andros. In the maps presented in this Chapter, Mangrove Cay and South Andros were grouped together as southern Andros for easier representation.

Total modern forest area for Andros is 1094.5 km^2, the largest in the archipelago (Table 2.3). However, only 21.3% of the island is covered with pine forests; a low number in comparison with Abaco or Grand Bahama (46.3% and 48.6%) (Figure 2.10). Forest is also more fragmented when compared to those islands (Figure 2.11).

The largest block of forest on the island is situated in northern Andros, between Owen's Town and Nichol's Town (Figure 2.22). This block measuring circa 155.3 km^2 was logged between 1966 and 1969. Good regeneration followed when logging activities ceased (Henry 1974); and forest loss in this area corresponds mainly to agricultural plots located between San Andros Airport and Owen's Town (Figure 2.23). Mangrove Cay was also logged during the early 1970s. The area visited during this research showed good regeneration.

An estimated increase of 19.4% in pine forest area from 1971 to 2008-10 has been observed (Table 2.3). This expansion can be noticed throughout the island. Changes happened mainly in South Andros pine fragments and in northern Andros in the vicinity of several settlements, e.g. Nichol's Town, Mastic Point, Staniard Creek and Cargill Creek (Figures 2.23 and 2.26). Other small fragments of pine forest have been lost or expanded throughout Andros Island, as can be observed in the same figures. Small pockets of pine forest amongst wetland are found in the west side of Andros and bigger blocks in the central and east parts of the island.

This island is less developed and touristic than Abaco, Grand Bahama and New Providence. Most towns and settlements are small and along the coast. Only one road in Mangrove Cay and a newly opened road in South Andros provide limited access to the pine forest in the southern part of Andros. Northern Andros pine forests are accessible through main roads or secondary roads opened during the logging period.

Several active water wells are located in the pine forests of northern Andros, being the main source of fresh water for this island and New Providence.

Protected areas of pine forest found in northern Andros include the Blue Hole National Park and the recently expanded West Side National Park. The first is situated west of Staniard Creek in northern Andros, and covers an area of 134.5 km^2 and the latter is the largest one found in the Bahamas covering an area of 5260.9 km^2 along the west side of Central Andros, Mangrove Cay and South Andros (The Bahamas National Trust 2012). Old-growth pine forest is only found in South Andros.

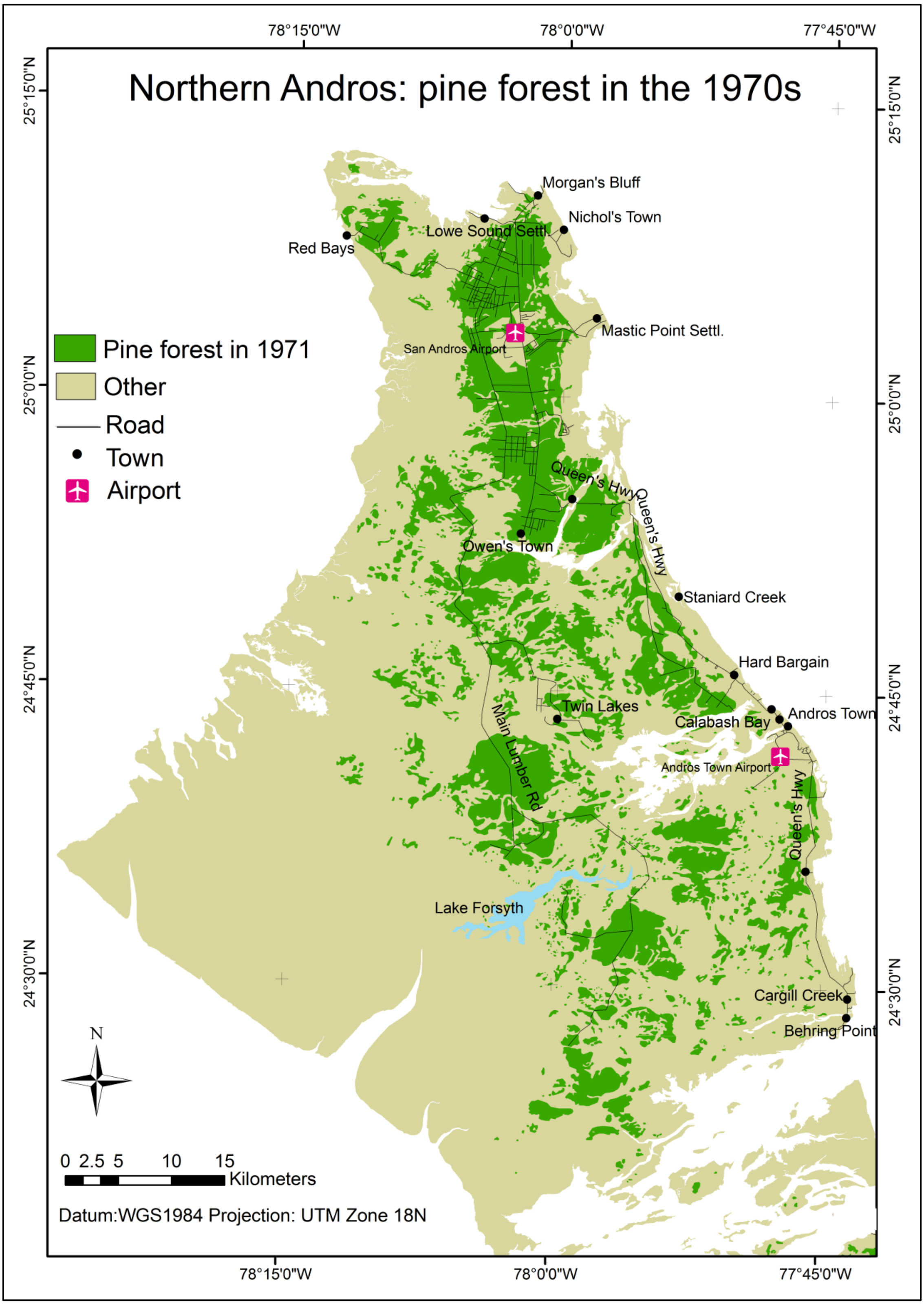


Figure 2.21. Pine forest area in northern Andros in the 1970s. Pine forest area digitized from historical maps in ESRI® ArcMap™ v.9.2 and 9.3. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

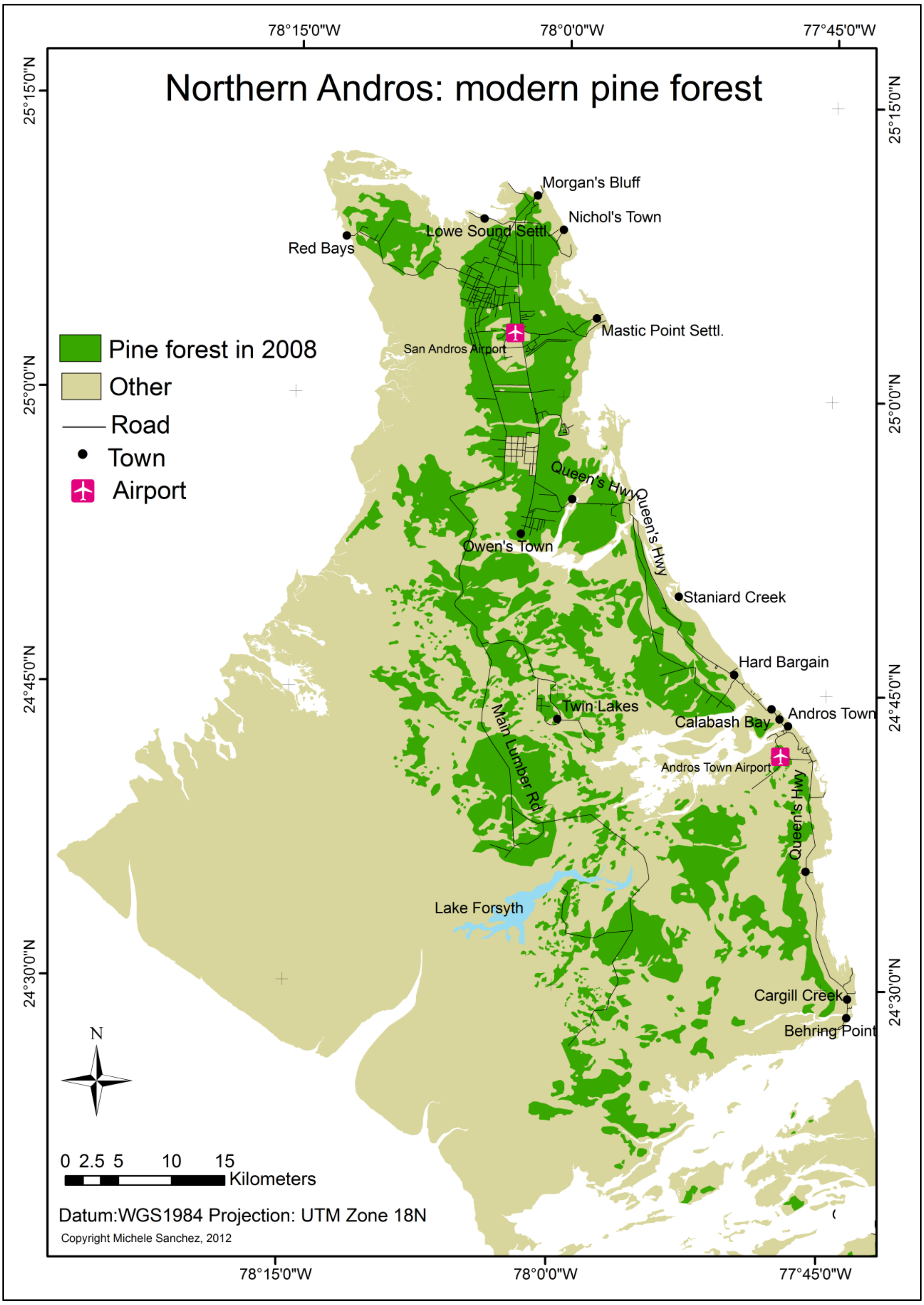


Figure 2.22. Modern pine forest area in northern Andros. Pine forest area digitized from satellite imagery in Google™ Earth and ESRI® ArcMap™ v.9.2 and 9.3; and features refined through ground truthing in 2008. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

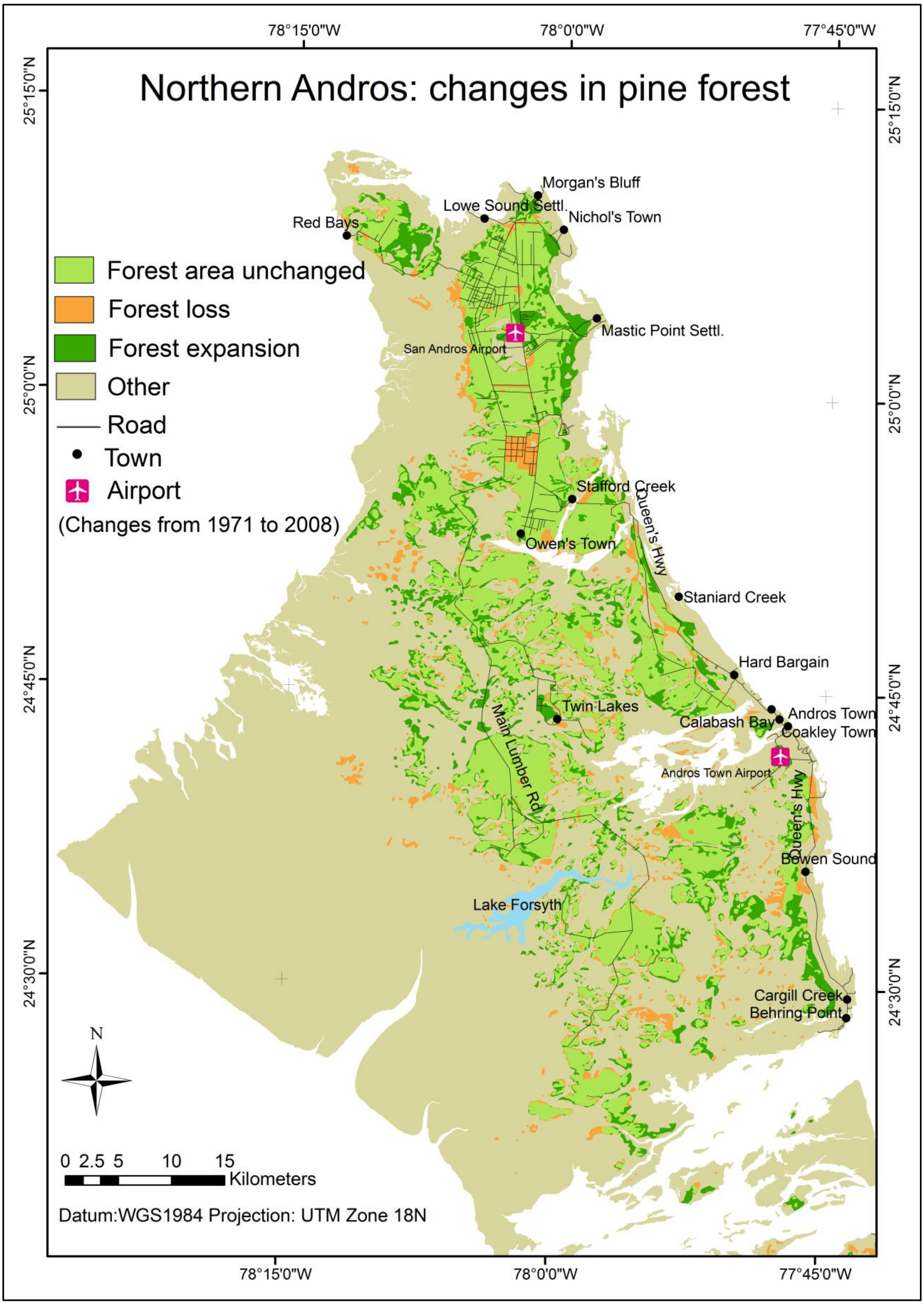


Figure 2.23. Changes through time to the pine forests of northern Andros. Unchanged forest area corresponds to areas of pine forest present during 1971 and 2008 surveys; forest loss to areas not present anymore in 2008 survey; and forest expansion to new areas not present or mapped in 1971 survey. ‘Other’ means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

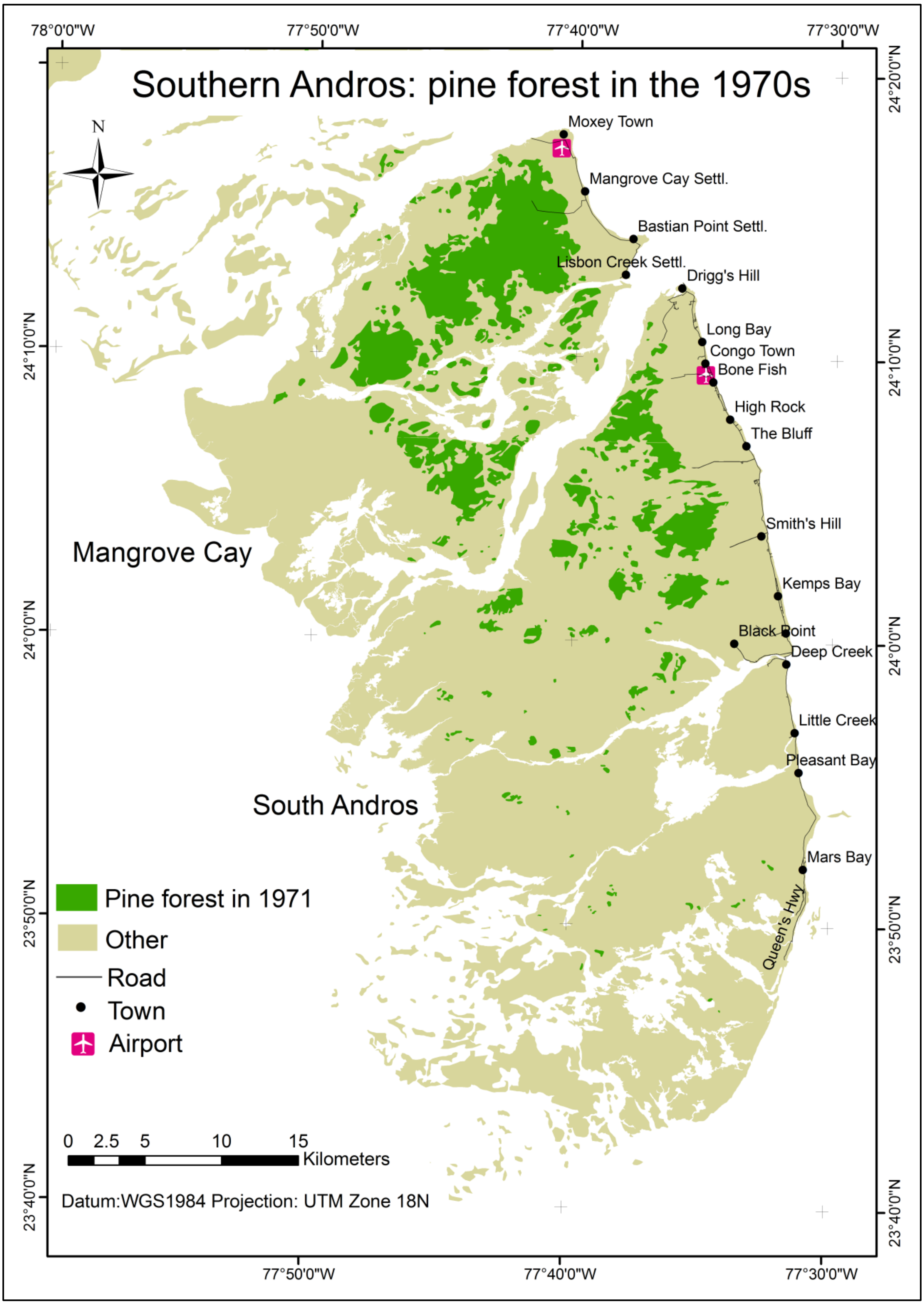


Figure 2.24. Pine forest area in southern Andros in the 1970s. Pine forest area digitized from historical maps in ESRI® ArcMap™ v.9.2 and 9.3. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

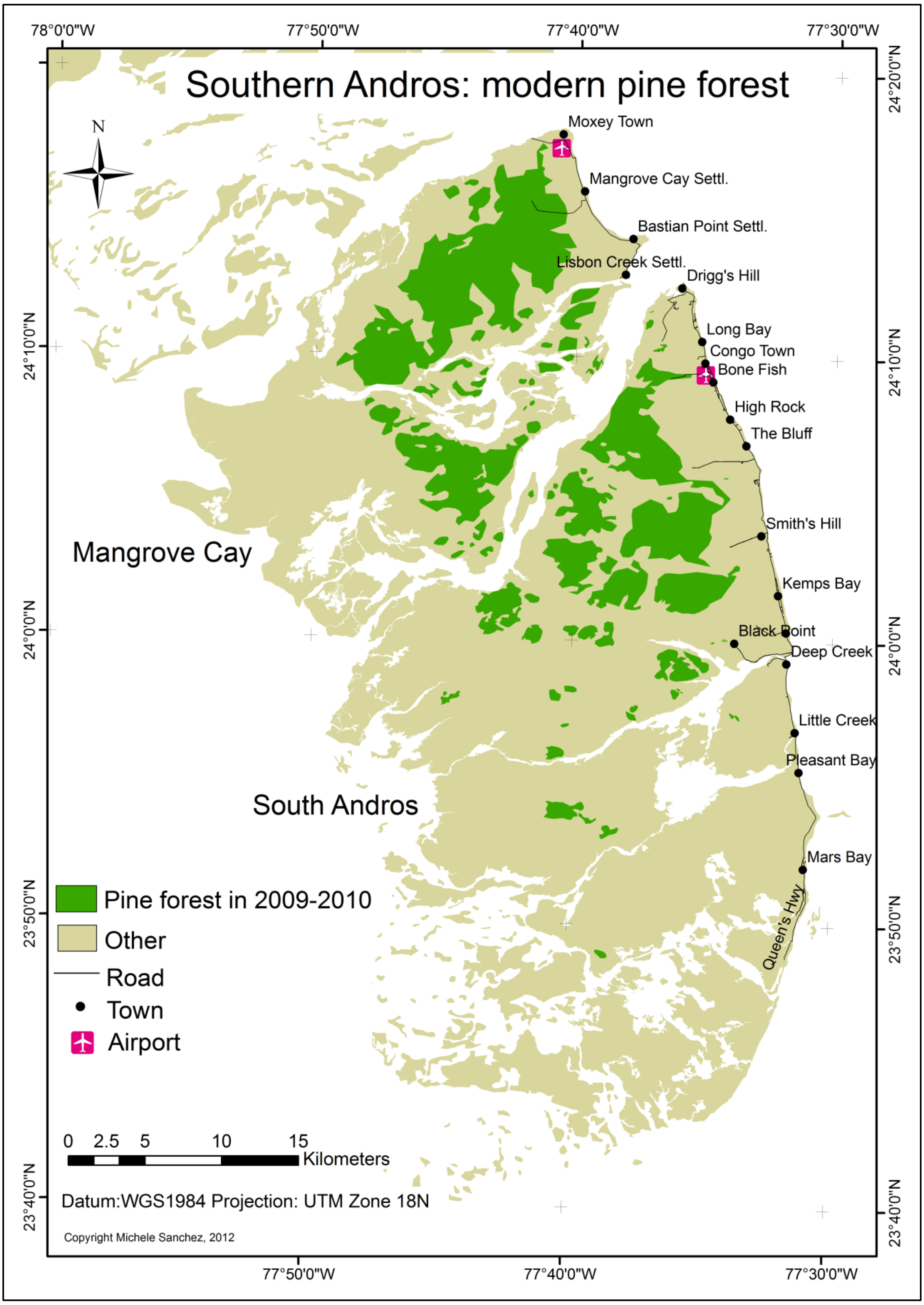


Figure 2.25. Modern pine forest area in southern Andros. Pine forest area digitized from satellite imagery in Google™ Earth and ESRI® ArcMap™ v.9.2 and 9.3; and features refined through ground truthing in 2009-10. ‘Other’ means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

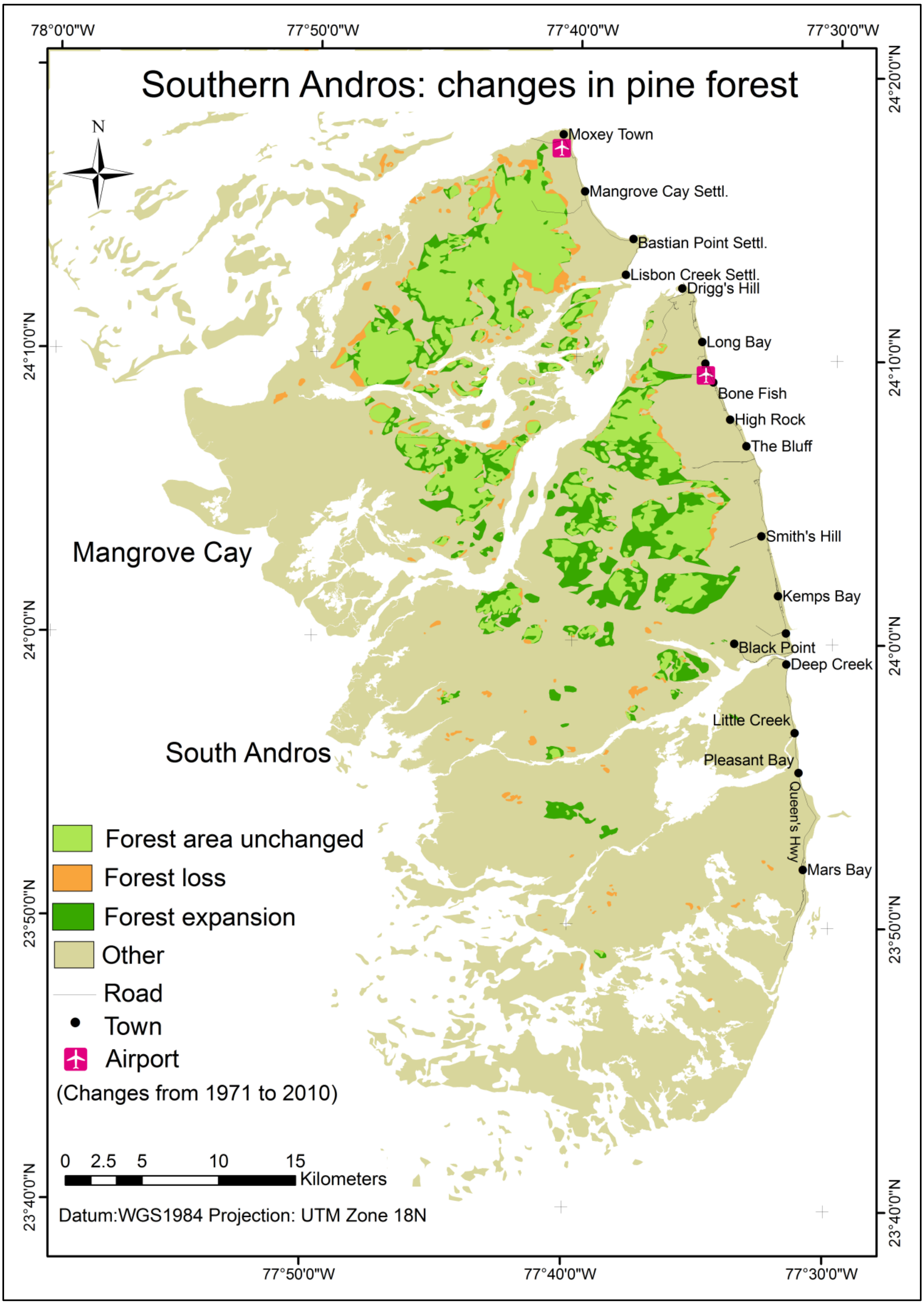


Figure 2.26. Changes through time to the pine forests of southern Andros. Unchanged forest area corresponds to areas of pine forest present during 1971 and 2009-10 surveys; forest loss to areas not present anymore in 2009-10 survey; and forest expansion to new areas not present or mapped in 1971 survey. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

c) Grand Bahama

Grand Bahama Island was for convenience divided in two in this chapter (referred to as western Grand Bahama and eastern Grand Bahama) for mapping purposes, due to date sources of the historical maps. Grand Bahama was considered as one unit for calculations of forest area.

Most of the island, from Freeport to McLean's Town, is covered in large tracts of pine forests (Figure 2.11). Many roads were opened and intensive logging practiced during the 1940s and 1950s. Nowadays, two main roads cut the island west to east (Warren J. Levarity and Grand Bahama Highways), but many secondary roads also give access to the pine forests. A total pine forest area of 606.2 km^2 on Grand Bahama, during the 1960s and 1970s, was estimated for the historical maps (Table 2.3). Total pine forest cover at that time was 64.6% (Figure 2.10). Since then, there has been a reduction of 24.8% in forest area, now of a size of 456.1 km^2 (Table 2.3) and total pine forest cover decreased to 48.6% (Figure 2.10).

Several recent areas of dead pine forest with no regeneration were observed during fieldwork in 2008-10 (Figures 2.28 and 2.31), most of them located in the west and north of the island. These areas account for a loss of 32.7% (49.1 km^2) of the total forest (150.1 km^2) since the 1960s and 1970s (Figures 2.29 and 2.32). It was observed that the blocks of dead pine trees visible in eastern Grand Bahama (Figure 2.31) were situated in low-line areas with seasonally flooded ponds, where sawgrass (*Cladium mariscus* subsp. *jamaicense* (Crantz) Kük.) is the dominant vegetation. All the eastern side of Grand Bahama is cut by natural tidal creeks and channels. Some forest loss and forest expansion was also observed in the several cays located at the end of eastern Grand Bahama (Figure 2.32).

No pine forest was recorded in the historical or modern maps beyond Deadman's Reef in western Grand Bahama, where most of the vegetation observed was broadleaf. Damage caused by a series of hurricanes in 2004 and 2005 (Table 1.1) was still visible in 2010 in the western extreme of the island. Freeport and surroundings, the main touristic area, has developed considerably since 1971 and much of the forest has been lost from Pinder's Point to Smith's Point (Figures 2.29 and 2.32). Several artificial canals were built in the area as the demand for boat docks grew (Figure 2.33). Other towns and settlements are small and located mostly along the coast.

Grand Bahama has two areas of protected pine forest. Rand Nature Centre with an area of 0.4 km^2 in Freeport and Lucayan National Park with an area of 0.16 km^2 to the west of Bevan’s Town near the south coast. No areas of old-growth forest were observed, although some old trees were spotted throughout the island.

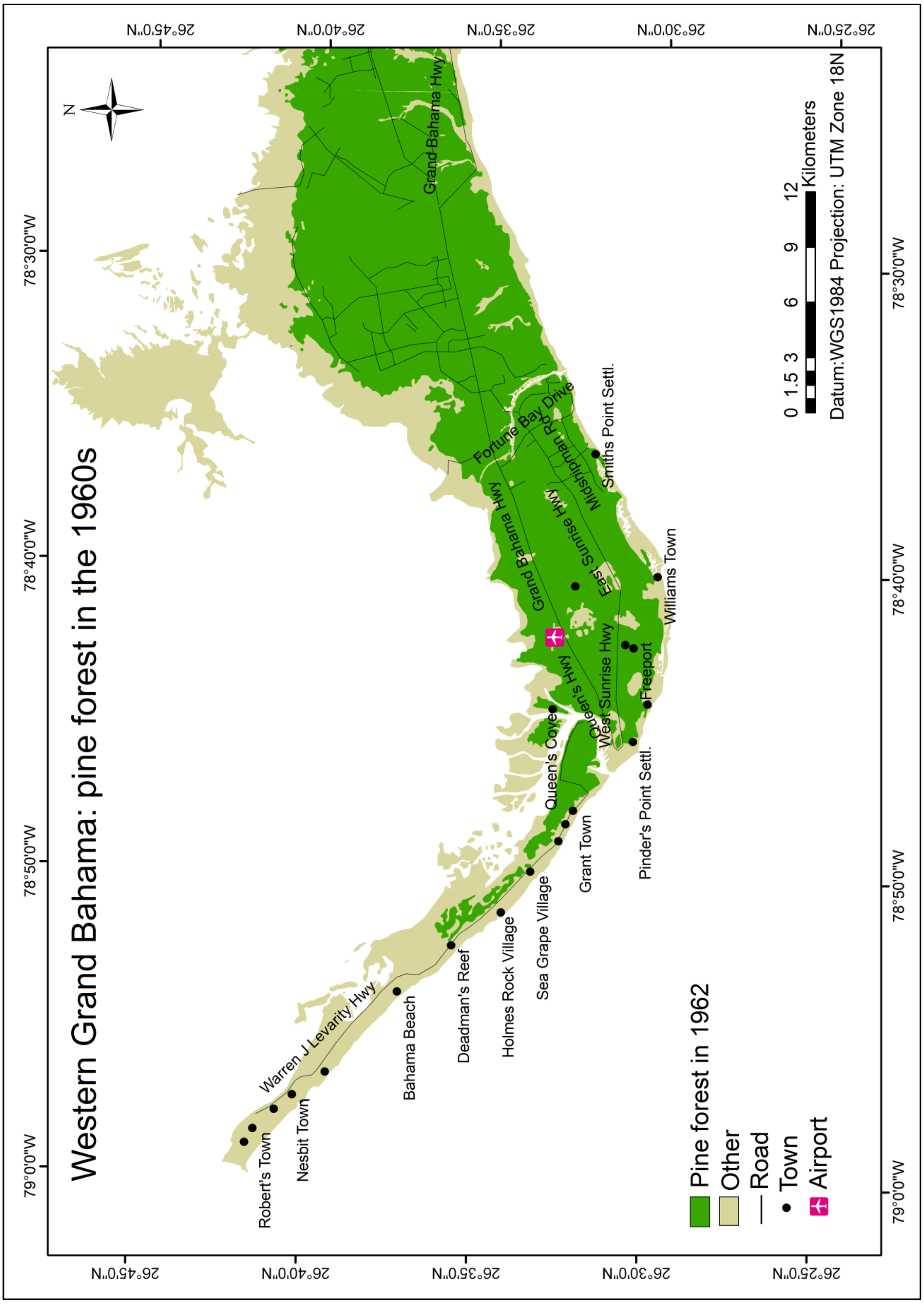


Figure 2.27. Pine forest area in western Grand Bahama in the 1960s. Pine forest area digitized from historical maps in ESRI® ArcMap™ v.9.2 and 9.3. ‘Other’ means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

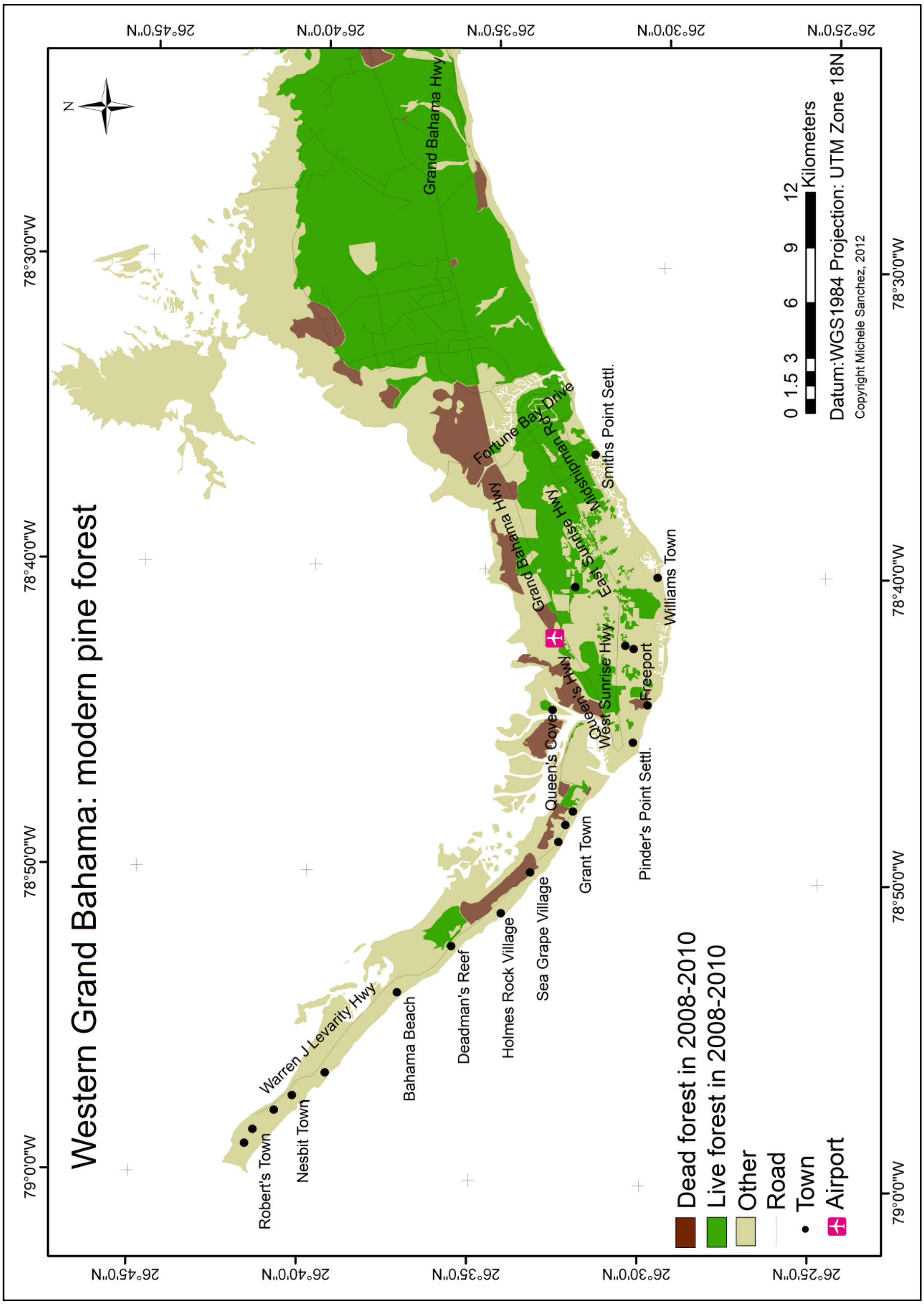


Figure 2.28. Modern pine forest area in western Grand Bahama. Pine forest area digitized from satellite imagery in Google™ Earth and ESRI® ArcMap™ v.9.2 and 9.3; and features refined through ground truthing in 2008-10. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

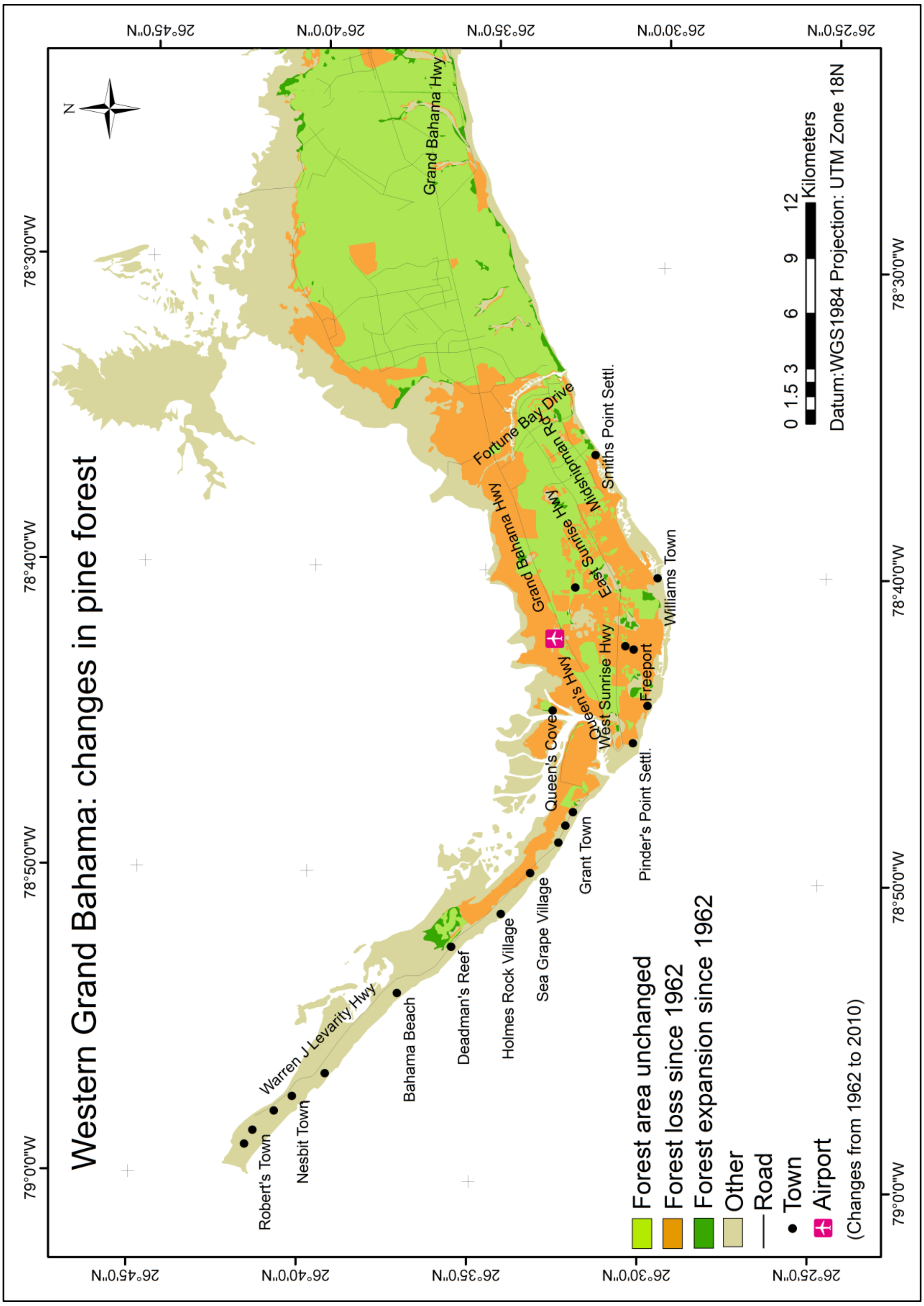


Figure 2.29. Changes through time to the pine forests of western Grand Bahama. Unchanged forest area corresponds to areas of pine forest present during 1962 and 2008-10 surveys; forest loss to areas not present anymore in 2008-10 survey; and forest expansion to new areas not present or mapped in 1962 survey. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

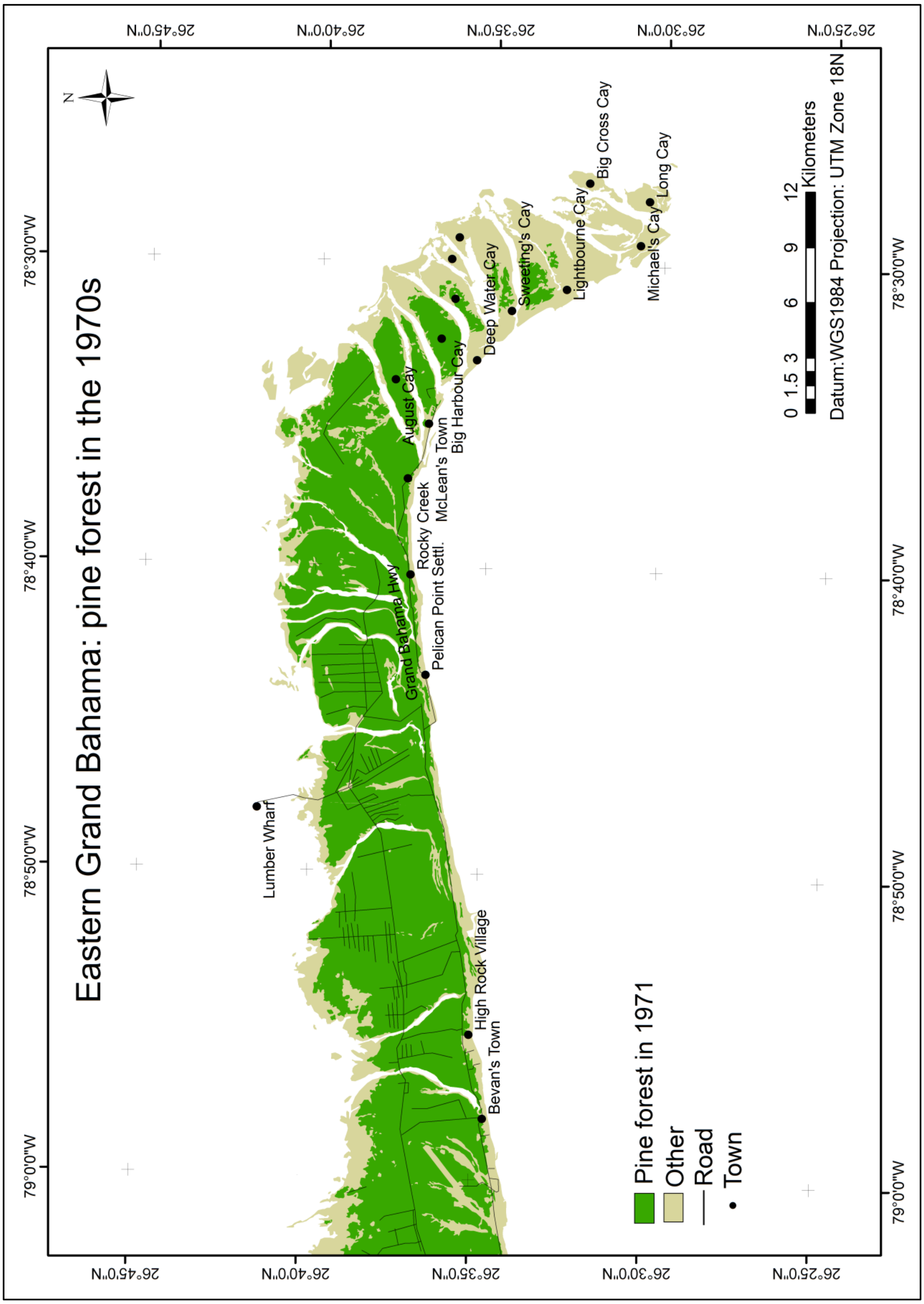


Figure 2.30. Pine forest area in eastern Grand Bahama in the 1970s. Pine forest area digitized from historical maps in ESRI® ArcMap™ v.9.2 and 9.3. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

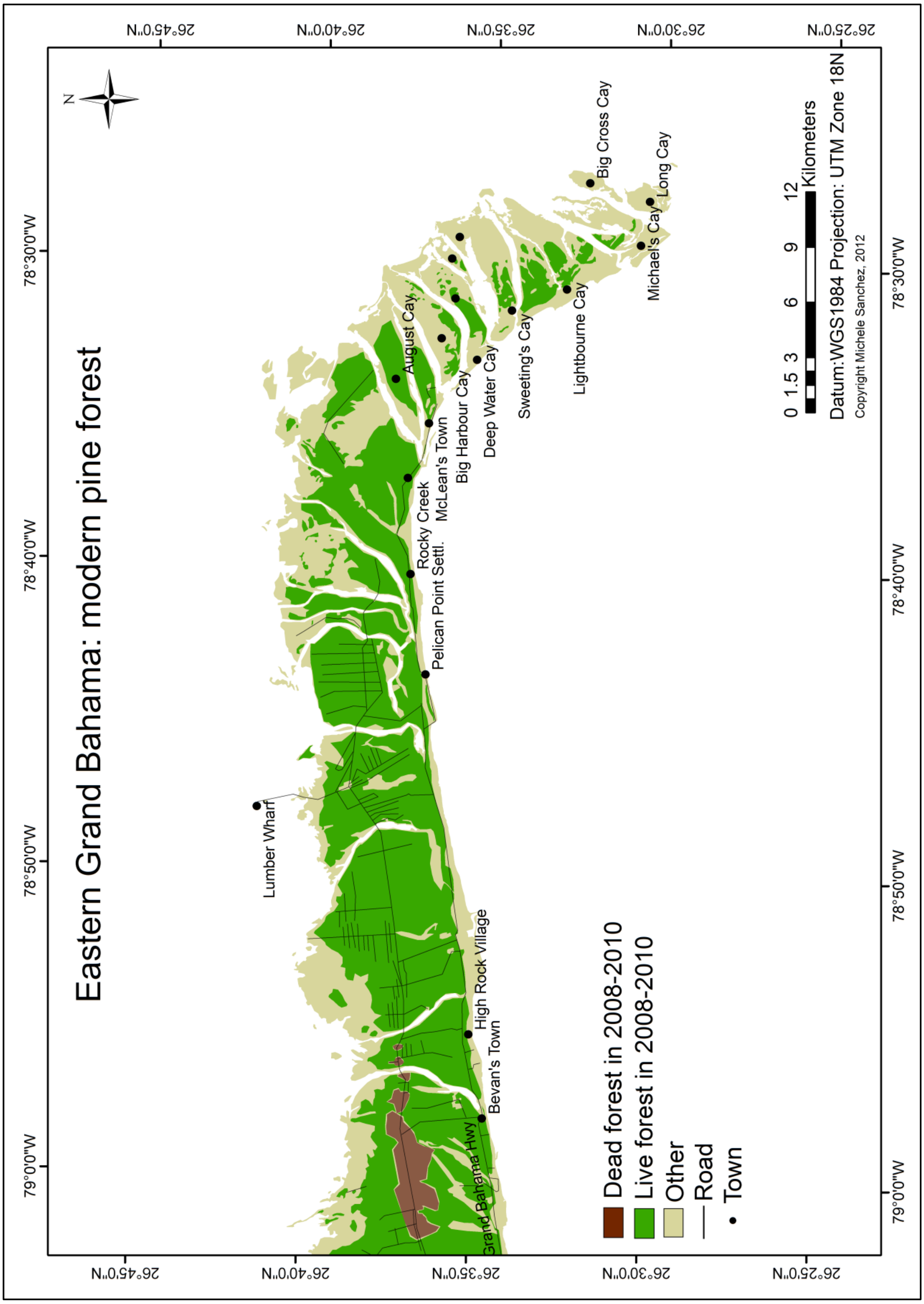


Figure 2.31. Modern pine forest area in eastern Grand Bahama. Pine forest area digitized from satellite imagery in Google™ Earth and ESRI® ArcMap™ v.9.2 and 9.3; and features refined through ground truthing in 2008-10. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

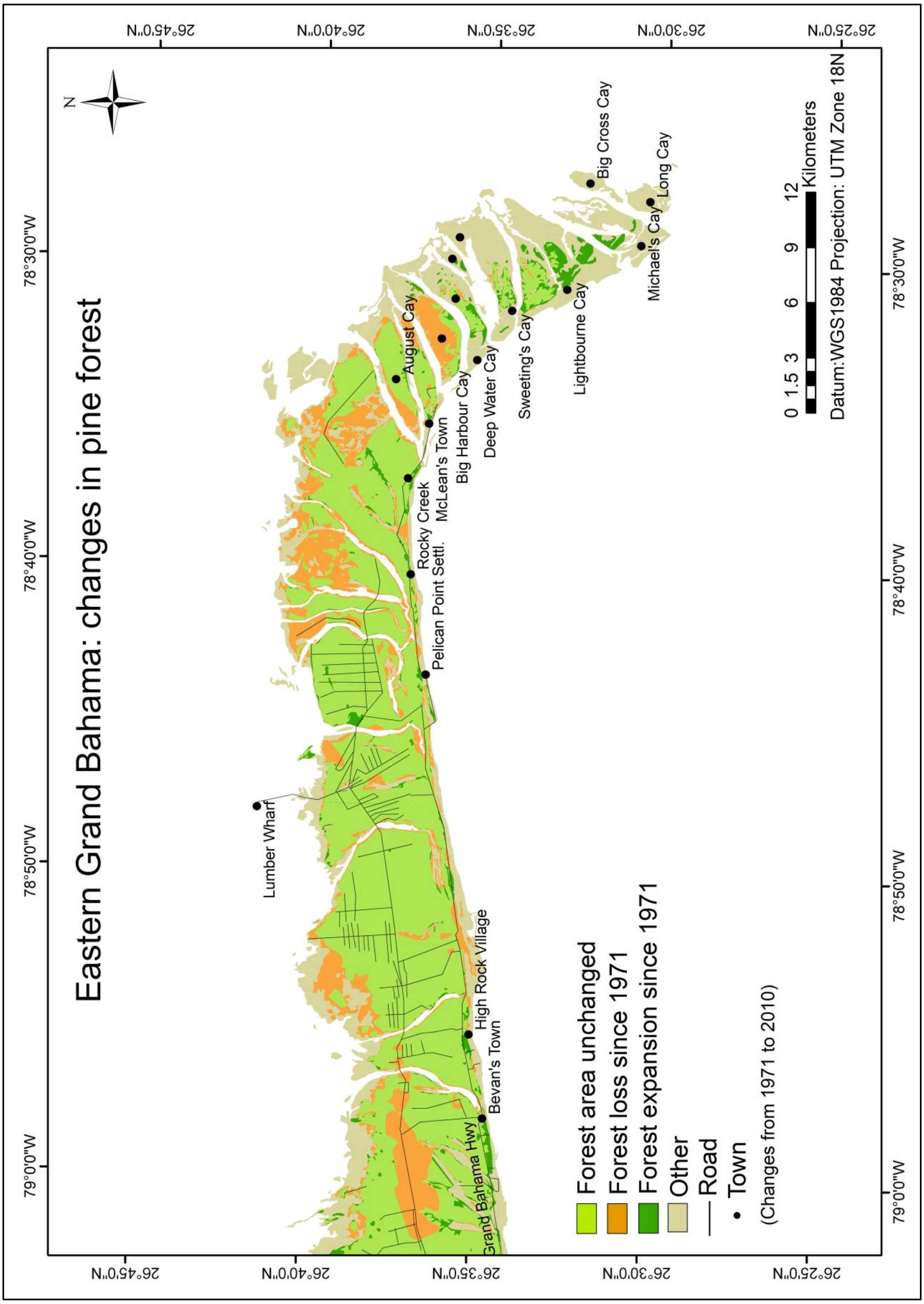


Figure 2.32. Changes through time to the pine forests of eastern Grand Bahama. Unchanged forest area corresponds to areas of pine forest present during 1971 and 2008-10 surveys; forest loss to areas not present anymore in 2008-10 survey; and forest expansion to new areas not present or mapped in 1971 survey. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

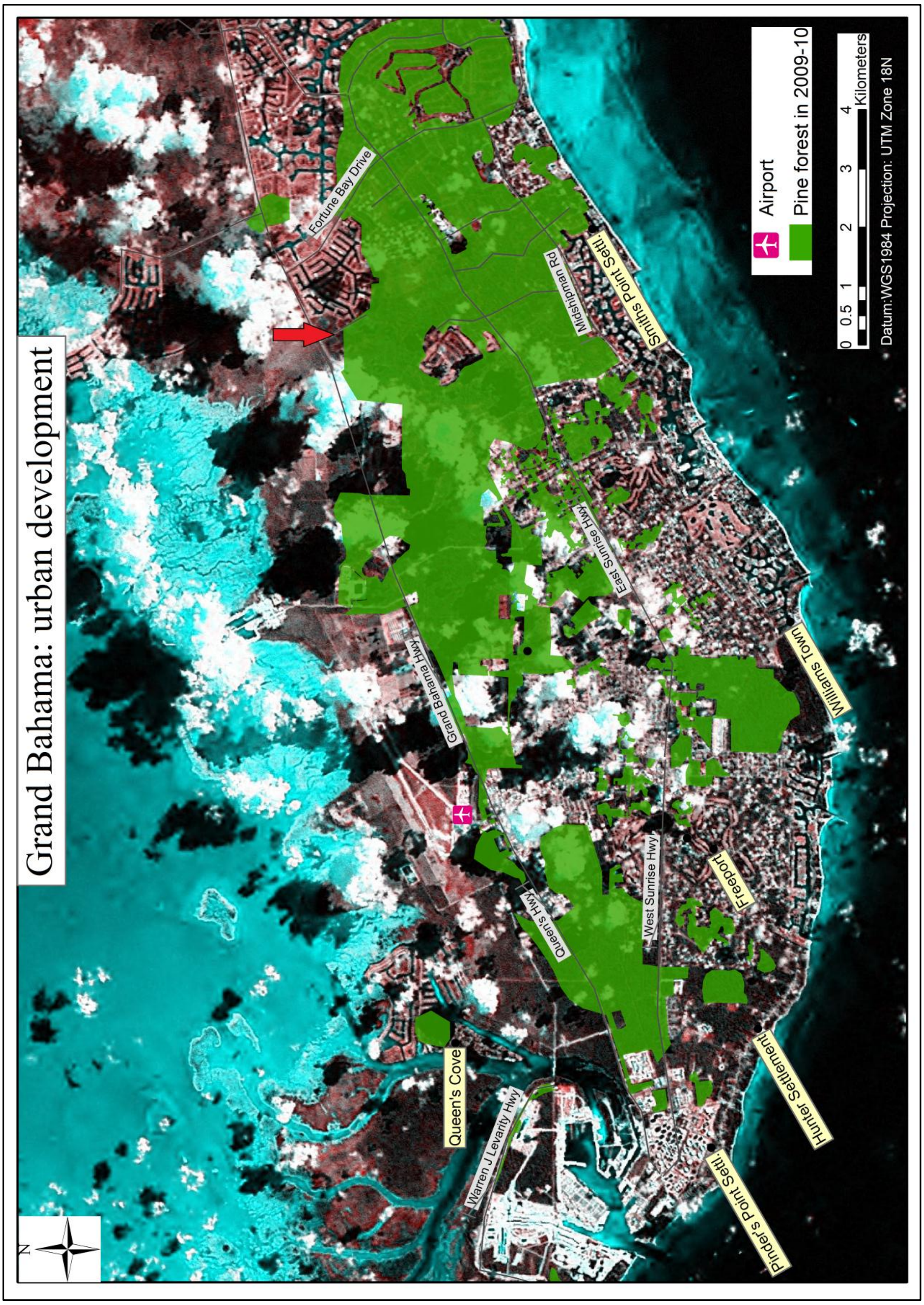


Figure 2.33. Urban development on Grand Bahama. Modern pine forest area represented in green. Satellite image with visible bands RGB 1,3,3 allowed easier visualization of the urban areas. Red arrow indicates area of dead pine forest around artificial canals in the Grand Lucayan Waterway.

d) New Providence

The capital city of the Bahamas, Nassau, is located on New Providence Island. This is the most developed and touristic island in the Bahamas; although, it is the smallest pine island. Most development is concentrated on its eastern side, where downtown Nassau is located, as can be seen in the satellite image in Figure 2.38. Urbanisation increased considerably since the 1960s and pine forest area decreased by 38.7% in only 13 years (from 1961 to 1974). Major losses during this time appear to have occurred south and east of Harold and Wilson Ponds, as well as south of Carmichael Road (Figures 2.34 and 2.35). Further forest losses continued since 1971, resulting in a total forest loss of 63.6% until 2009. Almost all forest located in the east part of the island and south of Carmichael Road have disappeared (Figures 2.37 and 2.38).

Modern pine forest is located mainly between the airport and Carmichael Road (Figure 2.36). It has an area of 24.8 km^2 and covers a total of 11.6% of the island (Figure 2.10). The main block of existing pine forest is situated north of Carmichael Road. It is part of the remaining Crown Land and it is where the water well fields for the city are located. This area has been mostly unchanged since 1961, apart from small areas of expansion recorded in 2008-9 (Figure 2.37). All areas visited showed good regeneration. Hardly any broadleaf forest remains on this island.

There are no large areas of protected pine forest on this island and no areas of old-growth forest can be found today. Some pine trees are found in The Harold and Wilson Ponds National Park and the Primeval Forest National Park in New Providence.

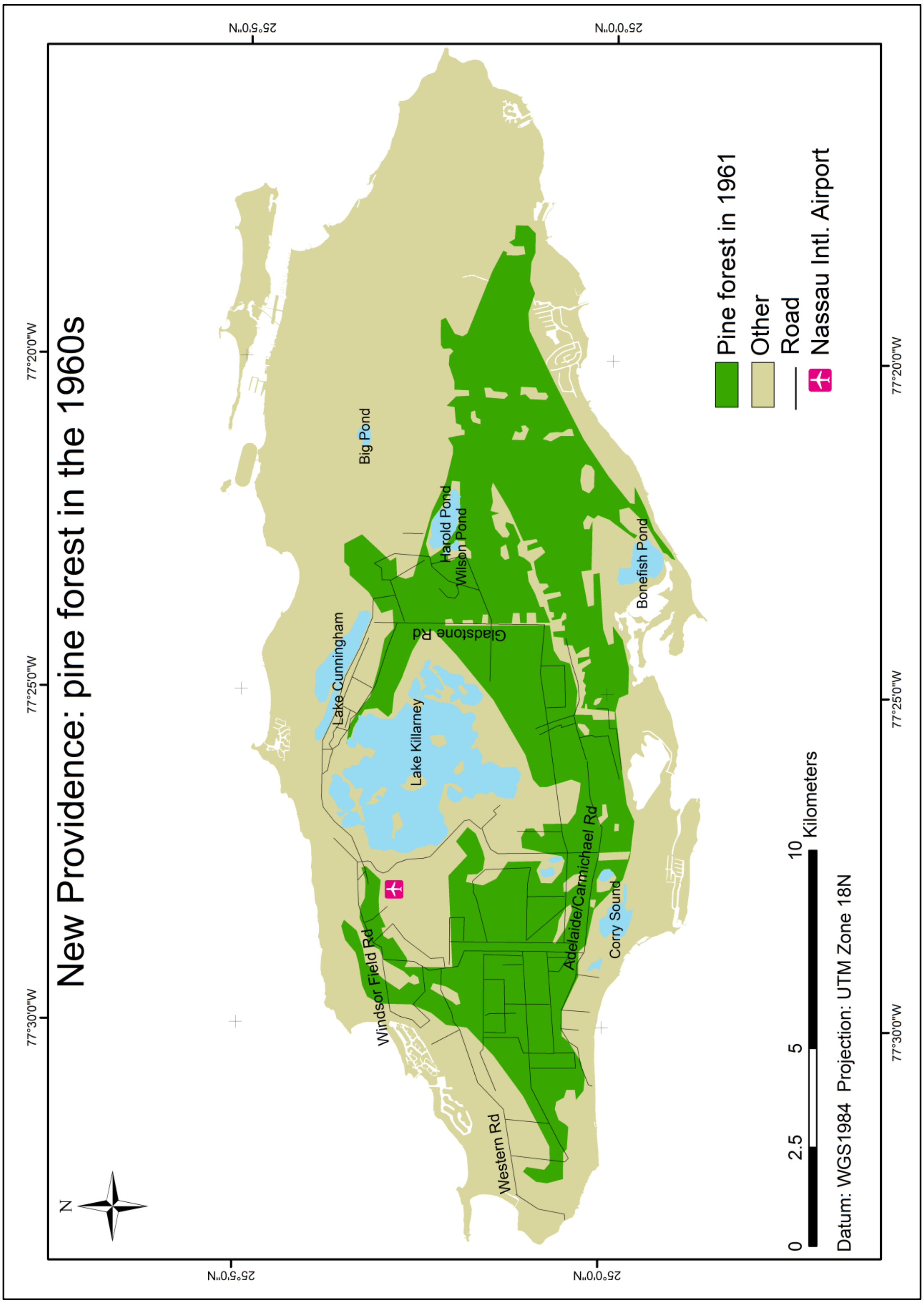


Figure 2.34. Pine forest area on the island of New Providence in the 1960s. Pine forest area digitized in ESRI® ArcMap™ v.9.2 and 9.3, after Bounds (1981). Feature 'Other' corresponds to other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented here are for reference only, and do not correspond to the totality present on the island.

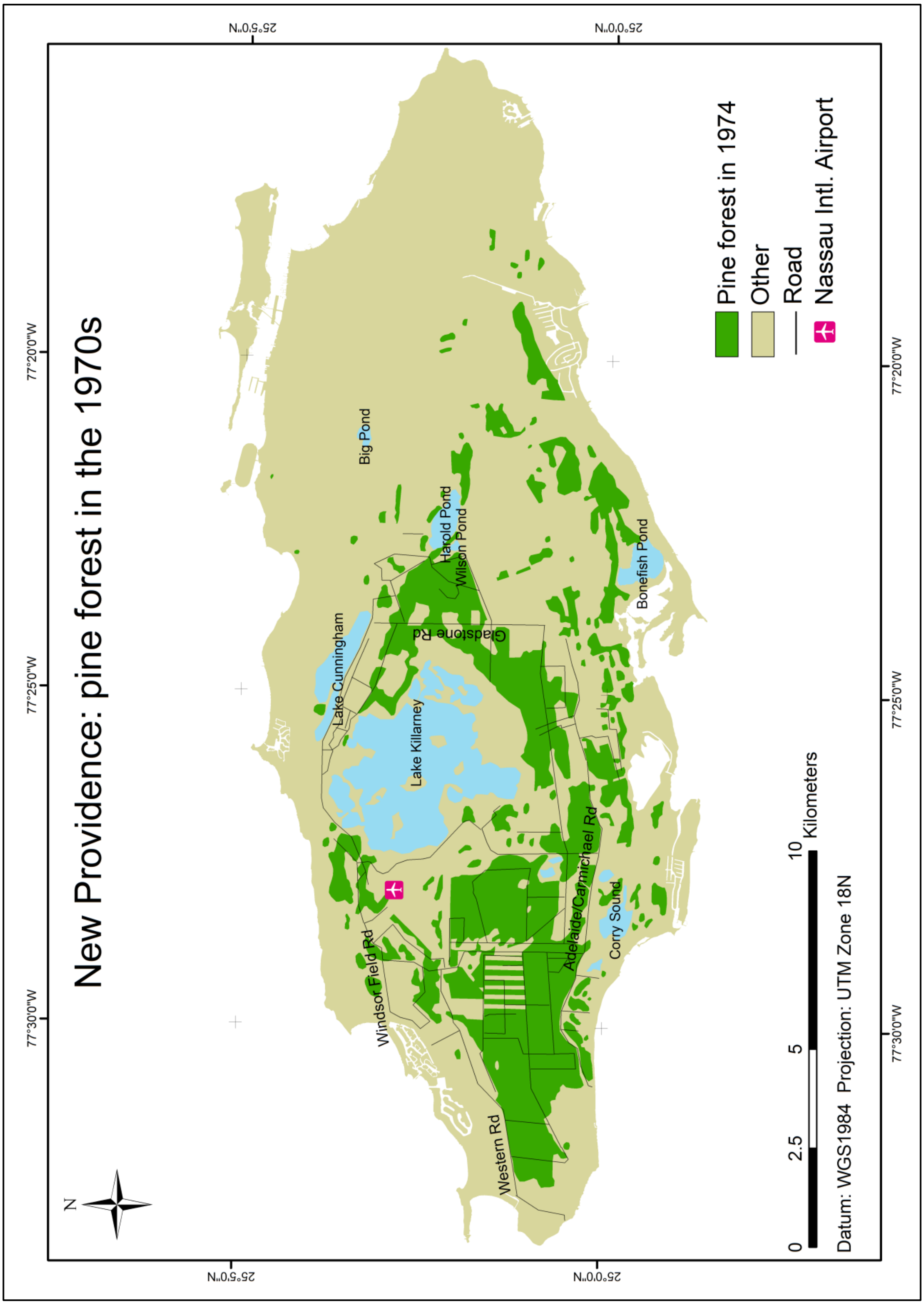


Figure 2.35. Pine forest area on the island of New Providence, Nassau in the 1970s. Pine forest area digitized from historical maps in ESRI® ArcMap™ v.9.2 and 9.3. Feature ‘Other’ corresponds to other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented here are for reference only, and do not correspond to the totality present on the island.

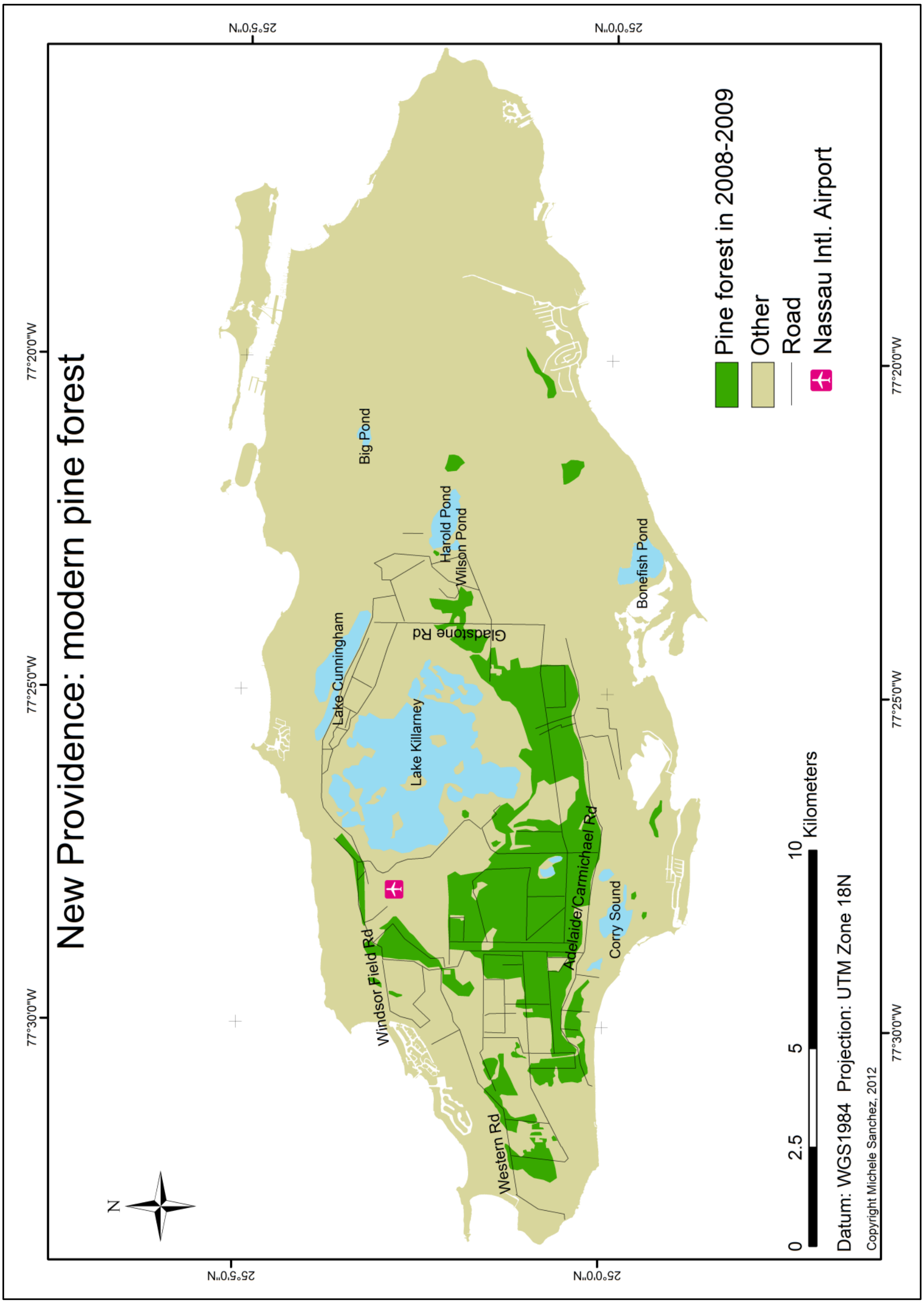


Figure 2.36. Modern pine forest area on New Providence, Nassau. Pine forest area digitized from satellite imagery in Google™ Earth and ESRI® ArcMap™ v.9.2 and 9.3; and features refined through ground truthing in 2008-9. 'Other' means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

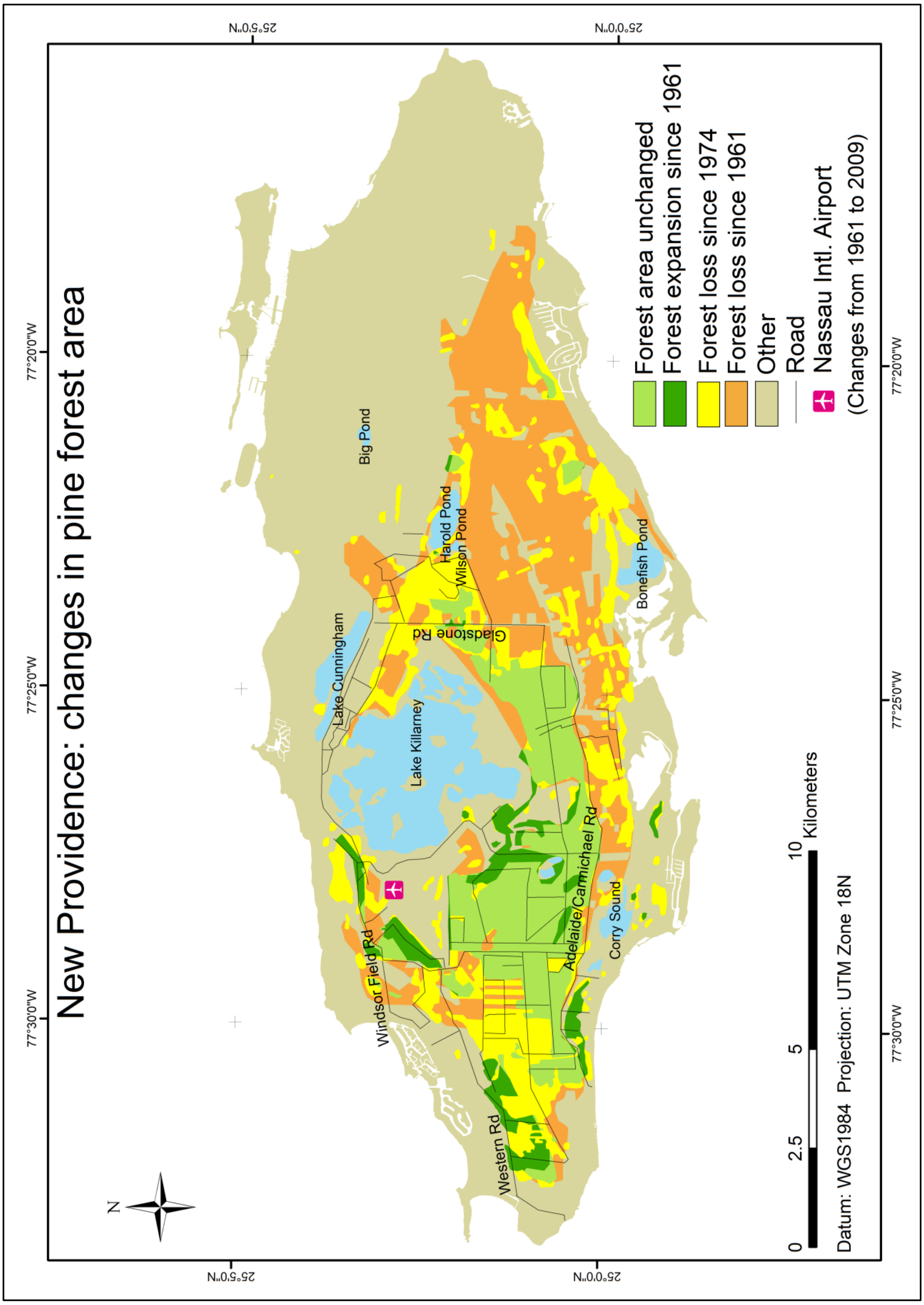


Figure 2.37. Changes through time to the pine forests of New Providence, Nassau. Unchanged forest area corresponds to areas of pine forest present during 1961, 1974 and 2008-9 surveys; forest loss to areas not present anymore in 2008-9 survey; and forest expansion to new areas not present or mapped in 1961 and 1974 surveys. ‘Other’ means other non-mapped features. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

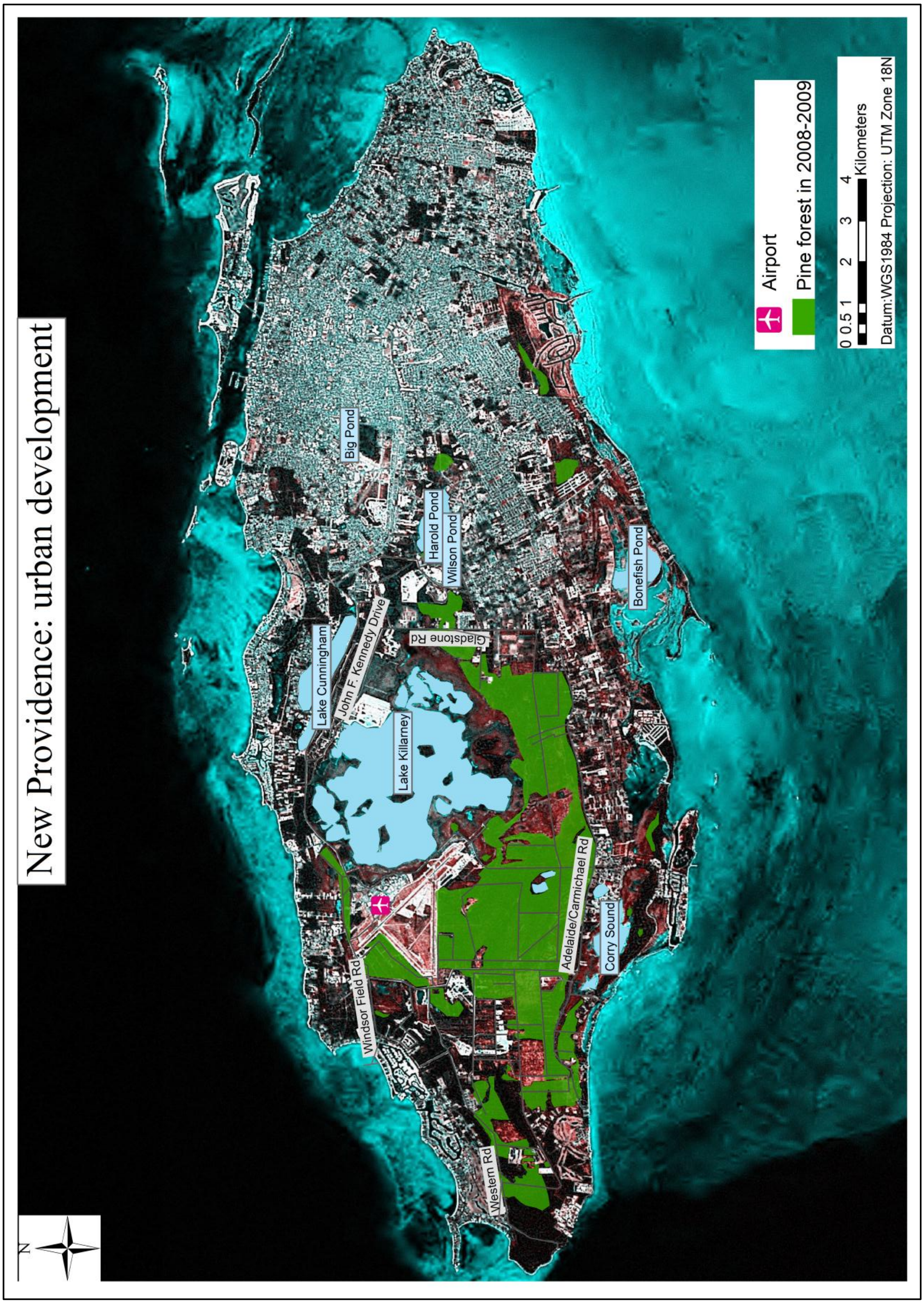


Figure 2.38. Urban development on New Providence, Nassau. Modern pine forest area represented in green. Satellite image with visible bands RGB 1, 3, 3 allowed easier visualization of the urban areas. Eastern side of the island is highly developed.

2.3.2.2. TCI

The area of the Turks and Caicos Islands is much smaller than the Bahamas and so is its pine forest of 13 km^2 (Table 2.3). This pine forest is smaller than the one found on New Providence, which is the smallest pine forest area in the Bahamas. Most of the islands, with the exception of Providenciales and Grand Turk, have undergone very little development in modern times.

Pine forests are found on the islands of Middle Caicos, North Caicos and Pine Cay. Middle Caicos has the largest total pine forest area in TCI, with 11.5 km^2 (Table 2.3). However, this is equivalent to only 4.1% of the total land area of this island (Figure 2.10). On the other hand, Pine Cay, the smallest pine island, has the largest percentage of pine cover in the TCI, with 29.7%. North Caicos has the smallest pine forest and pine forest cover per island since 2009, respectively 0.4 km^2 and 0.2% (Table 2.3 and Figure 2.10). North and Middle Caicos main vegetation types are broadleaf and salt marshes, and pine trees only grow in the central part of these islands (Figure 2.40).

Historical pine forest area for the TCI is not available, but it seems that all pine forests are old-growth forests and no commercial exploitation has occurred. Major known changes to pine forest area occurred from 2009 to 2010 on North Caicos, after a natural fire (Green 2011). An area of 4.3 km^2 has burned completely and no visible regeneration has been recorded since then (Malumphy *et al.* 2012), accounting for a reduction in 91.5% of North Caicos pine forest area and 24.8% of the total pine forest area for the TCI (Table 2.3 and Figure 2.39).

Severe reduction in the number of live pine trees has occurred in all the pine forests in the TCI since 2005, due to the spread of the invasive exotic scale insect *Toumeyella parvicornis*, as previously described in Chapter 1, section 1.5.4. However, a certain level of forest regeneration is present in all areas, with the exception of the 2009 burnt forest area on North Caicos. Therefore, pine forest area and pine forest cover per island shown in this research still consider the whole area previously reported by Salamanca *et al.*(2010). It was observed during this research that Pine Cay seems to have more healthy trees than the other islands and good regeneration.

There is no road access to most of the pine forests of North and Middle Caicos. Little development in modern times has happened in either of these islands, with the majority of it concentrated along the north coast. Pine Cay has a good network of roads throughout the island, one resort and scattered private homes. The only protected area with pine trees present in the TCI is situated in Conch Bar Caves National Park, which lies northeast of Middle Caicos airport. This park covers an area of 0.8 km^2; however, very few pine trees can be found there.

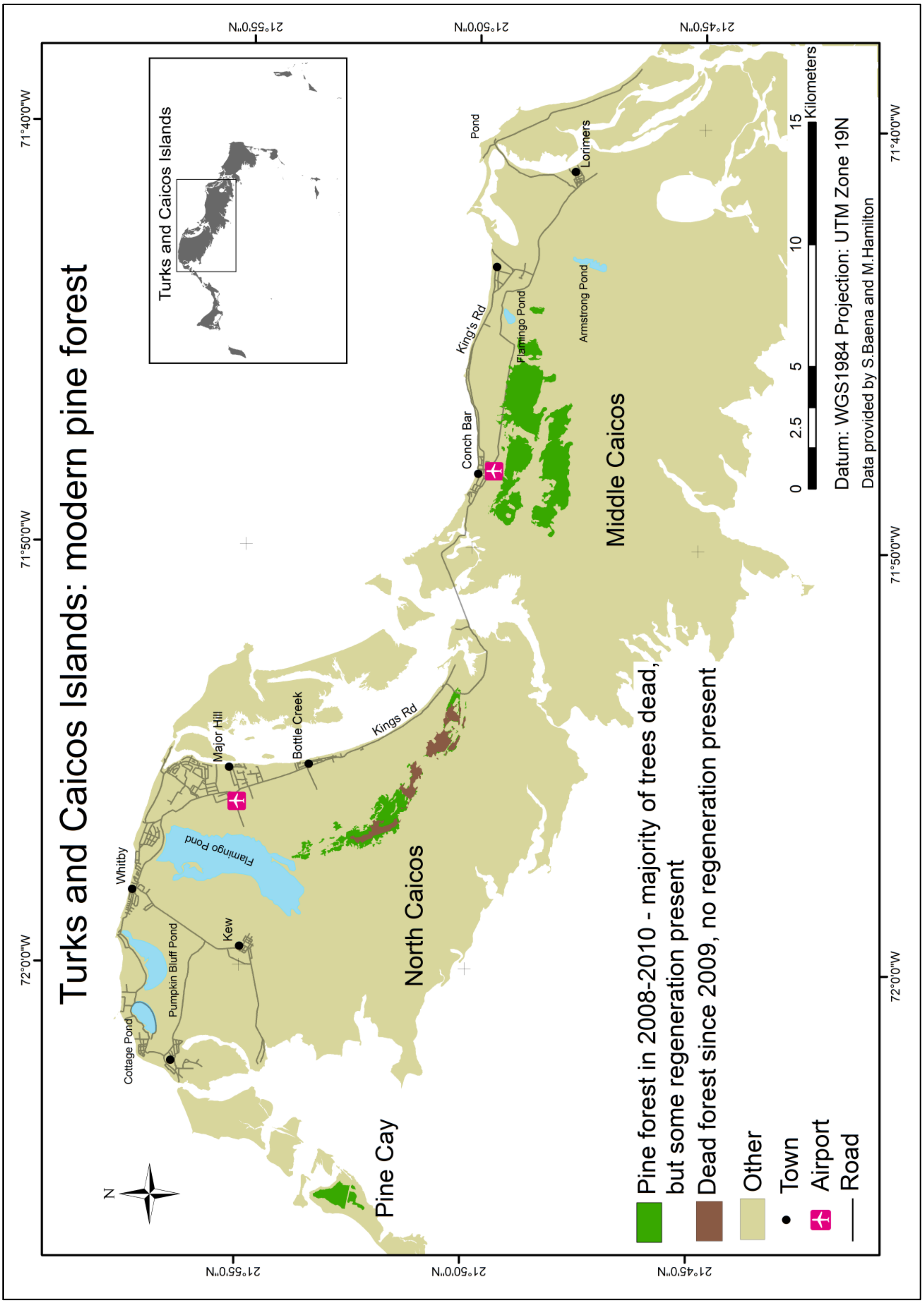


Figure 2.39. Modern pine forest area in the Turks and Caicos Islands. Map produced in ESRI® ArcMap™ v.9.2 and 9.3. Data provided by S.Baena and M. Hamilton, Kew. ‘Other’ means other non-mapped features, e.g. vegetation types and urban areas. Towns and roads represented are for reference only, and do not correspond to the totality present on the island.

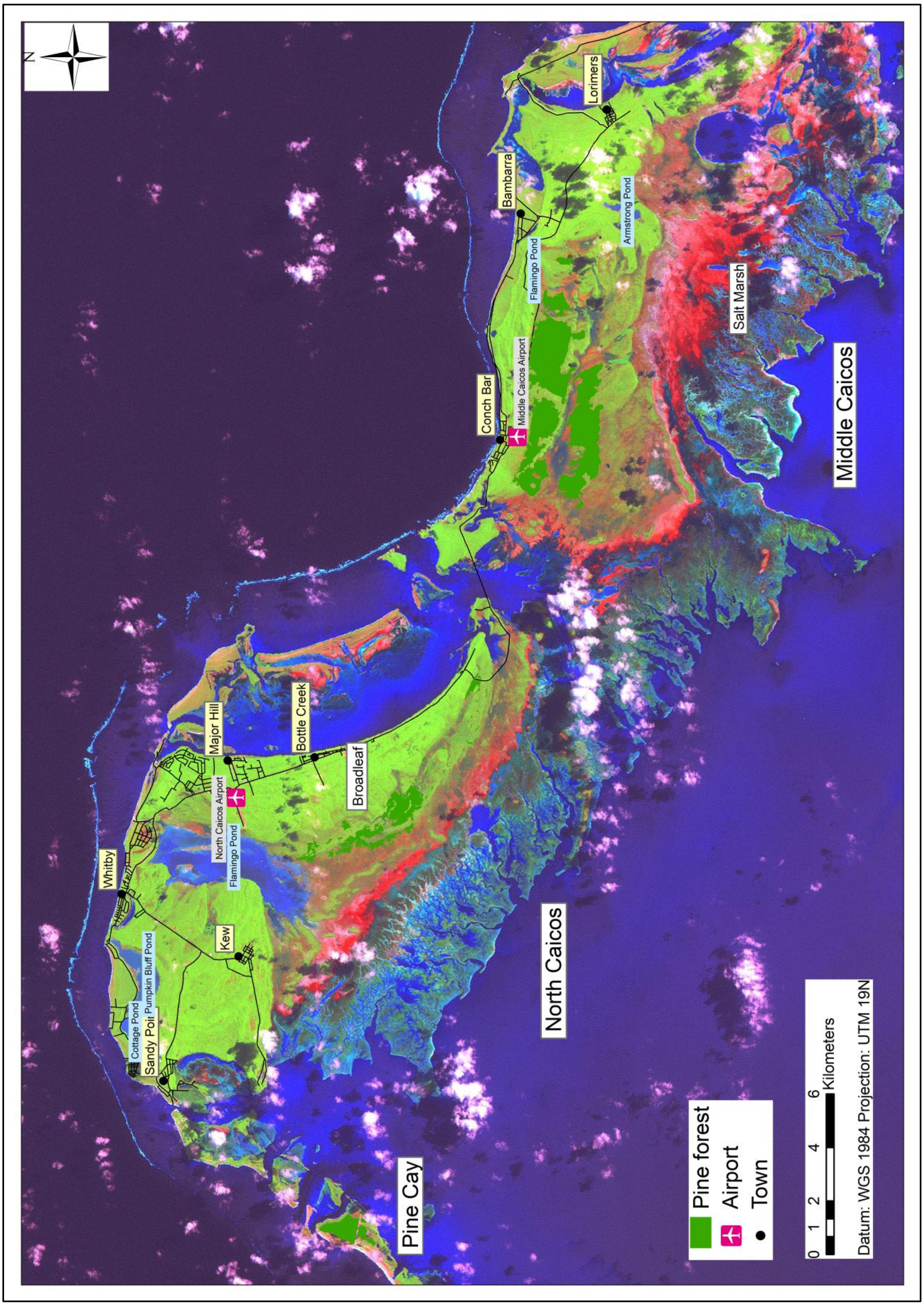


Figure 2.40. Pine forests and other vegetation types shown on satellite image of the TCI. Satellite image with visible bands RGB 1, 2, 3; showing salt marshes in pink, broadleaf vegetation in light green and pine forest in dark green.

2.3.3. Simulation of sea level rise in the archipelago

All the pine islands in the Bahamas and the TCI show inundation zones with only 1m rise of the current sea level (Figure 2.41). However, only in the TCI there is total flooding of land at this level. Flooding of the coastal areas is present in most islands. Andros Island also shows severe inundation in the south and west with an increase in 1-3m of the current sea level. Inundations could also affect the north, west and east extremes of Grand Bahama. Although most of Abaco seems unaffected even by higher sea levels, some damage to the coast and west part of Great Abaco is estimated. Damage to New Providence seems to be less intense than in the other pine islands.

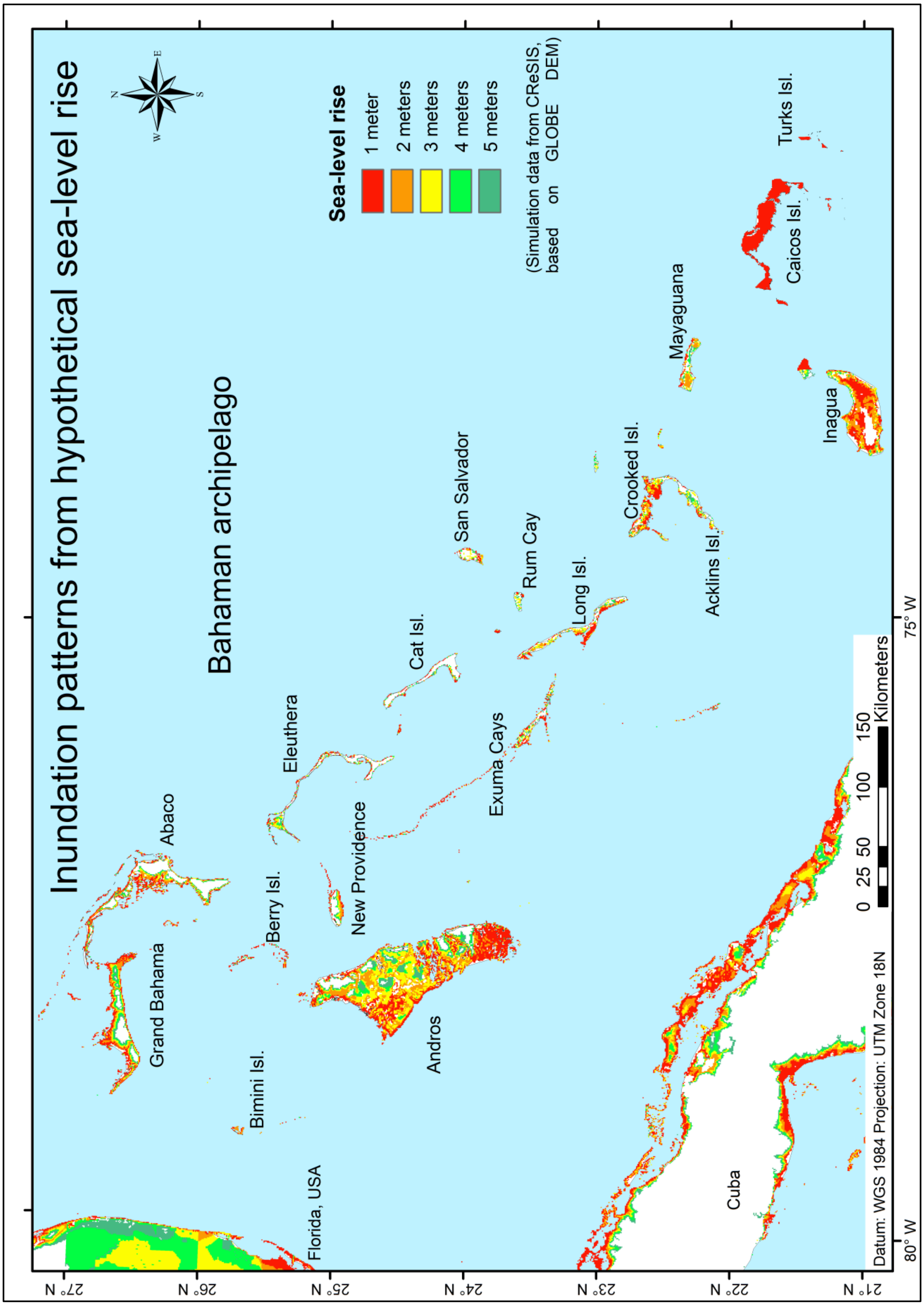


Figure 2.41. Simulation of sea-level rise and inundation zones in the Bahaman archipelago. Data obtained from CReSIS is based on GLOBE DEM and GIS raster analysis.

2.4. Discussion and Conclusions

Map accuracy and errors

The comparison of maps from different sources and in different scales has been made easier with modern technology, such as GIS. However, it is important to be aware of errors resulting from differences in drawing scale, projections, surveying, and estimation of boundaries (Foote and Huebner 2006). Goodchild (1989) states that subjective boundaries involving transition areas between vegetation types and the scale at which vegetation is mapped can be sources for mapping errors. The different scales found in the historical maps used to calculate and represent historical pine forest area, the scale used to map modern pine forest and the subjective delimitation of boundaries could have led to over- or under-estimation of changes in forest area through time. Very small fragments of forest expansion or forest loss found in the comparative maps of changes through time are probably a result of these differences in scale and mapping standards, instead of real changes in the vegetation. Nevertheless, the importance of these maps for the graphical representation of the trends in changes of forest area and analysis of the data is still high and powerful.

The thematic accuracy for the maps of modern pine forest (between 83.6% and 96.2%) is within the values reported by Horning *et al.* (2010) of 80-90% thematic accuracy for conifer/hardwood class, and >90% for forest/non-forest class, when Landsat TM images are used (Table 2.1). Problems such as definition of classes and their boundaries, and the sampling of accuracy points can decrease the reliability in the confusion matrix to judge accuracy; however, this is still a commonly used testing method (Foody 2002). Vegetation boundaries, as mentioned before, are usually not well defined, which can lead to lower thematic accuracy in the map (Foote and Huebner 2006). Other sources of inaccuracy found were the age and quality of the imagery available for mapping. The imagery available in Google™ Earth varies widely among areas and image sources, as shown in Figure 2.3. Vegetation types and their boundaries are more difficult to estimate in areas covered by cloud or with low resolution. In order to achieve higher accuracy, aerial photography of the studied area or extensive ground truthing throughout the whole islands would be necessary. However, availability, costs and extent of the study area have forbidden this approach.

Extensive field work was carried out in fragmented forests in urban areas, e.g. Nassau (New Providence) and Freeport (Grand Bahama), in order to check boundaries, identify vegetation types and detect changes in forest area in more recent years. Such areas are changing constantly with development and forest is usually fragmented in small patches and more difficult to detect in the satellite images. Presence of other tall trees with similar canopy shape to pines in urban areas can also mislead interpretation. All these factors were present on New Providence, where the highest over-estimation of forest area before ground truthing occurred, as shown in Table 2.2. Errors in the other islands were probably due to cloud cover, image resolution and recent changes in vegetation. Therefore, ground truthing was essential for refining the maps of modern pine forests and increasing their accuracy. Having access to the maps and data in the field also helped greatly with orientation, identification of important areas for ground truthing and maximization of ground covered during field work.

Positional accuracy calculated for the maps of modern pine forest, based on a scale of 1:18,000, was slightly lower than the standard values recommended by the United States Geological Survey (USGS) for mapping scales of 1:12,000 and 1:24,000, respectively, +- 10.1 m and 14.2 m (Subcommittee for Base Cartographic Data 1998). However, overall accuracy for the x-axis and y-axis had smaller figures (Table 2.1). Differences between the reference point and ground point on the islands of Abaco and New Providence were < 8 m. Higher values (<17.3 m) observed for the islands of Grand Bahama and Andros could have been caused by distortions in the projection. Both islands are located in two UTM zones, 17N and 18N. It is known that areas closer to boundaries between two UTM zones show higher distortion than areas near the central meridian (Kennedy 2000). Taking into account that the GPS positional accuracy was 10m and the quality of the imagery used for the reference points was low, the accuracy level achieved for Abaco and New Providence was good and for Andros and Grand Bahama still acceptable.

Simulations of sea level rise

The simulations of sea level rise and inundation obtained from CReSIS allowed the visualisation of low-lying areas and future impact of climate change on these forests. However, they were based on GLOBE DEM raster images, which have a vertical precision of circa 10 to 18 m (Hastings and Dunbar 1998) and cells measured circa 1 km. Most land in the archipelago has an elevation <20 m, although a few ridges and cliffs are present in some islands (Sealey 1985). The highest point in the Bahamas is located in Cat Island with 63 m elevation; and in the TCI on Providenciales with 49 m elevation, but none of these islands have pine trees. Although little inundation is shown for Cat Island in Figure 2.41; the island of Providenciales shows complete inundation with only 1m sea level rise. Taking into account precision of the elevation model and low elevation of the islands, in addition to cell assignment process during GIS raster analysis, it seems that part of the inundation zone present in the simulations is an over-estimation. This is certainly true for the TCI, where all land was inundated with only 1m rise in sea level, despite the presence of higher elevations on the islands. This resulted from large cell size, distribution of the DEM cells across the islands and the first steps of reassignment during raster analysis. Use of SRTM data (NASA Shuttle Radar Topography Mission) 30 or 90 m cell sizes would increase vertical precision considerably and probably produce a more realistic simulation for small islands. However, simulations using such data were not readily available; they would demand extra time and resources and were beyond the objectives of this research. Although CReSIS simulations seem not to be true for the whole extent of the islands, it is important to consider that pine forests are located in areas of low elevation and certainly will suffer the consequences of sea level rise through inundation or salinization of the fresh water lenses.

Pine forest distribution and size in the Bahaman archipelago

A general expansion of forest was observed for *P caribaea* var. *bahamensis* in the archipelago (Table 2.3). Estimated pine forest area during the 1960s and 1970s was circa 2011 km^2. Previous estimates of pine forest area for the Bahamas were 2023 km^2 (March 1949, Kellogg 1951) and 1782 km^2 (Greaves 1978), which are in accordance with the estimate from this research.

The extent of occurrence of these pine forests has not changed, i.e. from northern Bahamas to the Caicos Islands. However, previous observations of pine forests in the Berry Islands (Northrop and Northrop 1902) and in Great Inagua (Farjon and Styles 1997) were not confirmed by this research or other work (Miller, W. 1920, March 1949, Henry 1974, Greaves 1978). It would require further research to know if pine trees were originally present in these islands or if they have been planted for exploitation by the local communities. However, it seems that whatever the reason they are not present there.

The majority of the pine forests in the archipelago are found in the Bahamas and forest cover per island is more than four times higher there than in the TCI (Table 2.3). It seems that larger islands in the northern Bahamas have more favourable conditions for pine growth and establishment than the smaller and more southern islands in TCI. Andros, which is the largest island in the archipelago, is also the one with the largest pine forest area. Higher rainfall, cooler temperatures and deeper fresh water table seem to be key factors limiting forest distribution, as discussed in Chapter 1 section 1.2.2. It is unknown why pine forests are not present in the rest of the Bahamian islands, but some assumptions can be inferred. Great Inagua, in the southern Bahamas, is larger than New Providence; however, more exposed to the prevailing winds and salt spray, and with most of its extension covered in salt ponds (Figure 1.2, Chapter 1). Most of the other islands are small or narrow; thus, with higher exposure to salt spray, lower rainfall and less fresh water available (Sealey 1985). Therefore, challenging conditions for pine establishment and growth would be present, i.e. low fresh water availability, exposure to salt spray, drought and high temperatures. Changes in the sea level and climate in this region during the glacial and inter-glacial periods could also have affected forest distribution and composition, as discussed in Chapter 1 section 1.2.4. Whatever the reason, modern pine forests in the Bahaman archipelago are restricted to only four of the islands in the northern Bahamas and three islands in the TCI.

The Bahamas

Pine forest loss was observed on New Providence, Grand Bahama and North Caicos, whereas the two largest islands in the archipelago, Abaco and Andros, had an increase in forest size (Table 2.3). In general, pine forest area for the Bahamas increased as a result of forest expansion in the largest islands with the largest forest tracts. The frequent occurrence of uncontrolled and human-ignited fires on Andros and Abaco, many of them during the dry season, has been observed during this research (Chapter 4 section 4.3.3) and also by others in the past (Myers, R. *et al.* 2004, Miller, A. C. 2007). Such fires reduce the extent of the existing dry evergreen forest (coppice) and create gaps for the expansion and establishment of new areas of pine forests. Therefore, the increase observed in forest area in these islands.

The high occurrence of natural and seasonal fires in the northern Bahamas contributes to the maintenance of the pine forest ecosystem. Such fires prevent the undergrowth becoming overgrown and species composition changing to a dry evergreen forest, as previously mentioned in Chapter 1 section 1.4.2. Palaeobotanical data has also shown a relationship between population increase, fire increase and pine forest expansion on Andros and Abaco (Kjellmark 1996, Slayton 2010).

A discussion on the biogeography of the pine forests in each island is presented below.

Abaco

A great part of Abaco Island is suitable for pine forests, as observed from its high percentage of pine cover (46.3%). Its northern position and large size favour higher rainfall and cooler temperatures than those found in the more southern islands. Towns are small, with the exception of Marsh Harbour, and main urban development is situated along the coast. All these factors, in addition to the presence of fires, as explained before, help to maintain and expand the pine forest area, which has increased since 1967. Despite the general increase in forest area in Great Abaco, parts of its forests have been lost since the 1960s and 1970s (Figure 2.15). Some of these areas were replaced by agricultural land along the Great Abaco Highway and near Treasure Cay airport. It seems that the main changes in forest distribution on this island have been driven by urbanisation and fires, either natural or human-ignited, since its first settlements to this day (O'Brien *et al.* 2008, Slayton 2010).

In normal circumstances, forests of *P. caribaea* var. *bahamensis* show good regeneration after logging and fire. However, in certain circumstances (when fires are too frequent, gaps in the vegetation are too small, or seed source is impaired) regeneration can be low or non-existent (Henry 1974, Radabaugh 1974, Myers, R. *et al.* 2004, O'Brien *et al.* 2008). During the 1970s, areas of low or inexistent regeneration, mainly in southern Abaco, were observed by Henry (1974) (Figures 2.13 and 2.17). A combination of intensive logging, followed by hurricanes and frequent fires was catastrophic to parts of these pine forests, threatening more than 13% of the total forest area. Abaco was heavily hit by hurricane Betsy in 1965, with storm surges > 2m, and floods persisting for up to 13 days (Table 1.1). Henry (1974) reported the death of 95% of seed bearers in the area south of Crossing Rock by 1966; and high tree mortality due to desiccation and pine bark beetle (*Ips* sp.) infestations by 1967. He also mentioned the occurrence of 57 non-natural fires from 1965 to 1969. A large tract of forest was completely lost in South Abaco, as a result of flooding, increased soil salinity, pest attacks and death of trees and seedlings by fire. Wind damage and salt intrusion can enhance susceptibility of pine trees to insect attacks, as it was observed in the coastal forests of South Carolina after hurricane Hugo in 1989 (Gardner *et al.* 1992). Breda *et al.(2006)* suggested that droughts, in this case low availability of fresh water, reduce the production of carbohydrates, which are used in the production of secondary compounds, e.g. tannins and alkaloids. These compounds are involved in plant defence against insect and fungal attacks and their reduction can increase plant susceptibility to infestations and diseases. Billings *et al.* (2004) reported a positive correlation between non-natural fires in high density pine stands and beetle attacks on pine trees in Central America. In El Salvador, two outbreaks of southern pine beetle (*Dendroctonus frontalis*) were observed just one year after wildfires occurred in a local pine forest. Platt *et al.* (2002) have also shown that dry-season fires increased pine mortality after hurricane events, altering the wood structure of trees and making them more susceptible to pest attacks. Increased amounts of dead wood and extra litter on the soil surface after storm surge events can cause high intensity fires with high fuel loads. These fires can spread through large areas of the pine forest with devastating effects. Another of these events in 1999 killed many acres of forests on Abaco after the storm surge caused by hurricane Floyd in 1998 (Myers, R. *et al.* 2004, Wunderle Jr. *et al.* 2007).

Although forests are now present in most of the areas affected by storm surge in the past (Figures 2.14 and 2.18), it is uncertain if natural regeneration or restoration took place. Henry suggested in 1974 that the area south of Crossing Rock should be re-seeded with seed collected in Little Abaco or Andros, but no further information is available to confirm if any natural regeneration occurred or his suggestion was carried out. However, nowadays the forest is dense and even-aged, suggesting that some sort of re-seeding took place. Further discussion on this matter will follow in Chapter 3 section 3.4 and Chapter 4 section 4.4.

Andros

Andros pine forests share a similar history to that of Abaco, with major influences on pine distribution attributed to urbanisation and fire and an increase in forest area since 1971. Intensive logging took place in northern Andros from the 1950s to the 1970s; however, circa 5% of the pine trees in each plot were left intact to ensure regeneration (Snyder *et al.* 2007). It seems that regeneration was good following logging activities, according to observations by Henry (1974) and Greaves (1978). The possibility of logging being carried out in Mangrove Cay during the 1970s was also considered in his report. Although there is no further evidence of this fact, it is noticeable that the pine forest stand visited during this research is even-aged, and all the trees are probably less than 30 years-old. This suggests that logging did take place at some stage. Good regeneration of forests on Andros was probably due to less intensive logging than on Abaco and the preservation of small blocks of forest, which increased seed source. In addition to that, no major hurricanes have hit the island during or shortly after logging (Tables 1.2 and 1.3, Chapter 1) and no pest attacks have been reported. Secondary forest is even-aged and have almost double the density of trees per hectare than that found in the old-growth forest in the same island (Snyder *et al.* 2007). The combination of high forest density and the frequent fires which have been observed during this research and by others (Radabaugh 1974, Myers, R. *et al.* 2004) could lead to lower regeneration and susceptibility to pest attacks in the future, as previously mentioned.

Forest expansion near many settlements is probably a result of intentional or accidental forest fires. A large area of forest was lost to agricultural plots in northern Andros (Figure 2.23). As mentioned in Chapter 1 section 1.5.3, the pine forest area provides ideal conditions for agriculture with its levelled surface, fertile soil and fresh water supply. Losses of smaller fragments of forest throughout the island were also observed. These losses or small expansions could have resulted from areas being missed or ignored in the historical maps and included in the modern maps or vice-versa. Therefore, it is difficult to say if these changes have actually occurred or if they are just an artefact resulting from the comparison of different map scales and subjective vegetation boundaries.

In South Andros, mainly, where no logging took place and the forest is old-growth, changes are probably a result of mapping differences. Forest area is small and fragmented in comparison to northern Andros. Habitat is reduced by large expanses of tidal flats and marshes to the west and south, and old-growth coppice (dry evergreen forest) followed by dry scrub to the east (Sealey 1985, Myers, R. *et al.* 2004). Suitable habitat for pine forests would have been reduced since ancient times, as records of wetlands in the western part of South Andros date back to circa 7005 yr BP (Bourrouilh-Le Jan 2007) and a shift from pine forest to wetlands was recorded for northern Andros and Abaco during the same period (Kjellmark 1996, Slayton 2010), as described in Chapter 1 section 1.2.4. Precipitation is also lower there than in more northern islands (Chapter 2 section 1.2.3). Increased soil salinity and reduced rainfall could have affected survival rates and restricted forest expansion in South Andros, resulting in the present pine forest distribution.

Although regeneration has been good on Andros, the prediction of sea level rise of only 1m seems to cause severe inundations on this island (Figure 2.41). Further increases in sea level from 2-3m would affect most of the island and have a devastating effect on its pine forests. Thanks to a recent expansion of the West Side National Park on Andros, Mangrove Cay and South Andros pine forests are now protected (The Bahamas National Trust 2012). This was an important step in preventing imminent damage to the largest area of old-growth forest remaining in the Bahamas, since the opening of a new road giving direct access to the forest in South Andros in 2010 and pressure from the local community to use part of the forest for agriculture.

Grand Bahama

Grand Bahama is mostly covered in pine forests, with the exception of its western and eastern extremes. Pine trees can grow very close to the coast on the southern side of the island, as fresh water lenses of up to 14m are found only 200m from the coast and there is some protection from the predominant winds (Whitaker and Smart 1997). Some changes in forest area in the eastern cays were observed (Figure 2.32). The most extreme point containing pine trees in the east part of the island is Michael’s Cay. Some pine tree death was observed in Big Harbour and August Cays during field work; however, levels of regeneration could not be checked due to lack of time and difficulty of access. It is uncertain if forest expansion in these cays really happened or they resulted from differences in mapping scale and extent of survey, as previously discussed. Grand Bahama Island becomes very narrow and exposed in the west and pine forests are sparse, until they finally give way to the broadleaf forest beyond Deadman’s Reef (Figure 2.29).

Grand Bahama pine forests recovered well after logging activities during the 1940s and 1950s; despite some reports of losses by fires (Henry 1974, Radabaugh 1974). They used to cover circa 64.6% of the island during the 1960s and 1970s. No major hurricanes occurred on the island during or shortly after logging (Table 1.2). Fires, if present, were not extensive, due to the presence of many swamps and tidal creeks.

Several small fragments of forest, mainly in raised terrain, were left uncut during logging (Henry 1974). All these factors contributed to the presence of enough seed bearers, seedlings and soil seed bank reserve to regenerate the cut pine forest.

Most significant changes observed in the pine forests of Grand Bahama occurred in more recent years. Pine forest was lost to urbanisation mainly in the Freeport area, from Pinder's Point to Smith's Point, as seen in Figure 2.29. It became highly fragmented in this area after the construction of new buildings and canals (Figure 2.33). These artificial canals when penetrating deep inland can cause seepage of the sea water into the fresh water lenses, as has been observed in the Grand Lucayan Waterway (Sealey 1985). An area of pine death just south of the Grand Bahama Highway near Fortune Bay Drive can be seen in Figures 2.28 and 2.33 (indicated by a red arrow). This area contains many secondary canals derived from the Grand Lucayan Waterway. It is known that some salinization of the fresh water table has occurred near this canal due to seepage in the past (Sealey 1985). It is possible that storm surge and higher sea level could have reduced fresh water and slowed drainage, contributing to pine tree death in the area.

The majority of pine tree death observed on the island and represented in the maps of modern pine forest (Figures 2.28 and 2.31) occurred after 2005. Grand Bahama was hit by hurricane Jeanne in 2004 and Wilma in 2005; the latter causing a storm surge of up to 4m in some areas (Table 1.2). These hurricanes had devastating effects on the pine forests of Grand Bahama, especially on its western and northern parts. Hurricane Wilma also affected the pine forests of the Florida Keys, in the USA, killing more than 80% of the pine trees in the lower elevations (Ross *et al.* 2009). This high level of mortality was not only attributed to the storm surge, but also to the drought that followed after the hurricane season. The drought prevented the dilution of the salts deposited in the soil, turning it hyper saline. In addition, drainage after storm-surge was possibly slowed by a higher sea level. A similar situation could have happened on Grand Bahama, where the storm surge drained slowly in the low areas near marshes and between tidal creeks, killing the trees and seedlings. The presence of salt-tolerant species, i.e. sawgrass, and no seedlings or saplings in many of these areas was observed during field work and could indicate that salt concentration in the soil is still high. Gardner *et al.* (1992) noticed higher mortality after storm surge in transition areas between forest and marshes in North Inlet, South Carolina. Trees and shrubs within 10m of this transition zone lost

80-100% of their leaves two weeks after the storm, while pine trees located 50-150m away lost only 10-50% of their needles. Flooding and increased salinity in the soil can cause water stress, reduce growth, alter gas exchange, reduce photosynthesis, cause the senescence of leaves and shoots and disrupt the Nitrogen cycle (Gardner *et al.* 1992, Kozlowski 1997). In Florida, it was observed that tree mortality of *Juniperus virginiana* L. var. *silicicola* (Small) A. E. Murray increased in the two years following storm surge (Williams, K. *et al.* 2003). Seedling survival and regeneration after storm surge is usually very low. An experiment with seedlings of *P. caribaea* var. *hondurensis* and *P. elliottii* var. *elliottii* has shown that seedlings of the former were more susceptible to waterlogging than those of the latter (Lewty 1990). The duration of the floods and their periodicity can have different effects on pine regeneration. Another study on the effects of storm surge on pine forests in South Carolina has observed that pine seedlings could only get established two years after the event, even though the first seedlings appeared a year before that (Gardner *et al.* 1992). Thus, regeneration depends not only on the soil condition but also on the availability of fresh water, considering that seed source is available. No visible regeneration is occurring so far in many areas of dead forest on Grand Bahama (Figures 2.28 and 2.31), possibly because of these factors.

Besides hurricanes, climate change and sea level rise could also affect the low-lying areas near the coast and reduce forest area in the future. These areas are more subjected to inundations if the sea level rises, as shown in Figure 2.41, affecting existing trees and regeneration. Only a small part of the pine forest (0.56 km^2) is currently protected in the Lucayan National Park and Rand Nature Centre, leaving the majority of the forest subjected to further development or exploitation.

New Providence

New Providence Island has been inhabited, probably since the 11th Century or earlier (Steadman *et al.* 2007, Marvel 2008). Its pine forests have been used mainly in construction and for charcoal production, as seen in Chapter 1 section 1.5.1 and nowadays they are all secondary growth. On this island, forest removal was highly driven by urbanisation and a great part of the pine forest (63.6%) has been lost in the past 47-48 years. Population increased 75.4% from 1953 to 1963, 25% from 1963-1970 and 107.7 % from 1970-2000. A census carried out in the year 2000 shows that almost 70% of the total Bahamian population (210,832 inhabitants) live on New Providence

(The Commonwealth of the Bahamas 2008). Modern pine forest covers only 11.6% of the island, and is mainly located in its western part (Figure 2.36). Bounds (1981b) has observed an increase in urban land, agricultural land and amenities (e.g. airport, golf courses, etc.) on this island from 1960 to 1981. This was accompanied by a decrease in pine forest and broadleaf forest, but no decrease in swamp-marsh areas. It seems that this is the case nowadays, as lower urbanisation is present in the swamp-marsh land in the south part of the island (Figure 2.38). These areas are seasonally flooded and saline; thus, not good for agriculture or construction. Heavier urbanisation occurred in the eastern part of the island, where the port and historic centre are situated.

Most of the unchanged area of pine forest is located north of Carmichael Road and south of the airport (Figure 2.37). The preservation of this area was probably due to the existence of fresh water wells, which supply part of the fresh water for the city, in addition to its Crown Land status. Fresh water resources are scarce and valuable in both the Bahamas and the TCI and worth preserving. Very small fragments of expansion of modern forest or loss of historical forest present in the maps are probably artefacts of the comparison of maps in different scales, as explained before, and not real changes. Some remnant fragments of forest from the 1960s and 1970s are still observed throughout the island. These are probably at higher risk of disappearing in the near future due to their size and the high demand for land by developers.

Higher sea level in the future does not seem to have a great impact on this island, according to Figure 2.41. The presence of ridges up to 37m in the west, north and east of the island (Sealey 1985) could have caused errors in the estimation of overall height, since cells were large (1 km) and island size is small (circa 30 km wide by 10 km long). Nonetheless, pine forests are mostly located in the south of the island in low lying areas, which could be affected by sea level rise in the future. The lack of protection of the pine forests on the island could lead to further reduction in area and forest health.

TCI

Pine forest cover in the TCI (2.7%) is much smaller than in the Bahamas (28.4%). As discussed previously, climate and water availability play a vital role in the expansion and maintenance of pine forests in the archipelago. The islands are small in size, with low relief, and exposed to the prevailing winds; all factors that contribute to a dry and hot climate. Fresh water is also scarce and most of the southern coasts of the islands have large extensions of salt marshes. High temperatures, low rainfall and a lack of fresh water can limit plant growth and establishment (Proctor 1955) and could have influenced the distribution of the pine forests in the TCI. Islands on the southeast side of the Caicos Bank and in the Turks Bank, i.e. South Caicos and Grand Turk, have lower average annual rainfall (550mm) than islands in the central chain (750-800mm). Rainfall in the central chain increases from southeast (East Caicos) to northwest (North Caicos) (Sears and Sullivan 1978). In North and Middle Caicos pine trees are only found in the central part of the islands, where they are protected from salt spray, rainfall is higher and fresh water table is deeper (Figure 2.40). On the other hand, it seems that conditions are more favourable for pine growth on Pine Cay, where the percentage of pine cover per island is higher than that of Andros (Figure 2.10). Although this is the smallest pine island in the archipelago, it is the only one where the substrate is composed of consolidated aeolian sand, instead of limestone rock. An experiment on *P. taeda* has shown that trees growing on sandy substrate in controlled conditions have deeper roots and higher resistance to drought than the ones growing in loam (Hacke *et al.* 2000). Green's (2011) study of the TCI pine forests has shown that heavy infestations of pine tortoise scale are present in all of the pine islands. However, Pine Cay had the highest proportion of live trees and showed better regeneration than the other islands. Further discussion on forest decay and regeneration in the TCI will follow in the Chapter 4.

The major change in forest area observed in the TCI occurred on the island of North Caicos, which lost 91.5% of its forest in 2009 due to an escaped agricultural dry-season fire (Figure 2.39) (Malumphy *et al.* 2012). Slash and burn agriculture is still practiced in these islands, as explained in Chapter 1 section 1.5.3. Apart from reducing forest area through clearing of the land, they also pose additional threats due to escaped non-seasonal fires. Hurricanes Hannah and Ike hit the TCI in 2008, causing floods and storm surge in many islands (Table 1.2, Chapter 1). Very few live trees were present in North Caicos forests at that time due to the pine tortoise scale infestation. Many seedlings and weakened trees were also probably killed by the floods in low lying areas. Fuel loads in the pine forests were high, as a result of tree death due to the insect infestation, snapping and uprooting of trees during hurricanes and a high number of *Sabal palmetto* palms in the undergrowth (Green 2011). When fire started, it spread fast throughout the pine forests, due to the high fuel loads present and general lack of moisture in the vegetation during the dry season, killing mature trees and seedlings. A similar situation to the one that occurred on Abaco during the 1960s seems to have occurred here too. The effects of pests, flooding and fire resulted in the death of seedlings and trees. As a result, the lack of seedlings and seed source led to the absence of recruitment. Regeneration of pine forests depend mostly on seed source and good growing conditions (Ross *et al.* 2009), which were both non-existent on North Caicos.

The pine forests in the TCI are in high risk of disappearing in the near future. High fuel loads are present in all the pine forests; there are frequent hurricanes in the region, high levels of insect infestation, high tree mortality and low regeneration. Besides, simulation of increased sea level rise, as seem in Figure 2.41, indicate that the pine forests in the TCI are at high risk of inundation in the future. Nicholls *et al.* have suggested a rise in 0.5m to 2m in the global sea level by 2100, if emission levels continue high and temperature increases by 4°C. Despite possible errors in the simulation of sea level rise for the TCI, pine forests in this region are located in low-lying areas which are prone to the effects of salinization of the water table due to sea level rise.

Main conclusions

- It was possible to estimate modern forest area and its location in the Bahaman archipelago using satellite imagery. Field work proved essential for testing and increasing map accuracy. It was also fundamental in the observation of recent changes to the pine forests and in the understanding of ecological factors influencing past and modern pine distribution and survival.
- The analysis of historical maps in conjunction with modern forest maps produced during this research allowed the observation of trends in forest loss or expansion throughout the archipelago, despite small errors caused by differences in mapping scale and surveying.
- A small increase in pine forest area (circa 5%) was observed in the Bahaman archipelago. Pine forest expanded in the Bahamas since the 1960s and 1970s, especially due to pine forest maintenance and expansion caused by fires on Abaco and Andros. However, reduction in the forest area happened in all the other Bahamas islands and in the TCI.
- Urban areas or agricultural plots have replaced part of the forested areas in the Bahamas, mainly in New Providence which lost circa 64% of its pine forests since 1961. Meanwhile, pine forest loss in the TCI was caused primarily by an insect pest (*Toumeyella parvicornis*) infestation. North Caicos lost almost all of its pine forest after an accidental high intensity fire followed by storm surge, having nowadays the smallest pine cover per island (0.2%).
- Pine forest regeneration was good after logging, when conditions were favourable and seed source was available. However, hurricanes in conjunction with drought, non-seasonal fires and pest attacks had catastrophic effects on the regeneration of previously logged areas in the Bahamas. The presence of seed bearers has proved essential for the occurrence of natural regeneration after storm surges and high intensity fire events.
- Many pine islands face risks of inundation with current predictions of sea level rise, what can cause severe damage and possibly extinction of some pine forest populations across the archipelago.

Chapter 3 . Conservation Genetics

3.1. Introduction

This chapter will focus on the genetics of natural populations of *Pinus caribaea* var. *bahamensis* across the distribution range for this variety (Bahamas and Turks and Caicos Islands, TCI), using microsatellites as tools for detecting genetic diversity, gene flow and demographic patterns.

Microsatellites, also called simple sequence repeats (SSRs) or short tandem repeats (STRs), are tandem repeats of one to six nucleotides, usually found in non-coding regions of the genome called introns (Zane *et al.* 2002). High mutation rates ranging from 10^{-6} to 10^{-2} per generation have been observed in these regions, mainly as a consequence of replication slippage (misalignment during DNA replication) or recombination (Schlötterer 2000, Navascués and Emerson 2005). Most microsatellite loci are assumed to be selectively neutral as they are little or not directly involved in the expression of phenotypes, providing a good estimate of migration without the influence of natural selection in most cases (Silvertown and Charlesworth 2001). Even microsatellite loci under selection could still be used to estimate genetic structure if selection pressure has been stable for decades or centuries and they have not diverged significantly (André *et al.* 2011). These regions are also highly polymorphic (Zhang and Hewitt 2003), making them useful tools in detecting genetic variation through gene flow between and genetic drift within populations (Vendramin *et al.* 1996, Schmidt *et al.* 2000, Ouborg *et al.* 2010). Co-dominant nuclear microsatellites, i.e. those where all alleles in a locus can be identified and scored, are commonly used to estimate gene flow, genetic variability, spatial distribution of genetic variance, genetic distance between populations, heterozygosity and mating system (Sunnucks 2000, Broquet and Petit 2009).

Selectively neutral co-dominant nuclear microsatellite markers and haploid plastid markers have been used in this research, due to differences in their inheritance modes in conifers. Plastid DNA is paternally inherited in pines allowing estimates of pollen flow (Powell, W. *et al.* 1995). Gene flow via pollen is usually different from that via seed. In conifers plastid microsatellites usually show lower genetic structure and differentiation than nuclear microsatellites, because of its uniparental inheritance, non-recombinant genome and slow mutation rates. These characteristics also make it possible to detect common ancestral haplotypes and infer expansion patterns (Provan *et al.* 2001, Petit *et al.* 2005, Whittall *et al.* 2010). Nuclear DNA, on the other hand, is recombinant, has high rates of base substitutions and usually shows higher evolution rates than plastid DNA (Wolfe *et al.* 1987, Zhang and Hewitt 2003). As nuclear microsatellites are biparentally inherited they can indicate gene flow through seed dispersal. They are important tools in detecting the genetic structure, genetic diversity and migration patterns of a species (André *et al.* 2011). Therefore, the analysis of plastid and nuclear microsatellites for a given species can reveal past and present patterns of gene flow between and within populations, its genetic diversity and structure.

Several studies using plastid and nuclear microsatellites in pines have been carried out in the past decades. Plastid microsatellites were used to demonstrate demographic expansions of *P. canariensis* C.Sm. in the Canary Islands following volcanic activity (Navascués and Emerson 2005) and historical gene flow and population differentiation in *P. sylvestris* L. in Bulgaria (Naydenov *et al.* 2005). Both plastid and nuclear microsatellites were used to demonstrate low gene flow between forest fragments, higher genetic diversity within than between fragments and high genetic similarity in individuals <15m apart in *P. elliottii* var. *densa* in Florida, USA (Williams, D. A. *et al.* 2007). A more recent study showed limited gene flow, reduced migration and high genetic differentiation between populations of *P. henryi* Mast. and *P. tabuliformis* Carrière in China through nuclear microsatellite analysis (Liu *et al.* 2012).

Cross-specific markers were used in this research. Conserved DNA sequences flanking microsatellite regions are common in conifer genomes, allowing the use of markers developed for one species in the amplification of regions in related species (Vendramin *et al.* 1996, Echt *et al.* 1999, Selkoe and Toonen 2006). Conifer genomes contain highly repetitive DNA without recent polyploidization, and many triplet repeats are conserved across taxa, which makes them good candidates for marker transferability (Kutil and Williams 2001). Good cross-species transfer rates were observed in outbreeding and long-lived species (Barbará *et al.* 2007b), like *P. caribaea*. In addition, the use of cross-specific markers can reduce research costs and save considerable time being spent on primer development (Vendramin *et al.* 1996, Selkoe and Toonen 2006).

No comprehensive study of natural populations of *P. caribaea* var. *bahamensis* in the Bahamas and the TCI has previously been conducted and population structure, spatial patterns of gene flow and genetic variation are still unknown. Observed morphological differences between Bahamas and TCI populations are also not known to be derived from phenotypic plasticity or genotypic variation.

Species-level studies have been carried out previously by other researchers, but none of them has included samples from populations in the TCI or looked specifically at population dynamics and structure in the Bahaman archipelago. *Pinus caribaea* var. *bahamensis* showed considerable genetic distance to *P. caribaea* var. *hondurensis* in research using isozymes on Central American pine species (Matheson *et al.* 1989) and genetic similarities with *P. caribaea* var. *caribaea* in a provenance study by Zheng and Ennos (1999). Dvorak *et al.* (2005) revealed the population structure and gene flow between populations of *P. caribaea* var. *hondurensis* in Guatemala and Belize using allozymes. A more recent study (Jardón-Barbolla *et al.* 2011) using molecular markers, estimated divergence times and gene flow among the three varieties of *P. caribaea* (vars. *caribaea*, *hondurensis* and *bahamensis*). It found that there were no shared haplotypes among the varieties, but vars. *bahamensis* and *hondurensis* were more closely related to each other than to var. *caribaea* and estimated divergence times coincided with glacial periods.

The use of information on the genetic diversity of the species and better understanding of its biology and spatial structure can assist species management and conservation policies. Genetic diversity is key to the adaptation of species to new and changing environments. Identification of the genetic structure in a species and of hotspots of genetic diversity can help managers and policy makers in the selection of populations or specific locations for conservation action, future management units or re-introduction programmes (Fay *et al.* 1999, Fraser and Bernatchez 2001, Diniz-Filho *et al.* 2008, Kramer and Havens 2009). This topic will be explored further in Chapter 5.

During the past 50 years in the Bahamas and 10 years in the TCI, most pine forests suffered reductions in population size and area due to pests, logging, development and storm surges (Chapter 2 section 2.4). However, in long-lived species like pines, the effects of fragmentation may take decades or even centuries to be noticed (Kramer *et al.* 2008). Disturbances or stochastic events could lead to reduction in population size and genetic depauperation, as shown in other studies (Frankham 1996, Spielman *et al.* 2004, Kettle *et al.* 2007). The effects of inbreeding and population size will be discussed for the present data in section 3.4. An additional threat to the pine forests in the Bahaman archipelago is sea-level rise due to climate change, as previously explored in Chapter 2 section 2.4. Most pine islands have a maximum elevation of 40 m (Sealey 1985) and most pines are found in lower elevations. The fresh water table is usually no deeper than 14 m, varying considerably with the amount of rainfall and tidal fluctuations (Whitaker and Smart 1997). The predicted rise in temperature and sea level (Yin *et al.* 2009) could increase evapotranspiration losses and cause a reduction in the fresh water table, increasing drought and salinity. Detrimental effects on population size through drought and salinity were observed in the past for *P. elliottii* var. *densa* in southern Florida (Ross *et al.* 2009), as previously discussed (Chapter 2 section 2.4, Grand Bahama).

Several questions are still unanswered, and these will be the main focus in this chapter. Do plastid and nuclear regions have similar levels of variability and dispersal patterns? Are loci in linkage equilibrium? What is the population structure in this variety? Is the mating system random and outcrossing in all populations? Are pines in the Bahamas and the TCI genetically different? Are populations genetically distinct from each other? Have historical fragmentation and reduction in population size affected genetic diversity? Is the *ex-situ* collection representative of wild populations?

3.2. Material and Methods

3.2.1. Population sampling

A total of 436 samples was collected from wild populations of *P. caribaea* var. *bahamensis* in the Bahaman archipelago (Table 3.1, Figures 3.1 and 3.2). Islands visited for sample collecting in 2008 and 2009 were Abaco (including Little and Great Abaco), North and Central Andros (northern Andros), Mangrove Cay, Grand Bahama and New Providence in the Bahamas and Middle Caicos, North Caicos and Pine Cay in the TCI. Samples were also collected in 2009 from an *ex-situ* nursery situated at the Middle Caicos Conservation Centre (MCCC). In 2010, collecting was restricted to the island of South Andros.

Samples consisted of two or three needles cut into small pieces and dried in 40-50g of silica gel (28-200 mesh, grade 12) with circa 5% added methyl violet coloured indicating silica gel (6-16 mesh, grade 12). The indicating silica gel changes colour from orange when dry to green when saturated. Sealable plastic bags containing a ratio of 10:1 silica/leaf allow for drying the fresh material in circa 12-24 hours and preserving the DNA for future analysis. Samples were checked after 24 hours and, if dry, most silica was removed from the bag using a sieve to ensure plant material was not lost. Samples can be easily preserved and shipped overseas in this way (Chase and Hills 1991). Silica gel could also be re-used, if necessary. Each sample was geo-referenced using handheld computers with built-in GPS (as specified for ground truthing methods in Chapter 2 section 2.2.1.3) and given a unique identification number.

A stratified sampling strategy was set out to maximise collections across the whole range and distribution area in each island for this variety, taking into account time, access and resources. Petit *et al*. (2005) showed that estimates of genetic distance between populations (G_{ST}) were mostly affected by the number of populations sampled across the distribution range of a species rather than the number of individuals sampled per population. Therefore, sampling from all possible populations would be advantageous in detecting regional variation. Sampling quadrats of 10×10 m were used to record associated data and target trees for collection of DNA samples and herbarium vouchers. One pine tree was sampled for DNA in each quadrat. Sampled trees had an average height of 7.1 m, ranging from 1.5 m to 18.9 m. Material collected from *ex-situ* collection in the TCI consisted of seedlings. Most of the quadrats were plotted along transects following the longer axis of the pine forest area. The distance between quadrats ranged from 10-200 m for islands with small fragmented forest area and/or precarious and difficult to access trails (New Providence, South Andros, Mangrove Cay and all three islands in the TCI). The distance between quadrats was 3.2 km apart for islands with a large extent of forest (Grand Bahama, northern Andros and Abaco). If no pine trees were present in a plotted quadrat, the quadrat was skipped and sampling was done in the next quadrat that contained one or more pines. Several individuals were sampled in each population in order to capture variation at the population level. A sampling distance usually >100 m was observed to minimise the sampling of siblings. The same distance was observed by García Quintana (2006) during his research on morphological and ecological parameters in *P. caribaea* var. *caribaea.* Studies have reported most seed fall within 15-48 m from the parent tree for *P. halepensis* (Nathan *et al.* 2000) and 40-60 m for *P. pinaster* (González-Martínez *et al.* 2006). Williams *et al.* (2007) demonstrated high genetic similarities for individuals up to 15 m apart for *P. elliottii* var. *densa* populations in Florida, USA. DNA was also extracted from 59 samples collected in 2007 and 2009 from the TCI wild populations. These samples were collected by the UK Overseas Territories Programme staff members (Kew) with TCI colleagues. The final number of individuals sampled per population, with latitude and longitude data for populations and regions, is shown in Table 3.1.

Although *P. caribaea* var. *bahamensis* is the only pine taxa recorded for the Bahaman archipelago in the literature (Henry 1974, Farjon and Styles 1997), herbarium vouchers, two or more per population, were collected in order to verify field identification of the species and improve collections at Kew. After drying, mounting, labelling and scanning, the specimens were added to the Kew Herbarium collection and serve as vouchers for the genetic samples added to the Kew DNA bank.

Location	**Latitude Range**	**Longitude Range**	**N**	**N_{nu}**	**N_p**	**$Area_F$**	**$Area_{ISL}$**
Bahamas							
Northern Abaco	26.3160°N-26.9158°N	77.0597°W-77.8007°W	31	21	28	325.9	836.3
Southern Abaco	25.8982°N-26.0748°N	77.2191°W-77.2564°W	20	16	20	216.7	335.4
Grand Bahama	26.5373°N-26.6864°N	78.1091°W-78.6734°W	50	36	44	456.1	938.7
Mangrove Cay	24.2505°N-24.2522°N	77.6759°W-77.6924°W	20	6	20	126.1	451.0
New Providence	25.0083°N-25.0395°N	77.3822°W-77.4793°W	50	46	49	24.8	212.9
Northern Andros	24.5136°N-25.1330°N	77.8479°W-78.2047°W	49	33	47	867.0	3796.4
South Andros	24.1140°N-24.1877°N	77.6080°W-77.6226°W	40	20	39	101.4	889.2
Total	**24.1140°N-26.9158°N**	**77.0597°W-78.6734°W**	**260**	**178**	**247**	**2118.0**	**7459.9**
Turks and Caicos							
Middle Caicos	21.8118°N-21.8245°N	71.7522°W-71.7931°W	81	65	75	11.5	277.9
North Caicos	21.8376°N-21.8429°N	71.8913°W-71.9120°W	79	54	69	0.4(4.7)•	206.1
Pine Cay	21.8720°N-21.8873°N	72.0884°W-72.0913°W	75	59	71	1.1	3.7
Total	**21.8118°N-21.8873°N**	**71.7522°W-72.0913°W**	**235**	**178**	**215**	**13.0(17.3)•**	**487.7**
Overall	**21.8118°N-26.9158°N**	**71.7522°W-78.6734°W**	**495**	**356**	**462**	**2131.0(2135.3)•**	**8128.4**

Table 3.1. Sampling and population data for genetic analysis. Latitude/longitude range-minimum and maximum in decimal degrees for sampling areas (WGS 1984, UTM 18N in the Bahamas and 19N in the TCI), N-number of individuals sampled, N_{nu}-number of samples used for nuclear microsatellite analysis, N_P-number of samples used for plastid microsatellite analysis, $Area_F$-estimated forest area in km^2 and $Area_{ISL}$ total island area in km^2. •Forest area before 2010 is shown in brackets.

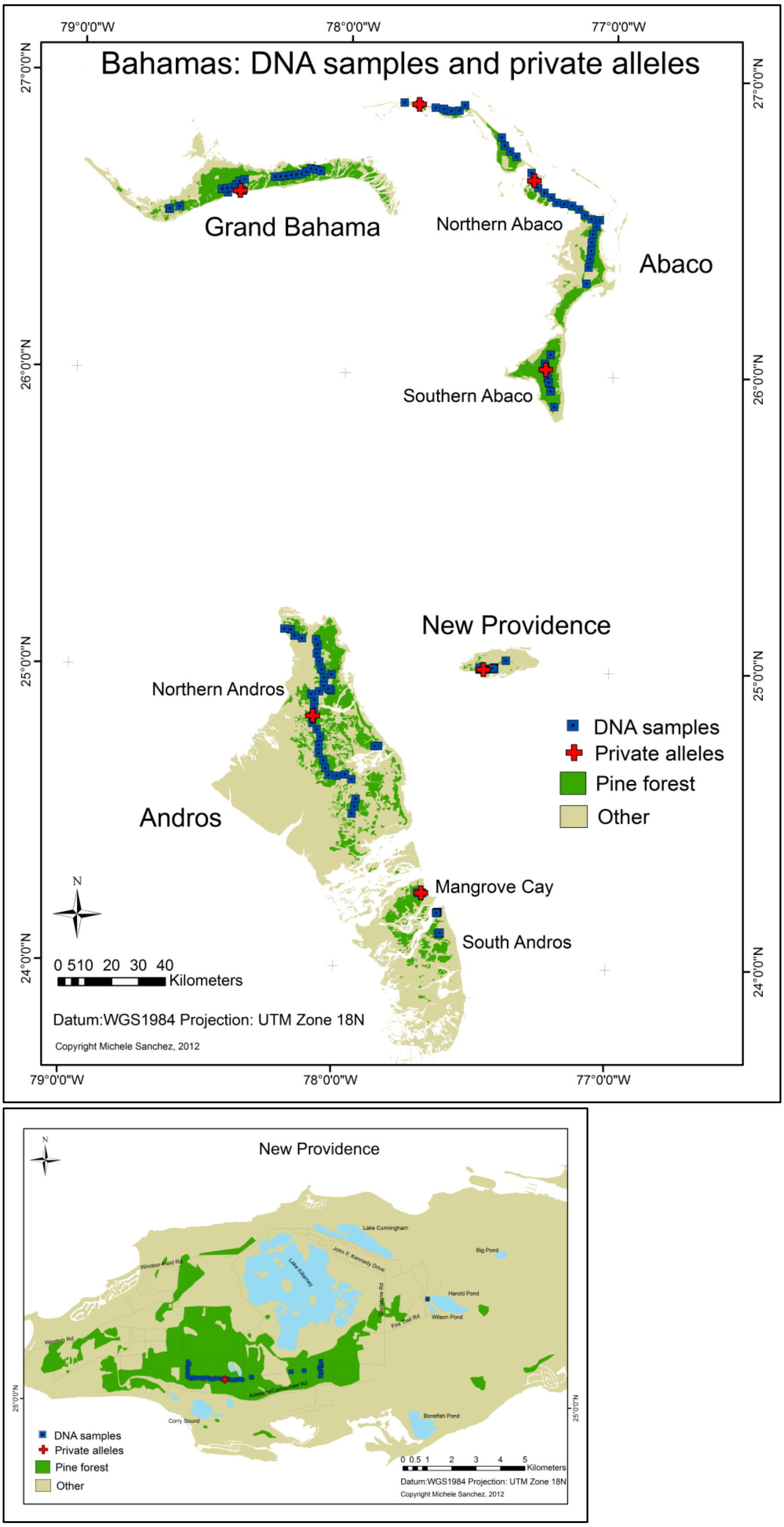


Figure 3.1. DNA samples and private alleles in the Bahamas. Upper figure shows sampling points (DNA samples) and location of private alleles on all islands; below detail map of New Providence.

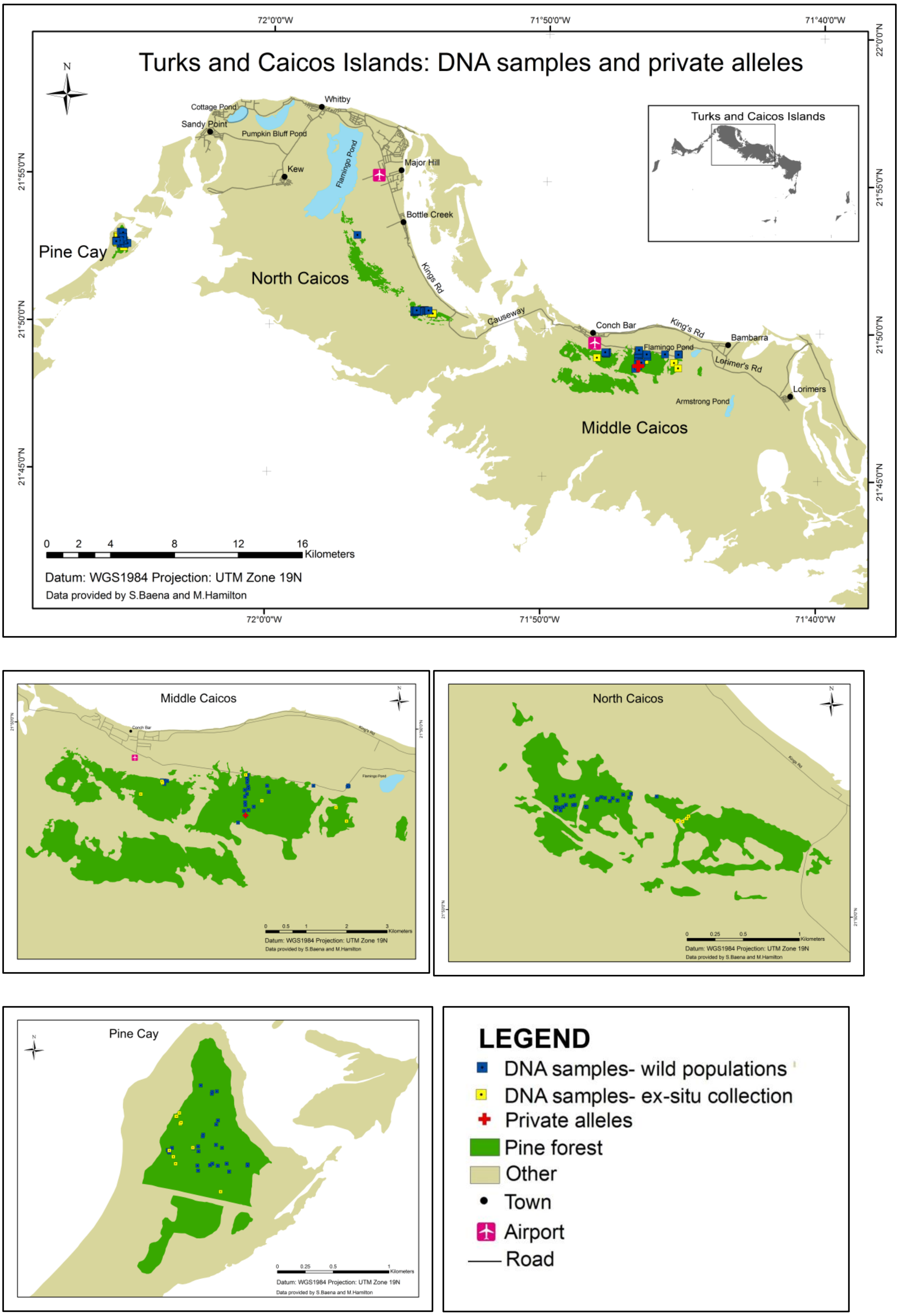


Figure 3.2. DNA samples and private alleles in the Turks and Caicos Islands. Upper figure shows sampling points (DNA samples) on all islands and the only private allele found (Middle Caicos population); below detail for each island. Yellow squares represent original location in the field of samples now in the *ex-situ* collection.

3.2.2. Molecular analysis

3.2.2.1. DNA extraction

DNA extraction followed a modification of Doyle and Dickson (1987) and Saghai-Maroof *et al.* (1984) protocols. Silica-dried pine needles, 20-30 mg per sample, were broken into small pieces and mixed with sterilised fine sand in an Eppendorf® tube, and then ground to a fine powder using a Retsch Mixer Mill MM301. Immediately after that, 750 µl of 2×CTAB buffer (cetyl trimethyl ammonium bromide) and β-mercaptoethanol (mixed in a proportion of 1 ml: 4 µl and pre-heated to 65°C) were added to the ground sample and heated to 65 °C for 20 min. This step was followed by removal from the heated bath and addition of 750 µl of SEVAG (24 chloroform: 1 isoamyl alcohol). The aqueous layer containing DNA was transferred to a new Eppendorf tube after agitation and centrifugation. Precipitation of the DNA occurred after addition of -20 °C absolute ethanol (99.7%, double the volume of aqueous layer) and at least 24 hours at low temperature (-20 °C). After centrifugation and removal of the remaining liquid, the DNA pellet was washed with 750 µl of 70% ethanol. The final steps consisted of removing all ethanol, drying the DNA pellet and re-suspending it in 100 µl of TE 0.1 Buffer (10 mM Tris-HCl, 0.1 mM EDTA). The DNA band could then be visualized through agarose gel electrophoresis with ethidium bromide (EtBr). Samples were stored in a -20 °C freezer. Before utilisation, they were thoroughly thawed and mixed. Total DNA of some samples that failed to amplify in two or more primer reactions was purified further with a QIAquick PCR Purification Kit (QIAGEN), following the manufacturer's protocol.

One DNA sample from each island was selected for long-term storage in the Kew DNA bank, except for Abaco which had two samples selected. Total cellular DNA extraction for these samples followed a modification of CTAB isolation procedures by Doyle and Dickson (1987) and Saghai-Maroof *et al.* (1984), similar to that explained above. Differences consisted of larger amounts of leaf material (70-100 mg per sample) and reagents (10 ml of 2×CTAB buffer /40 µl β-mercaptoethanol; 10 ml of SEVAG and 4 ml of 70% ethanol for washing) being used. The DNA pellet was resuspended in 4 ml of EtBr/CsCl (ethidium bromide/caesium chloride) solution, instead of TE buffer. Samples were further purified by density gradient and EtBr removed with dialysis, following the protocol of Csiba & Powell (2006). Samples were also run on an agarose gel before storage in a freezer at -80 °C. Kew DNA bank numbers and provenance for these samples are: 28743 (Pine Cay); 28744 (Middle Caicos); 28745 (North Caicos); 28746 and 28747 (Abaco); 28748 (New Providence); 28749 (Grand Bahama); and 28750 (Andros).

3.2.2.2. Polymerase Chain Reaction (PCR)

Nuclear Microsatellites

Five nuclear microsatellite markers developed by Elsik *et al.* (2000) for *P. taeda* PtTX2123, PtTX3013, PtTX3019, PtTX3025 and PtTX3034 (codes as in the original reference) were chosen as they have proved useful for cross-specific amplification in previous works (Kutil and Williams 2001, Shepherd, M. *et al.* 2002, Nelson *et al.* 2007, Williams, D. A. *et al.* 2007) and have shown polymorphism in *P. caribaea* var. *hondurensis* (Shepherd, M. *et al.* 2002, Shepherd, M. *et al.* 2003).

A three-primer protocol consisting of an unlabelled forward primer with a M13 tail at its 5′ end, a reverse primer and the universal FAM (6-carboxy-fluorescein)-labelled M-13 primer was adopted for the amplification of nuclear products in this research because of its cost-effectiveness and previous success in other species (Schuelke 2000, Barbará *et al.* 2007a, Palma-Silva *et al.* 2007). Total DNA was quantified using an Eppendorf® Bio Photometer and then diluted to 5 ng/µl with sterile deionised water.

Amplifications by PCR were achieved using primers PtTX2123 and PtTX3013 and a Perkin-Elmer GeneAmp 9700 thermo cycler with a touchdown PCR programme. The touchdown PCR starts with a high annealing temperature above the melting temperature of the primer and subsequent reduction of 0.5 to 1 °C per cycle for 10-15 cycles; therefore, increasing specificity and product yield (Korbie and Mattick 2008). The touchdown programme described by Burke *et al.* (2002) was used for product amplification with a final annealing temperature of 46 °C. The 10 µl standard reaction contained: 5 ng DNA, 1 µl 10×NH_4 Bioline Taq buffer, 0.2 µl Bioline 50 mM $MgCl_2$, 0.1 µl 10 mM dNTPs, 0.5 U Promega GoTaq® Hot Start Polymerase, 5 pmol forward (F) primer, 10-100 pmol reverse (R) primer, 1 pmol of universal FAM-labelled M13 primer (Applied Biosystems) and sterile deionised water.

Optimization was required for three other primers PtTX3019, PtTX3025 and PtTX3034 due to low product yield. Low product amplification could be the result of non-optimal conditions for the polymerase enzyme, inadequate quantities of reagents or primer specificity (Roux 1995). Changes of annealing temperature, number of cycles and quantities of reagents were carried out in order to optimize reactions. The best results were achieved by the following modifications to the touchdown program: final annealing temperature increased by 2 °C (48 °C) and increased number of cycles (32 cycles). Double quantity of universal FAM-labelled M13 primer (2 pmol) was also added to the standard reaction for primer PtTX3019. Reaction for primer PtTX3025 had 50% more reverse primer (15-50 pmol) and universal FAM-labelled M13 primer (1.5 pmol) than the standard reaction. The primer PtTX3034 reaction had double the amount of GoTaq® Hot Start Polymerase (1U), and 10 mM dNTPs (0.2 µl). The quantity of 50 mM $MgCl_2$ was increased to 0.25 µl for reactions with the last two primers.

Another four nuclear primer pairs, NZPR6 and NZPR1 developed by Fisher *et al.* (1998) for *P. radiata* D.Don and PtTX3020 (Elsik *et al.* 2000) and PtTX3018 (Shepherd, M. *et al.* 2002) developed for *P. taeda*, were tested in initial stages of the research. Primers NZPR6 and PtTX3020 failed to amplify and NZPR1 and PtTX3018 lacked polymorphism and had low product yield. Therefore, these primers were not selected for further analysis.

Plastid Microsatellites

Plastid microsatellite markers Pt9383, Pt15169, Pt30204, Pt36480, Pt71936 and Pt87268 were selected for amplification. These markers, developed by Vendramin *et al.* (1996) from the plastid genome of *P. thunbergii* Parl., have proved useful for cross-specific amplification (Vendramin *et al.* 1998, Clark, C. M. *et al.* 2000, Bucci *et al.* 2007, Williams, D. A. *et al.* 2007, Vendramin *et al.* 2008). Forward primers were labelled with 5' end fluorescent dyes FAM or JOE (6-carboxy 4', 5'-dichloro-2', 7'-dimethoxy fluorescein), which allowed later visualisation of products in a fluorescence-based DNA electrophoresis system (ABI PRISM® 3100 Genetic Analyser, Applied Biosystems).

Microsatellite regions were amplified by PCR using a Perkin-Elmer GeneAmp 9700 thermo cycler with the following programme: 2 min denaturing at 94 °C; 30 cycles of 0.5 min denaturing at 93 °C, 1 min annealing at 48°C, 1min extension at 72 °C; and 8 min final extension at 72 °C, with subsequent cooling to 4°C. The standard 10 µl reaction solution used for samples collected in 2008 was: 0.4 µl of DNA extract (purified or not), 9 µl Thermo Scientific 1.1× PCR Master Mix with 1.5 mM $MgCl_2$, 0.2

µl BSA 0.4% (bovine serum albumin), 2 pmol FAM- or JOE-labelled F primer, 2-20 pmol R primer.

Samples collected in 2007 and 2009 failed to amplify with the standard reaction solution. Levels and presence of secondary metabolites present in plant tissue, such as phenolic compounds, are known to inhibit PCR (Khanuja *et al.* 1999). Dilution of the DNA extract and an optimization of PCR solution were sufficient to overcome this problem. The optimized protocol was as follows: 0.5 µl of diluted DNA (1 part DNA extract: 1 part of sterile deionised water) or 0.4 µl of purified DNA, 1 µl 10×NH_4 Bioline Taq Buffer, 0.2 µl Bioline 50mM $MgCl_2$, 0.1 µl 10 mM dNTPs, 0.5U Promega GoTaq® Hot Start Polymerase, 2 pmol FAM- or JOE-labelled F primer, 2-20 pmol R primer, sterile deionised water.

Sequencing of microsatellite fragments was carried out in the early stages of the research to check if variation in the number of bases was due to insertions or deletions in the microsatellite region itself and not a non-template base addition by the polymerase (Clark, J. M. 1988). Size of the repeats was also checked. Alleles with a small number of repeats have shown lower mutation rates, as they are more stable and less prone to errors in re-alignment during replication (DNA replication slippage) (Dieringer and Schlötterer 2003).

DNA extracts were purified with a QIAquick PCR Purification Kit (QIAGEN), following the manufacturer protocol. Target regions (two samples for all six primers) were amplified through PCR using purified DNA, unlabelled primers and 1.1× Thermo-Start ReddyMix™ Master Mix with 1.5 mM $MgCl_2$. Final products were amplified again using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the following PCR programme: 0.1 min at 96 °C; 26 cycles of 0.05 min at 50 °C, and 4 min at 60 °C, with subsequent cooling to 4 °C. Product purification followed the BigDye® Terminator v3.1 Cycle Sequencing Kit protocol from Applied Biosystems (with ethanol/EDTA/3M sodium acetate) and sequencing was performed in an ABI 3730 DNA Analyser (Applied Biosystems). Forward and reverse sequences were read and aligned by eye using SEQUENCHER v.4.1.2 (Gene Codes) and PAUP* v.4 software.

3.2.2.3. Genotyping

PCR products were visualized through agarose gel electrophoresis. The PCR product containing DNA is loaded into an agarose gel stained with ethidium bromide which intercalates with DNA and allows its visualization under UV light. DNA itself is negatively charged and moves through the gel towards the positive charge. Larger fragments migrate at slower rates, and thus bands with different fragment sizes are visible and can be scored against a standard 'ladder' (molecular weight size marker). Thus, it is possible to check fragment size and estimate concentration of PCR product, according to location of the band in the gel and its intensity. Comparing the intensity of the DNA band with other PCR products previously run, gives an estimate of the dilution required for genotyping. PCR products were then diluted with sterile deionised water, if necessary.

Diluted or undiluted PCR product (1 µl) was added to a solution of 10 µl of Hi-Di™ formamide and 0.2 µl of GeneScan™-500 ROX™ Size Standard (Applied Biosystems), de-natured for 3 min at 95 °C and then analysed on an ABI PRISM® 3100 Genetic Analyser (Applied Biosystems). Multiplexing was carried out for plastid microsatellites only, adding PCR products from two primers with different sizes and label colour (e.g. Pt9383 JOE-labelled, 84-86bp, with Pt71936 FAM-labelled, 144-146bp) to the same well in a PCR 96-well plate. DNA fragments from PCR products, containing a fluorescent label, could be precisely sized against the internal lane size standard (ROX). Peaks were analysed with GeneScan® Analysis Software and Genotyper® Software v.3.7 (Applied Biosystems), determining size of fragments and number of alleles.

3.2.3. Statistical Analysis

Nuclear Microsatellites

Most samples (353) used for statistical analysis were scored for all loci, apart from three samples, for which there were missing data for some of the loci. These were, however, added to the dataset due to the presence of private alleles. Private alleles are alleles which only occur in one population. The number of private alleles is an important parameter in measuring genetic diversity, hence their inclusion in the analysis (Kalinowski 2004). The total proportion of missing data was <5% for all loci, and no locus was excluded from any of the statistical analysis (Table 3.1).

Samples were divided in three hierarchical levels for analysis: ‘regional’, ‘inter-population’ and ‘intra-population’. The regional level comprised the Bahamas (1) and the Turks and Caicos Islands (2) regions. The populations from region 1 were: northern Abaco (including Little Abaco and northern part of Great Abaco), southern Abaco (southern part of Great Abaco), northern Andros (North and Central Andros), South Andros, Mangrove Cay, Grand Bahama and New Providence. Populations from region 2 were: Middle Caicos, North Caicos and Pine Cay. Numbers of samples per population are given in Table 3.1 and the spatial locations are shown in Figures 3.1 and 3.2.

The decision to divide Andros in three populations (northern Andros, South Andros and Mangrove Cay) and Abaco into two populations (northern and southern Abaco) was taken for the following reasons: populations were separated by sea (Andros) or a distance >25 km (Abaco); populations have shown significant genetic distances from each other in preliminary analysis; and finally, a subdivision between these populations was inferred in simulation models based on allele frequencies and spatial patterns in Geneland software (Guillot *et al.* 2005a, Guillot *et al.* 2005b, Guillot 2008, Guillot *et al.* 2008, Guillot and Santos 2009, 2010) (Figure 3.7).

A Bayesian model using the Markov chain Monte Carlo (MCMC) method was run in Geneland in order to detect and visualise spatial patterns in the data. The MCMC method locates high-likelihood portions of space, identifying genetic discontinuities and inferring number of populations. Different chain lengths and burn-in steps (running period in the chain before sampling starts) were tested to maximise performance (Guillot *et al.* 2005a, Guillot *et al.* 2005b, Excoffier and Heckel 2006). MCMC simulation models were run with K (unknown number of populations) from 1 to 10, 10^6 iterations with thinning of 1000 and 10^5 iterations with thinning of 100, spatial coordinates for all samples, uncorrelated data and 200 to 600 burn-in steps. Ten multiple independent runs were used for each model to find the highest probability and check for consistency in number of populations. Runs with different parameters produced similar results and K=3 for all runs, which indicate that the run was long enough to detect all variability in the dataset. The model chosen for post-processing was the one that showed highest probability (87%), K=3, 10^5 iterations with thinning of 100 and 500 burn-in steps. Maps of posterior probability of population membership and MCMC showing number of populations along the chain are represented in Figure 3.7.

This simulation model gives an estimate of the number and spatial organization of populations, taking into account genetic and geographical data. It assumes that all populations are in Hardy-Weinberg equilibrium, there is linkage equilibrium among loci and populations are separated by genetic discontinuities (Guillot *et al.* 2005a). These can be unrealistic assumptions for many real situations, in which migration, drift and selection are present in populations. An overestimation of the number of populations can occur when genetic discontinuities are derived from isolation by distance. Errors in interpretation can also occur when kin-clustering and inbreeding are present. In this study, this model was used as an auxiliary tool to visualise genetic discontinuities and help in the interpretation of data along with other parameters. Results of F-statistics, geographical and historical factors, the presence of linkage disequilibrium, inbreeding and small deviations from Hardy-Weinberg equilibrium corroborated the partial acceptance of the model and the division of the data into more populations than suggested in the simulation.

The software CREATE v.1.33 (Coombs *et al.* 2008, © 2007 Jason A.Coombs) was used to create and convert input files for genotypic data to be run in different software, i.e. Arlequin, Fstat and Genetix. Hierarchical analyses were performed in Arlequin 3.1 (Excoffier *et al.* 2005), Fstat 2.9.3.2 (Goudet 1995, 2002) and GeneticStudio (Dyer 2009, ©2002-2008 R.J. Dyer) software. AMOVA (analysis of molecular variance) was performed in Arlequin 3.1, in order to detect genetic structure and distribution of variability throughout the hierarchy. Total variance is partitioned into covariance components and the sums of squared deviations among regions, among populations within regions and within populations is calculated. This method uses number of mutations between molecular genotypes (unique combination of alleles for all loci for diploid nuclear DNA), instead of variance in gene frequencies. It also uses the correlation of uniting gametes as a function of variance (Weir and Cockerham 1984, Excoffier *et al.* 1992, Weir 1996).

F-statistics was used to infer population structure (F_{ST}), genetic differences between populations (pairwise F_{ST}), mating system and inbreeding (F_{IS} and F_{IT}).

According to Wright (1965), $F_{ST}=(F_{IT}-F_{IS})/(1-F_{IS})$, where F_{IT} shows correlation between gametes of individuals in relation to the entire population, F_{IS} refers to uniting gametes of individuals within one population relative to that of the entire population, and F_{ST} to the correlation of random gametes from different individuals within a population relative to the total gametes of all populations. F_{ST} varies from 0 to +1, with +1 being the maximum differentiation between populations and highest population subdivision. F_{IS} (inbreeding coefficient) and F_{IT} (total inbreeding coefficient) vary from -1 to +1, with positive values indicating homozygote excess and inbreeding. Mating is random when F_{ST} equals F_{IT}. Deviations of F_{IS} from zero were tested as described in Table 3.2 , indicating tendencies to inbreeding (positive values) or outbreeding (negative values) in the population (Wright 1965).

The fixation index F_{ST} for pairs of populations was calculated through AMOVA in Arlequin 3.1. This index indicates the probability that the gametes come from the same or different populations. It is calculated by an adaptation of Wright's formula (1951, 1965), based on genetic covariance derived from differences in genotypes among populations, within populations and in different populations within and between groups (Table 3.3). Small and negative values obtained for some pairs (<-0.010) are an artefact of the calculations, probably computational errors in the software, and are equivalent to lack of structure or differentiation $F_{ST}=0$, so they were converted to zero (Meirmans 2006). The null hypothesis of no genetic difference between pairs of populations, based on pairwise F_{ST} values, was calculated using 26082 random permutations of haplotypes among populations (Table 3.2). Higher permutation values were tested (up to 10^5) to check for changes in *P* values, but these did not alter any of the results. Thus, the standard permutation (26000) recommended by the software manual was used. A confidence limit of 95% or higher ($P<0.05$) was chosen for all statistical tests. Therefore, a *P* value <0.05 rejects the null hypothesis.

Total F_{ST}, F_{IS} and F_{IT} indices for the entire population were calculated in Fstat 2.9.3.2 and Genetix 4.05.2 (Belkhir *et al.* 1996-2004) based on Weir and Cockerham (1984) and Weir (1996). Total indices were estimated per locus over all samples. F_{IS} was also estimated per population over all loci, and used as parameter for estimation of inbreeding in each population. The maximum number of permutations allowed by the software (10000) was used to check significance level and accept or reject the null hypothesis (Table 3.2).

Nei's unbiased genetic distance $\hat{G}_{ST}$ was calculated in Genetix 4.05.2 to infer genetic distance between pairs of populations. It refers to the genetic distance based on the allelic frequency of the multi-allelic loci, taking into account allelic differences per locus and heterozygosity levels (Nei 1978). It is a variation of G_{ST} measure of the genetic diversity of populations corrected for sampling size and population structure (Nei and Chesser 1983). It varies from 0 to +1, with 0 indicating no genetic distance between populations. Small negative values (<-0.012) were replaced by zero, as suggested by Nei (1978), as they are computational errors. The formula used for the calculations is presented in Table 3.3 and details of statistical tests used to accept or reject the null hypothesis in Table 3.2. The maximum number of random permutations allowed by the software (10000) was used in the statistical tests.

Genetic flux between pairs of populations *Nm* was calculated according to Wright's formula (1969), as shown in Table 3.3. This parameter gives an indication of gene flow and migration between two populations. Genetic flux cannot be calculated when $F_{ST}=0$, in this case *Nm* was represented by 'inf' in Table 3.6, indicating high gene flux.

Nei's unbiased gene diversity (*uHs*) was calculated in Fstat 2.9.3.2. based on Nei (1973) and Nei and Chesser (1983). It represents expected heterozygosity under Hardy-Weinberg equilibrium within subpopulations, adjusted for sample size. This parameter indicates genetic diversity in a population and can be used to compare diversity across populations or between regions. Nei estimate of genetic diversity (H_T) was also calculated for each locus over all populations and over all loci to give an idea of amount of genetic diversity detected by each locus and total diversity of the entire population (Table 3.3). Allelic richness also referred to as the allelic diversity or mean number of alleles per locus was also calculated with Fstat. This parameter measures the frequency distribution of alleles at a locus standardized to the smallest population size by rarefaction, so that comparisons between populations of different sizes can be done without bias (Leberg 2002). This parameter is complementary to gene diversity as it takes into account the number of different alleles instead of allelic frequencies. It is correlated to genetic differences and the potential of a population to respond to selection over a number of generations (Petit *et al.* 1998).

Genotype count per region and populations, population graphs and Mantel tests were performed in GeneticStudio software (Dyer and Nason 2004, Dyer 2007). Population graphs are useful in the analysis of intraspecific genetic structure using co-dominant markers, showing spatial correlation of genetic data. They provide a visual representation of how genetic covariance is distributed among strata and are complementary to AMOVA. Edges connect only populations (nodes) with a significant degree of genetic dependence, increased by the exchange of migrants. Paths (links between nodes) are linear and proportional to physical distance, resulting from the analysis of all populations simultaneously; thus they are useful in isolation by distance inference. Topologies (pattern of connections between populations within a graph) can also be used to determine patterns of gene flow. Populations under Wright's island model (Wright 1943), in which there is random breeding among subpopulations and migrants come randomly from the total population, show a compact topology. On the other hand, populations under Kimura's stepping stone model (Kimura and Weiss 1964), where populations are subdivided into colonies and migration is restricted to neighbouring colonies, show an elongated topology (Dyer 2007).

Additional analysis at intra-population level included comparisons of observed and expected heterozygosity and deviations from Hardy-Weinberg (H-W) equilibrium. The Hardy-Weinberg law describes a model in which allelic frequencies are constant throughout generations in a large outcrossing population with random mating and no migration, drift, selection or mutation. The frequency of two homozygous alleles p and q are $p+q=1$ in the parent generation, and $p^2 + 2pq + q^2=1$ in the offspring. If more than two alleles are present then $\sum_i p_i=1$, where p_i is the frequency of the ith allele (Silvertown and Charlesworth 2001, pg.54). Thus, expected heterozygosity is based on this equation and observed heterozygosity is calculated through allelic frequencies observed in the dataset. Populations showing equal or similar expected and observed heterozygosity for most loci are under H-W equilibrium. Deviations from this equilibrium indicate that mating in a given population is not random and selection, migration or mutations could be altering allelic frequencies. Calculations of observed and expected heterozygosity over all loci per population were performed in Arlequin 3.1 and are represented in Table 3.5 as H-W_{DEV}, meaning the difference between observed and expected number of heterozygotes. The null hypothesis tests were based on Guo and Thompson (1992) and Levene (1949) (Table 3.2).

The hypothesis of isolation by distance (IBD) was tested through regression analyses using matrix correspondence in GeneAlEx 6.41 (Smouse *et al.* 1986, Smouse and Long 1992, Peakall and Smouse 2006). This test compares two matrices, pairwise genetic differences between populations (F_{ST}) and geographical distances between populations in km. Geographical distances were obtained through a ‘haversine’ formula, which calculates the shortest distance over the surface of the Earth between two points, using the online application Movable Type Scripts (© 2002-2010 Chris Veness). Median longitude and latitude for each population were calculated and input into the formula to obtain distance in kilometres. Values can contain errors up to 0.3%, because calculations are based on a spherical Earth, instead of an ellipsoidal one (Veness 2010); however, precision was not imperative for IBD inference. Mantel tests (9999 permutations) checked significance of correlation between the two matrices, with a null hypothesis of no significant relationship. The parameter R^2 (squared correlation coefficient between observed and predicted values) infers the percentage of observed variability in genetic differences attributed to geographical distance (Norusis 2008).

Principal coordinates analysis (PCoA) is a useful tool in the inference of infraspecific genetic structure, showing major patterns of variation within a multivariate co-dominant dataset. It considers all individuals in the dataset, which are clustered into panmictic units or geographically distinct populations (Broquet and Petit 2009). GeneAlEx 6.41 was used for PCoA analyses (based on algorithm by Orloci 1978). This method requires a genetic distance matrix, in this case calculated as the sum of the squared size differences between two alleles. Data were standardized by dividing the distance data by the square root of n-1. The final graph (Figure 3.5) plots all individuals, colour labelled by population, on the axes which explain most of the existing variation, in this case 1 and 3. Fewer axes are needed to explain the variation, the more distinct the groups of individuals are from each other (Peakall and Smouse 2010).

Linkage disequilibrium refers to the non-random association of alleles. It is correlated to mutation, which can increase allele association, or recombination, which can decrease this association. Linkage disequilibrium also relates to population size, bottleneck and inbreeding (Flint-García *et al.* 2003). It was tested for pairs of loci in each population in Arlequin 3.1 through exact tests using contingency tables (Slatkin 1994). The null hypothesis of no association between two loci was tested as shown in Table 3.2. This parameter can be used to detect recombination and the demographic history of populations.

Plastid Microsatellites

All samples (495) were used in the statistical analysis for most parameters. Exceptions included analysis based on haplotype frequencies, such as AMOVA and Network, where a total of 462 samples with complete data were used instead. No locus had >6-8% of missing data for all populations. A breakdown of the number of samples per population is given in Table 3.1.

Analysis of AMOVA based on absolute frequency of haplotypes (unique combination of alleles at all loci for haploid plastid DNA) was carried out in Arlequin 3.1 to infer genetic structure. Calculations used the same hierarchical scheme as for nuclear microsatellites. The absolute frequency analysis is based on the number of haplotypes and number of individuals for each haplotype in each population. Genetic differentiation between populations was estimated by the parameter φ_{PT} via AMOVA in GeneAlEx 6.41. This parameter is based on the proportion of variance among populations relative to the total variance. It varies from -1 to +1 and is an analogue in haploid data for F_{ST}. Higher values indicate higher genetic differentiation (Peakall and Smouse 2010).

Allelic frequency and unbiased genetic diversity (*uh*) were also calculated in GeneAlEx 6.41. The uh is a measure of the genetic diversity in haploid individuals and is calculated by the formula $n/n\text{-}1(1\text{-}\sum p_i^2)$ where p_i is the frequency of the *i*th allele and n is the sample size (Peakall and Smouse 2010). It varies from 0 to 1, indicating probability of differentiation between individuals. Isolation by distance was inferred by regression analysis and Mantel tests, as explained for nuclear microsatellites. A population graph based on genetic covariance (GeneticStudio) was also used to show spatial correlation of genetic data, as explained for nuclear data. Both representations allowed for comparisons between plastid and nuclear data.

A network following the stepwise mutation model (SMM) using the median-joining method (Bandelt *et al.* 1999) was produced in Network 4.6.0.0 (© 2004-2011 Fluxus Technology Ltd, available at http://www.fluxus-engineering.com). Median-joining was chosen for being the standard method for multi-state data used in intraspecific phylogenetic analyses. Data input included haplotypes and their respective frequencies. An epsilon value of 40 was chosen, based on the mismatch distribution between haplotypes (value of 4 for maximum pairwise differences). This value represents the weighted genetic distance within the dataset. The more complex a network is, higher the epsilon should be to allow longer run time and increased precision. Only the most parsimonious tree is presented. It shows evolutionary patterns and ancestral haplotypes, whereas mismatch distribution reveals contractions and expansions of populations through time (Fluxus Technology Ltd. 2010).

Null hypothesis	**Parameters**	**Statistical test**	**Software**	**Reference**
No genetic difference between pairs of populations	Pairwise F_{ST}	Non-parametric permutation test (26082 permutations of haplotypes among populations)	Arlequin 3.1	Excoffier *et al.* 1992
No genetic distance between pairs of populations	Pairwise $\hat{G}_{ST}$	Exact tests (10000 permutations of individuals among populations)	Genetix 4.05.2	Nei and Chesser 1983
No inbreeding in the population (F_{IS}=0)	F_{IS} per population	Exact tests (10000 permutations among individuals within each population)	Genetix 4.05.2	Raymond and Rousset 1995
Populations are at Hardy-Weinberg equilibrium (random mating)	H-$W_{DEV=}H_O - H_E$ H_O (observed heterozygosity) H_E (expected heterozygosity)	Exact tests using a modified version of Markov chain (chain 10^6 steps, 10^4 dememorization steps)	Arlequin 3.1	Guo and Thompson 1992; Levene 1949
No linkage disequilibrium between pairs of loci	LD	Exact tests using contingency tables and Markov chain (chain 10^6 steps, 10^4 dememorization steps)	Arlequin 3.1	Slatkin 1994
No correlation between genetic differences and geographical distance (isolation by distance)	Pairwise F_{ST}; pairwise $\varphi_{PT;}$ pairwise geographical distance (km)	Regression analysis using matrix correspondence; Mantel tests (9999 permutations)	GeneAlEx 6.41	Smouse *et al.* 1986

Table 3.2. Null hypotheses and statistical tests used to accept or reject the null hypotheses for genetic data.

Genetic parameter	Indicator	Calculations	Range	Software	Reference	Applications
Analysis of Molecular Variance (AMOVA)	Hierarchical genetic structure	Sums of squared deviations among regions, populations within regions and within populations using number of mutations between haplotypes (nuclear data) or number of haplotypes and number of individuals for each haplotype in each population (plastid data)	0 to 100	Arlequin 3.1	Excoffier 1992, Weir and Cockerham 1984, Weir 1996	Detect distribution of genetic variance among different hierarchical levels. It can be used for haploid or diploid data
F statistics (F_{IT}, F_{ST}, F_{IS})	Population structure and genetic differentiation; inbreeding; mating system	$\mathbf{F_{ST}=(F_{IT}-F_{IS})/(1-F_{IS})}$	F_{ST} 0 to +1 F_{IT}, F_{IS} -1 to +1	Fstat 2.9.3.2 Genetix 4.05.2	Goudet 1995 Weir and Cockerham 1984, 1986	Allows comparison of genetic structure and heterozygosis among loci or at taxa level. It is used for diploid data.
Pairwise genetic differences (F_{ST})	Genetic differences between pairs of populations	$\mathbf{F_{ST}=\sigma^2_a+\sigma^2_b/\sigma^2_T}$ $\sigma^2_{T=}\sum\sigma^2a+\sigma^2b+\sigma^2w$. Total covariance due to differences among genotypes within populations (σ^2_w), among populations (σ^2_b) and between regions (σ^2_a).	0 to +1	Arlequin 3.1	Modification of Wright 1951, 1965 and Excoffier 1992	Identify patterns of genetic differentiation at population level. Allows direct comparison between population pairs. It is used for diploid data, but allows comparison with φ_{PT} for haploid data.
Pairwise genetic differences via AMOVA (φ_{PT})	Genetic differences between pairs of populations	$\boldsymbol{\varphi_{PT}=V_{AP}/(V_{AP}+V_{WP})}$ V_{AP}=variance among populations V_{WP}= variance within populations	-1 to +1	GeneAlEx 6.41	Excoffier 1992, Peakall *et al.* 1995 and Peakall and Smouse 2010	Identify patterns of genetic differentiation at population level. Allows direct comparison between population pairs. It is used for haploid data, but allows comparison with F_{ST} for diploid data.
Nei unbiased genetic distance per population pairs ($\hat{G}_{st}$)	Genetic distance between pairs of populations	$\boldsymbol{\hat{G}st=1-(Hs_{(nc)}/Ht_{(nc)})}$ $Hs_{(nc)}=(\tilde{n}/(\tilde{n}-1))*((Hs-Ho)/2\tilde{n})$ $Ht_{(nc)}=(Ht+Hs_{(nc)}/\tilde{n}*N)-((Hs-Ho)/(2\tilde{n}*N))$ *Ho*= arithmetic mean of observed heterozygotes over all subpopulations $\tilde{n}$ = harmonic mean number of individuals in the subpopulations *N*= number of subpopulations *Hs*= arithmetic mean of expected heterozygotes per locus in each subpopulation over all subpopulations *Ht*= arithmetic mean of expected heterozygotes for the total combined population over all loci	0 to +1	Genetix 4.05.2	Nei 1978, Nei and Chesser 1983	Identify genetic distance at population level. Allows direct comparison between population pairs. Similar to F_{ST}, but less susceptible to variation in sampling size between populations and small samples. It is used for diploid data.
Pairwise genetic flux (*Nm*)	Gene flow and migration between pairs of populations	$\mathbf{Nm=(1-F_{ST})/4*F_{ST}}$	0 to infinite (inf.)	Genetix 4.05.2	Wright 1969	Inference of gene flow between populations and isolation by distance. It is used for diploid data.
Nei unbiased gene diversity (uH_S)	Expected heterozygosity under Hardy-Weinberg equilibrium within subpopulations, adjusted for sample size	$\mathbf{uH_S=(2\tilde{n}/2\tilde{n}-1)*(1-\sum_k\overline{X2}_k)}$ $1-\sum_k\overline{X2}_k$= *expected* heterozygosity in the subpopulation $\tilde{n}$=harmonic mean number of individuals in the subpopulations	0 to +1	Fstat 2.9.3.2	Nei 1973, Nei and Chesser 1983	Allows comparison of genetic diversity at different hierarchical levels. It is used for diploid data.
Unbiased genetic diversity (*uh*)	Genetic diversity in the population	$\mathbf{uh=(n/n-1)*(1-\sum p_i^2)}$ $p_{i=}$ frequency of the *i*th allele *n*= sample size	0 to +1	GeneAlEx 6.41	Peakall and Smouse 2010	Allows comparison of genetic diversity at different hierarchical levels. It is used for haploid data.
Nei estimate of genetic diversity per locus over all populations (H_T)	Genetic diversity at each locus and total diversity for entire population	$\mathbf{H_T=(n/n-1)*(1-\sum^r_{i=1}p_i^2)}$ *r*= number of loci *pi*= frequency of *ith* allele at a locus n= sample size	0 to +1	Fstat 2.9.3.2	Nei 1978	Allows comparison of genetic diversity between taxa and show patterns of variation among loci. It is used for diploid data.
Principal coordinates analysis (PCoA)	Patterns of variation in the dataset considering all individuals	Genetic distance matrix based on sum of the squared size differences between two alleles. Standardized by dividing by square root of n-1	N/A	GeneAlEx 6.41	Orlocci 1978	Allows visualisation of variation in the dataset and identification of groups. It is used for haploid or diploid data.

Table 3.3. Statistical tests and formulae used to calculate genetic parameters.

3.3. Results

Nuclear and plastid microsatellite data were analysed separately and are divided in two main sections below.

3.3.1. Nuclear Microsatellites

3.3.1.1. Allelic diversity

All loci were polymorphic for all populations, with the number of alleles per locus varying from six to 11 and size from 147-284 base pairs (bp) as shown in Table 3.4.

Amplification through PCR produced a high yield of product for loci PtTX3013, PtTX2123 and PtTX3019, whereas a moderate quantity of product was obtained after optimization for PtTX3034 and PtTX3025. Only samples with clear, readable peaks were scored for allele presence and size. Samples with a low signal were amplified again and if amplification failed for most loci the DNA extract was purified further through column cleaning before new amplifications were performed. No sample failed completely at all loci; however, complete results for all loci could not be obtained for 28% of the samples.

Higher numbers of allele combinations were found for loci PtTX3034 (allelic range 26) and PtTX3025 (allelic range 18), both showing higher fixation indices (F_{IT}, F_{ST} and F_{IS}) and gene diversity calculated over all populations than the other loci (Table 3.4). The lowest value was found for locus PtTX3019. All loci contained private alleles.

Locus	**A_{range}**	**A**	**A_{genot}**	**F_{IT}**	**F_{ST} (θ)**	**F_{IS}**	**H_T**
PtTX3013	147-163	6	14	0.013	0.012	0.001	0.612
PtTX2123	207-219	7	12	0.04	0.087	-0.051	0.303
PtTX3034	208-234	10	32	0.193	0.115	0.089	0.803
PtTX3019	217-235	6	11	-0.055	0.044	-0.104	0.604
PtTX3025	266-284	11	25	0.415	0.270	0.198	0.748
Overall	**147-284**	**6-11**	**11-32**	**0.143**	**0.114**	**0.033**	**0.614**

Table 3.4. Genetic parameters for nuclear microsatellite loci over all populations. Estimation of F_{IT}, F_{ST} (θ) and F_{IS} (Weir and Cockerham 1984) per locus and overall. A_{range}-allelic range; A-total number of alleles; A_{genot}-number of allele combinations observed; H_T-Nei estimate of gene diversity over all populations.

3.3.1.2. Genetic Structure

Most nuclear microsatellites genetic variation (83%) was found within populations, rather than between regions (15%) or among populations (2%) (Figure 3.3, a). This result concurs with the high number of individuals with unique genotypes (284), against only 72 with shared genotypes (Table 3.5). Shared genotypes were present in all the TCI populations, but in only one population in the Bahamas (New Providence).

The total number of alleles per population varied from 18 (Mangrove Cay) to 28 (northern Abaco and Grand Bahama) (Table 3.5). Less than half of the total alleles observed for the populations had a frequency of <5%. Mean observed heterozygosity across all loci and populations (Ho=0.525) was slightly lower than expected (He=0.603), but did not depart significantly from H-W equilibrium. No significant inbreeding was observed at taxon level (F_{IS}=0.033, P=0.13). Similar F_{IT} and F_{ST} values over all loci for the species (0.143 and 0.114 respectively) are shown in Table 3.4. Total gene diversity over all populations (H_T) was 0.164 and allelic richness (A_R) 3.19 (Tables 3.4 and 3.5).

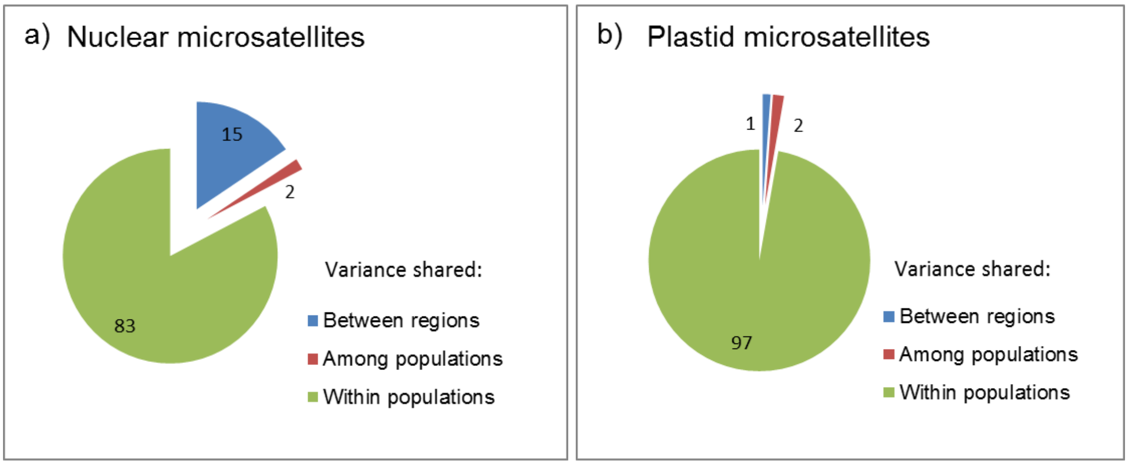


Figure 3.3. Analysis of molecular variance (AMOVA) among strata. AMOVA as a weighted average over loci for all individuals sampled (Arlequin 3.1, based on Weir and Cockerham 1984; Excoffier, Smouse and Quattro 1992; Weir 1996). Data for nuclear microsatellites (a) showed higher variance shared between regions than plastid microsatellites (b).

	Nuclear microsatellites											
Location	A_{nu}	A_{priv}	A_R	$A_{0.95}$	Gen_{shared}	Gen_{unique}	*uHs*	H-W $_{DEV}$	(*P* value^1)	F_{IS}	(*P* value^2)	Linked pair loci
Bahamas												
Northern Abaco	28	2	3.94	18	0	21	0.643	-0.014	(1.00)	0.023	(0.32)	1
Southern Abaco	21	1	3.31	17	0	16	0.539	0.033	(1.00)	-0.063	(0.18)	1
Grand Bahama	28	1	3.33	15	0	36	0.558	-0.035	(0.80)	0.063	(0.09)	0
New Providence	22	1	2.76	13	3	43	0.487	-0.044	(0.42)	0.090*	(0.04)	2
Northern Andros	25	1	3.11	13	0	33	0.544	-0.010	(0.84)	0.019	(0.33)	1
South Andros	20	0	3.19	16	0	20	0.554	-0.053	(1.00)	0.080	(0.10)	0
Mangrove Cay	18	1	3.60	18	0	6	0.604	-0.003	(0.52)	0.006	(0.37)	0
Mean	**23**	**1**	**3.32**	**16**			**0.561**	**-0.037**	**(0.96)**	**0.031**	**(0.17)**	**1**
Turks and Caicos												
Middle Caicos	21	1	2.90	14	2	63	0.562	0.017	(0.65)	-0.023	(0.23)	7
North Caicos	24	0	3.07	13	4	50	0.559	0.004	(0.44)	-0.012	(0.41)	7
Pine Cay	20	0	2.59	14	3	56	0.497	-0.056	(0.57)	0.113**	(<0.01)	4
Mean	**22**	**0**	**2.86**	**14**			**0.539**	**-0.015**	**(0.90)**	**0.027**	**(0.61)**	**6**
Mean Overall	**22**	**1**	**3.19**	**15**				**-0.067**	**(0.98)**	**0.033**	**(0.13)**	**3**
Total	**18-28**	**8**	**2.59-3.94**	**13-18**	**32**	**284**						

Table 3.5. Comparative table of genetic diversity for nuclear microsatellites per population, per region and overall. A_{nu}–total number of alleles, A_{priv}-private alleles, A_R–allelic richness, $A_{0.95}$ – alleles with a frequency ≥5%, Gen_{shared} –shared genotypes, Gen_{unique} –unique genotypes, *uHs*- unbiased gene diversity H-W_{DEV}-deviation from Hardy-Weinberg equilibrium (observed heterozygosity minus expected heterozygosity), F_{IS}-within-population inbreeding coefficient, *P* value^1- tests of significance for deviations from Hardy-Weinberg equilibrium based on a null hypothesis of no significant deviations (Markov chain with 10^6 steps, 10^4 dememorization steps), *P* value^2- value for test of significance with 10000 permutations within each population based on a null hypothesis of no difference from F_{IS}=0, Linked pair loci- number of significantly linked pairs of loci (*P* from 0 to 0.045). * $P<0.05$ and ** $P<0.01$.

Following hierarchical statistical analysis, results are displayed here at three levels: regional, inter-population and intra-population.

Regional

The dataset contained equal numbers of samples for the Bahamas and the TCI (178 for each region). Forest area, population sizes and total island area are considerably larger in the Bahamas than in the TCI (Table 3.1).

No statistical differences were found for mean number of alleles, mean allelic richness or number of more common alleles (frequency >95%); nevertheless, the last two parameters were slightly lower for the TCI (Table 3.5). The TCI also shared more genotypes among individuals (19) than the Bahamas (13), respectively circa 25% and 16% of the total number of individuals per region. Private alleles were present in most populations of the Bahamas, with the exception of South Andros, and in only one of the TCI populations (Table 3.5, Figures 3.1 and 3.2).

The TCI populations also had a higher number of linked pairs of loci than the Bahamas, but similar inbreeding coefficient F_{IS} (Table 3.5). Additionally, all TCI populations showed linkage disequilibrium between pairs of loci, against only four Bahamas populations. New Providence was the population with the highest number of linked pairs of loci in the Bahamas, and is also the smallest population.

No significant departure from H-W equilibrium was observed for any of the regions. However, New Providence and Pine Cay showed significant inbreeding coefficient F_{IS} (Table 3.5). All Bahamas populations were statistically different from the TCI populations, in terms of genetic differences and distances (Table 3.8). However, most populations did not show any significant differences within regions, except northern Abaco and Mangrove Cay in the Bahamas and Pine Cay in the TCI, with the latter being significantly different from all other populations. There was lower genetic structure within regions, demonstrated by low F_{ST} (Bahamas, 0.028 and TCI, 0.009), than at species level F_{ST} (0.114).

Regression analysis has also shown similar results, with lower genetic differences found between populations which are geographically closer (Figure 3.6). A significant correlation ($P<0.01$) between pairwise genetic differences F_{ST} and geographical distances was observed. There was a marked increase in genetic differences for distances between population pairs >600 km, corresponding to the distance between populations in the Bahamas and the TCI regions. However, no distinct pattern was observed for populations inside regions (300 km range), with no significant differences between the two most geographically distant populations in the Bahamas and the two closest. The lack of data for the range of 300 km to 600 km is due to absence of pine forests in this area.

Regional differences were also clear in a population graph based on genetic covariance and spatial structure, as shown in the graphical topology in Figure 3.4, b. All populations were linked, showing gene exchange within regions. Bayesian analysis has also grouped all TCI populations into one cluster (Figure 3.7, b). Principal coordinates analysis (PCoA) based on allelic differences shows grouping of individuals in the TCI and the Bahamas. Nonetheless, there was an overlapping region indicating some gene flow between regions (Figure 3.5).

Inter-population

Pairwise genetic differences between populations (F_{ST}) and Nei unbiased genetic distances ($\hat{G}_{ST}$) were estimated in order to infer genetic distance and genetic flux (*Nm*) between populations (Tables 3.6 and 3.7). The only population that was significantly different from all others was Pine Cay (Table 3.8). The other populations in the TCI (Middle and North Caicos) were genetically similar to each other. All TCI populations were genetically different and distant from Bahamas populations (Tables 3.6 and 3.7).

The Bahamas populations were mostly genetically similar to each other (New Providence, Grand Bahama, southern Abaco, northern Andros and South Andros) as shown by low and non-significant F_{ST} and $\hat{G}_{ST}$ values (Tables 3.6, 3.7 and 3.8). The same pattern was observed through Bayesian analysis, where all these populations belonged to the same cluster (Figure 3.7, c). Large genetic flux values (>1) were observed for all calculated pairs, apart from one (Pine Cay/Mangrove Cay). The highest genetic flux was observed between Grand Bahama, New Providence, South Andros and northern Andros, with values >161. Close links among these populations are also represented graphically in Figure 3.4, b.

Northern Abaco and Mangrove Cay were, however, genetically closer to each other and different from the rest of the populations (Tables 3.6, 3.7 and 3.8). This was also demonstrated in the maps of posterior probability, where they both belonged to cluster 2 (Figure 3.7, a). Both islands showed high allelic richness and unbiased gene diversity, with private alleles being present in both populations (Table 3.5 and Figure 3.1). On the other hand, New Providence, the population with the smallest area in the Bahamas (Table 3.1), had the lowest allelic richness and unbiased gene diversity. It also had the highest number of linked pairs of loci (Table 3.5).

	N.Abaco	S.Abaco	G.Bahama	N.Providence	N.Andros	Mangr.Cay	S.Andros	M.Caicos	N.Caicos	Pine Cay
N.Abaco		0.000	-0.010	0.004	-0.005	-0.007	-0.005	0.015	-0.001	0.035
S.Abaco	0.065**		0.017	-0.002	0.021	0.059	-0.026	-0.009	0.000	0.054
G.Bahama	0.063**	0.000		0.017	0.003	0.001	0.018	0.015	-0.001	0.017
N.Providence	0.096**	0.000	0.002		-0.002	0.015	-0.010	0.021	0.015	0.053
N.Andros	0.076**	0.000	0.003	0.000		-0.025	0.007	0.034	0.015	0.041
Mangr.Cay	0.027	0.109**	0.090**	0.122**	0.109**		0.025	0.059	0.018	0.040
S.Andros	0.076**	0.013	0.002	0.008	0.011	0.089**		0.009	0.009	0.051
M.Caicos	0.181**	0.153**	0.146**	0.186**	0.171**	0.189**	0.128**		-0.001	0.048
N.Caicos	0.173**	0.161**	0.155**	0.199**	0.182**	0.189**	0.141**	0.000		0.013
Pine Cay	0.195**	0.160**	0.160**	0.201**	0.187**	0.229**	0.164**	0.017**	0.011*	

Table 3.6. Matrix of pairwise genetic differences between pairs of populations for nuclear microsatellites. F_{ST} (below diagonal) and plastid microsatellite linearized φ_{PT} (above diagonal). Significant differences indicated by asterisk (*P<0.05, **P<0.01; see table 3.8 for exact P values)

	N.Abaco	S.Abaco	G.Bahama	N.Providence	N.Andros	Mangr.Cay	S.Andros	M.Caicos	N.Caicos	Pine Cay
N.Abaco		3.60	3.72	2.35	3.04	9.12	3.04	1.13	1.20	1.03
S.Abaco	0.101**		inf	inf	inf	2.04	18.75	1.38	1.30	1.31
G.Bahama	0.097**	0.000		160.01	95.54	2.53	109.40	1.46	1.36	1.31
N.Providence	0.122**	0.000	0.000		inf	1.80	29.55	1.09	1.01	0.99
N.Andros	0.117**	0.000	0.003	0.000		2.04	22.58	1.21	1.12	1.09
Mangr.Cay	0.044	0.16**	0.137**	0.146**	0.164**		2.56	1.07	1.07	0.84
S.Andros	0.124**	0.016	0.003	0.006	0.013	0.134**		1.70	1.52	1.27
M.Caicos	0.387**	0.253**	0.246**	0.291**	0.295**	0.375**	0.205**		inf	14.54
N.Caicos	0.361**	0.268**	0.264**	0.317**	0.322**	0.367**	0.231**	0.000		22.48
Pine Cay	0.341**	0.22**	0.232**	0.279**	0.282**	0.387**	0.233**	0.017**	0.01*	

Table 3.7. Matrix of pairwise unbiased genetic distance and genetic flux between pairs of populations. Unbiased genetic distance $\hat{G}_{ST}$ (Nei 1978) (below diagonal) and estimated genetic flux between pairs of populations Nm (Wright 1969) (above diagonal) for nuclear microsatellites. Significant differences in $\hat{G}_{ST}$ are indicated by asterisk (*P<0.05, **P<0.01) and exact P values are shown in Table 3.8.

	N.Abaco	S.Abaco	G.Bahama	N.Providence	N.Andros	Mangr.Cay	S.Andros	M.Caicos	N.Caicos	Pine Cay
N.Abaco		< 0.01	< 0.01	< 0.01	< 0.01	0.16	< 0.01	< 0.01	< 0.01	< 0.01
S.Abaco	< 0.01		0.84	0.57	0.74	< 0.01	0.15	< 0.01	< 0.01	< 0.01
G.Bahama	< 0.01	0.88		0.55	0.30	< 0.01	0.36	< 0.01	< 0.01	< 0.01
N.Providence	< 0.01	0.45	0.38		0.77	< 0.01	0.27	< 0.01	< 0.01	< 0.01
N.Andros	< 0.01	0.75	0.32	0.65		< 0.01	0.12	< 0.01	< 0.01	< 0.01
Mangr.Cay	0.11	< 0.01	< 0.01	< 0.01	< 0.01		<0.01	< 0.01	< 0.01	< 0.01
S.Andros	< 0.01	0.15	0.39	0.20	0.13	< 0.01		< 0.01	< 0.01	< 0.01
M.Caicos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01		0.51	< 0.01
N.Caicos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.53		0.04
Pine Cay	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.03	

Table 3.8. P values for pairwise genetic differences (F_{ST}) and unbiased genetic distance ($\hat{G}_{ST}$). P values for F_{ST} below diagonal (26082 permutations) and $\hat{G}_{ST}$ above diagonal (10000 permutations); according to the null hypothesis of no genetic differences between pairs of populations

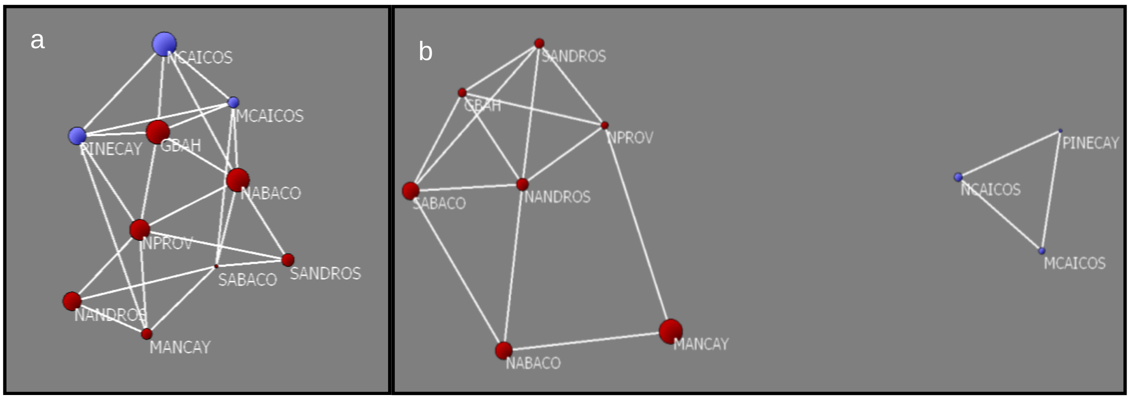


Figure 3.4. Population graph topologies for plastid (a) and nuclear (b) data based on genetic covariance (GeneticStudio). Bahamas populations are represented in red and TCI populations in blue. Abbreviations for populations are: GBAH-Grand Bahama, NPROV-New Providence, NANDROS-northern Andros, SANDROS-South Andros, MANCAY-Mangrove Cay, NABACO-northern Abaco, SABACO-southern Abaco; MCAICOS-Middle Caicos, NCAICOS-North Caicos and PINECAY-Pine Cay. Tighter plastid topology indicates genetic dependence and high gene flow among populations, whereas nuclear topology shows a split between regions due to genetic drift and isolation in the TCI.

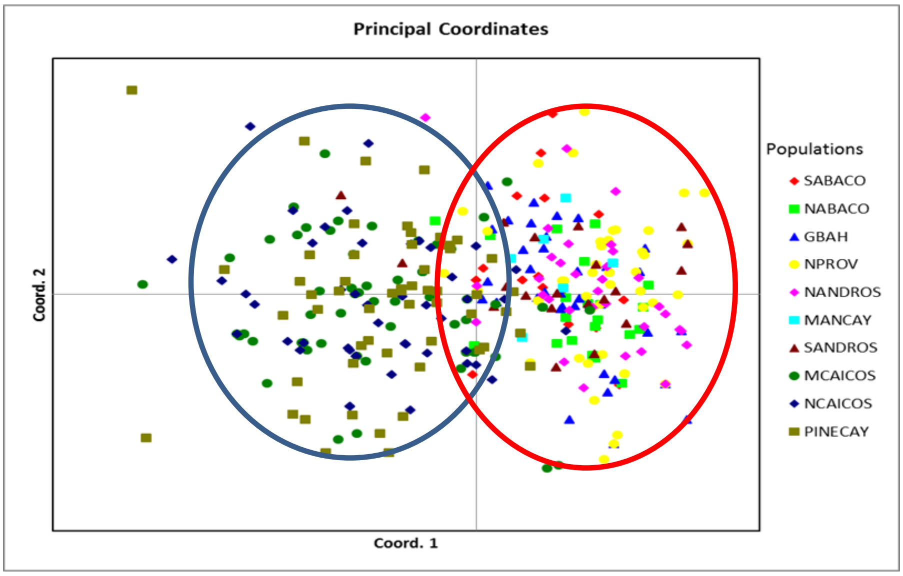
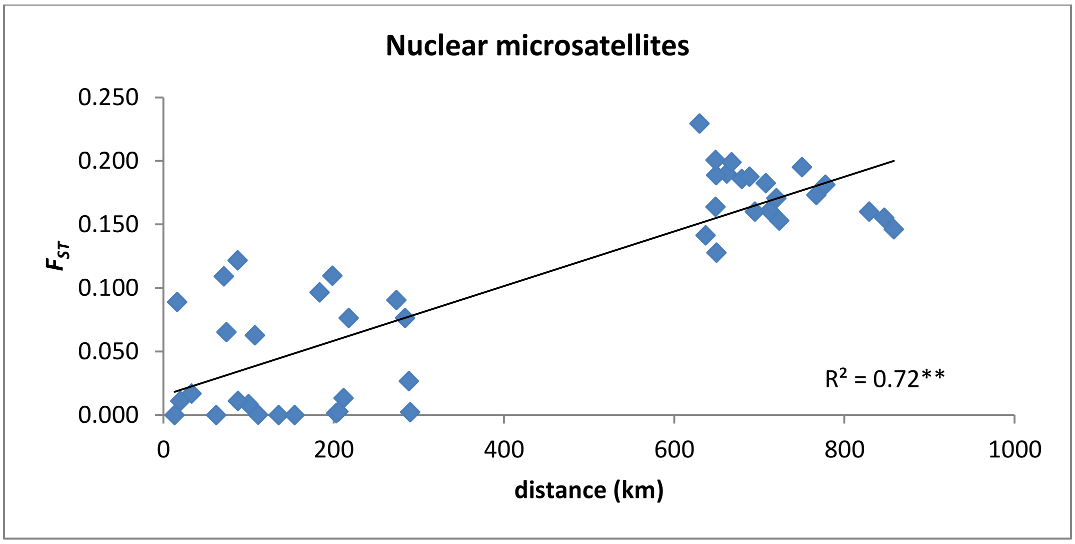


Figure 3.5. Principal coordinates analysis (PCoA) for nuclear microsatellites. PCoA via genetic distance matrix with data standardization for all populations (GeneAlEx 6.41). Population abbreviations are as in Figure 3.5. Results show all individuals sampled grouped in two main blocks, corresponding to the Bahamas (red) and TCI (blue) regions.



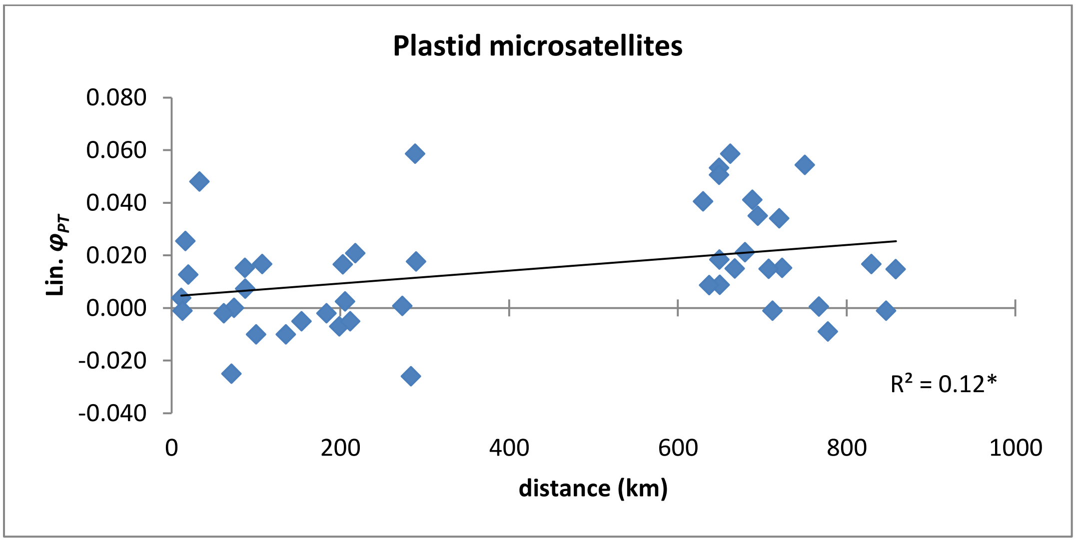


Figure 3.6. Isolation by distance model (IBD). IBD tested by regression analysis between geographical distance (km) and pairwise population genetic distance (F_{ST} for nuclear microsatellites and linearized φ_{PT} for plastid microsatellites) between pairs of populations (GeneAlEx 6.41). Significance through Mantel test (9999 permutations) was $P<0.01$(**) for nuclear data and $P=0.02$ (*) for plastid data. R^2 indicates variance explained by the model; in this case 72% of the genetic distance between pairs of populations can be attributed to the geographic distance between these populations for nuclear microsatellites and only 12% for plastid microsatellites.

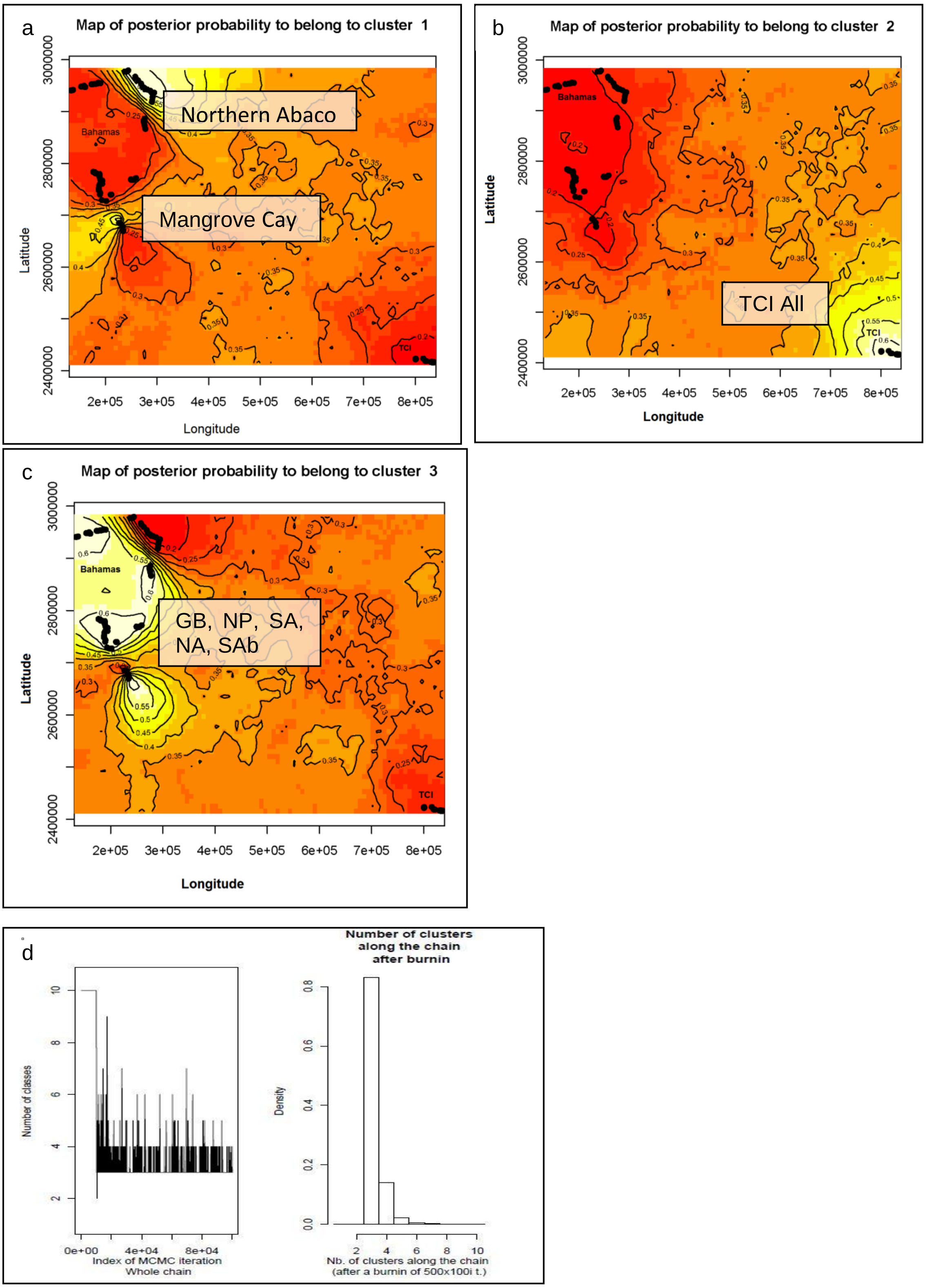


Figure 3.7. Bayesian analysis of populations and their spatial distribution. Simulations performed in Geneland software. Three populations were inferred through MCMC (number of populations along chain- plate d). Maps of posterior probability to belong to each population (cluster) are shown in plates a, b and c. High probabilities are indicated by lighter colours and dots represent sampled individuals. Cluster 1 show northern Abaco and Mangrove Cay; cluster 2 TCI islands; and cluster 3 Grand Bahama, New Providence, southern Abaco, northern Andros and South Andros.

Intra-population

Observed heterozygosity (*Ho*) was slightly lower than expected heterozygosity (*He*) for most populations, showing a negative value of H-W_{DEV} (Table 3.5). Exceptions were southern Abaco in the Bahamas and Middle Caicos and North Caicos in the TCI, which had a higher number of observed than expected heterozygotes. However, these differences were not statistically significant (Table 3.5).

Within-population inbreeding coefficient F_{IS} did not show departure from zero for the majority of populations (Table 3.5). However, significant inbreeding was observed for New Providence and Pine Cay populations.

3.3.2. Plastid Microsatellites

3.3.2.1. Allelic diversity

All sequenced microsatellite regions contained repeats of seven or more bases. Primers Pt36480, Pt71936 and Pt87268 amplified regions including mononucleotide repeats and Pt15169 and Pt30204 amplified regions including dinucleotide repeats (Table 3.9). No sequences were obtained for locus Pt9383.

Locus Pt36480 was the only monomorphic locus of the six loci tested. It was also the shortest microsatellite repeat with only seven base pairs. All other loci were polymorphic and had three alleles per locus with a variation of 1-2 bp (Table 3.4).

Amplification, after optimization, was good for all loci tested and product yield was high. Distinct allelic sizes and the use of two coloured labels (FAM and JOE) allowed multiplexing of PCR product during genotyping, with good and clear results.

Locus	**Location•**	**A_{range}**	**A**	**Repeat**
Pt36480	*psbJ* -*petA* intergenic region	141	1	$(T)_7$
Pt71936	IRF169	144-146	3	$(T)_{12}$
Pt87268	*trnI* (GAU)-*trnA* (UGC) spacer	160-162	3	$(T)_{11}$
Pt9383	*trnG* (UCC) intron	84-86	3	$(T)_9$ AG $(A)_{10}$•
Pt15169	*rps2*	104-106	3	$(C)_{14}(T)_{19}$
Pt30204	*clpP*	134-136	3	$(A)_8$ $(G)_6$

Table 3.9. Genetic parameters for plastid microsatellite loci over all populations. A_{range}- allelic range in bp, A- total number of alleles observed and Repeat- type of microsatellite repeat. • Data for *Pinus thunbergii* according to Vendramin *et al.* (1996).

3.3.2.2. Genetic structure

AMOVA analysis showed that most genetic variation (97%) was within populations, with little structure by populations and regions (≤ 3%) (Figure 3.3, b).

Twelve haplotypes with distinct allele combinations were identified for this dataset, which is a low number compared to 316 genotypes detected for nuclear microsatellites (Tables 3.5 and 3.10). The most common haplotype (H7) was shared by all populations and circa 80% of individuals (Figure 3.8). Lowest haplotype frequency was 0.22% for H3 and H10, which were found in only one population each, New Providence and North Caicos, respectively.

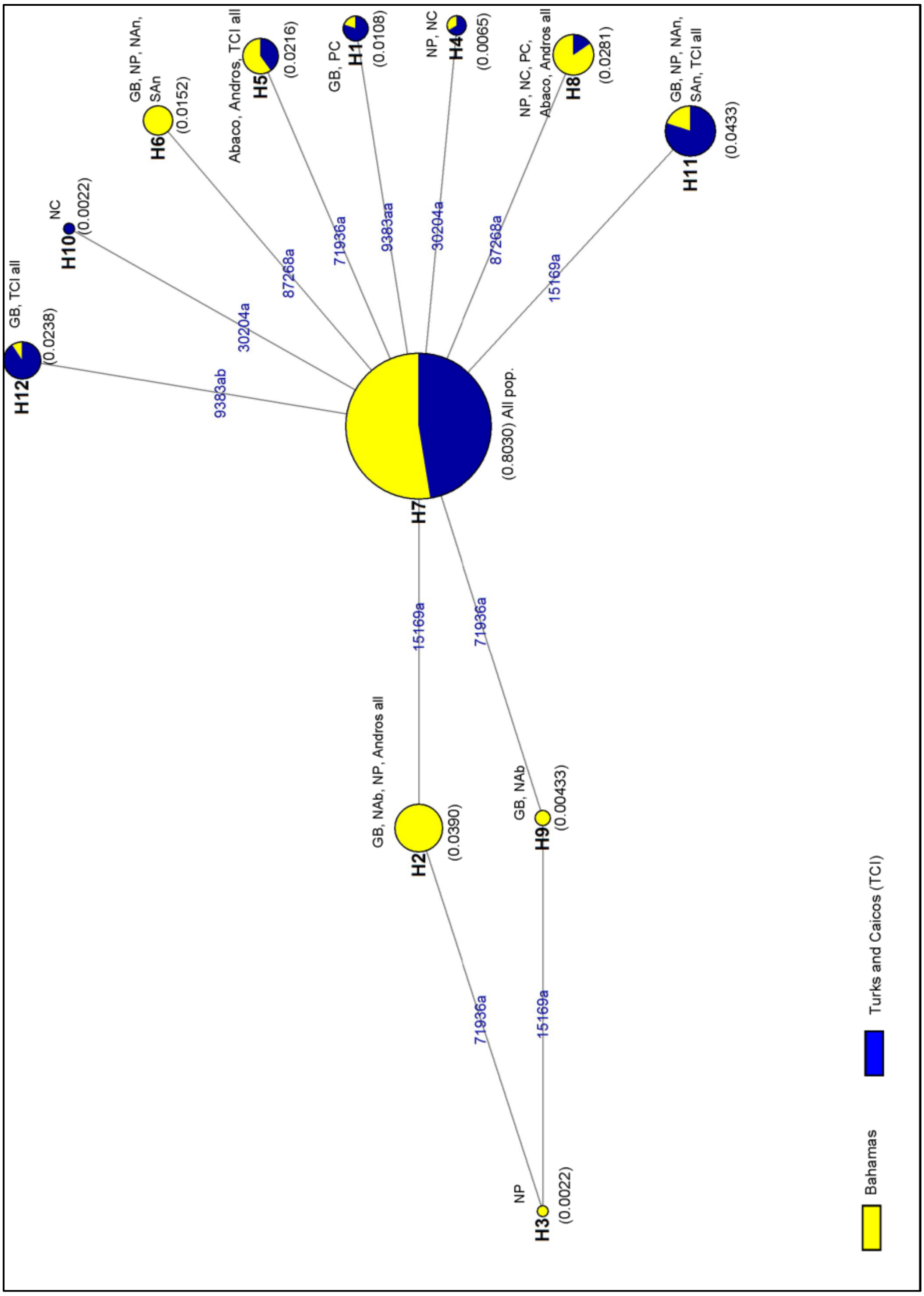


Figure 3.8. Plastid microsatellite network under stepwise mutation model (SMM). Network used median joining method (Network 4.6.0.0, based on Bandelt *et al*. 1999). Nodes size correspondent to haplotype frequency (shown in brackets below node). Populations containing haplotypes are shown beside or above nodes, with the following abbreviations: GB- Grand Bahama, NP- New Providence, NAn- northern Andros, SAn- South Andros, NAb- northern Abaco, SAb- southern Abaco; MC- Middle Caicos, NC- North Caicos and PC- Pine Cay. Numbers in blue correspond to mutations at primer loci.

	Plastid microsatellites				
Location	**P**	**A_p**	**Hap_N**	**Hap_{unique}**	***uh***
Bahamas					
Northern Abaco	50.0	10	5	0	0.056
Southern Abaco	33.3	8	3	0	0.033
Grand Bahama	83.3	13	7	0	0.061
New Providence	50.0	11	7	1	0.075
Northern Andros	50.0	11	6	0	0.081
South Andros	50.0	11	6	0	0.065
Mangrove Cay	50.0	9	4	0	0.089
Mean	**52.4**	**10**			**0.066**
Turks and Caicos					
Middle Caicos	66.7	10	4	0	0.033
North Caicos	83.3	13	7	1	0.066
Pine Cay	66.7	11	6	0	0.084
Mean	**72.2**	**11**			**0.061**
Mean Overall	**58.3**	**11**			**0.064**
Total	**33.3-83.3**	**8-13**	**12**	**2**	

Table 3.10. Comparative table of genetic diversity for plastid microsatellites per population, per region and overall. P-percentage of polymorphic loci, A_p–total number of alleles, Hap_N- number of haplotypes, Hap_{unique}-number of unique haplotypes, and *uh*-unbiased estimate of genetic diversity.

Following hierarchical statistical analysis, results are displayed at two levels: regional and inter-population.

Regional

Average number of alleles per population was similar for both regions, as was unbiased genetic diversity (Table 3.10). Locus polymorphism was slightly higher for the TCI (72.2%) than the Bahamas (52.4%).

The Bahamas had a higher total number of haplotypes (11) than the TCI (8) (Figure 3.8). It has also shown more variation at population level with only one shared haplotype (H7) shared by all populations against three shared haplotypes in the TCI (H5, H11 and H12). The number of unique haplotypes for the region was also higher for the Bahamas (H2, H3, H6 and H9) than the TCI (H1 and H10).

No major division in regions was observed for plastid microsatellites, as it was for nuclear microsatellites. All populations shared haplotypes at a certain level with each other. An interlinked topology in the population graph demonstrated high gene flow and dependence between populations, with populations from both regions grouped together (Figure 3.4, a).

Regression analysis showed significant (P=0.02) correlation coefficient between genetic variation (φ_{PT}) and geographical distance (Figure 3.6). However, only 12% (R^2=0.12) of this variation can be attributed to geographical distance. This is considerably lower when compared to nuclear microsatellites with 71% of variation related to geographical distance. Low and high φ_{PT} values were distributed uniformly among closer and more distantly separated populations.

Inter-population

Plastid microsatellite polymorphism was lowest for southern Abaco (33.3%) and highest for Grand Bahama and North Caicos (83.3%) (Table 3.10). The overall mean value of polymorphism observed in the populations (58.3%) was almost half of that observed for nuclear microsatellites (100%).

Pairwise genetic differences φ_{PT} was low for all pairs of populations (Table 3.6), however, no statistical test was available to test significance of genetic differences pairwise for this parameter. Highest values were observed for the Pine Cay population, which also had a high unbiased genetic diversity value (Table 3.10). These data are in agreement with those for nuclear microsatellites, which have shown genetic distance and differences from all other populations.

No private alleles were observed for any of the populations. The highest number of alleles occurred on Grand Bahama and North Caicos (both with 13), varying little among populations (Table 3.10). These two populations, along with New Providence, also had the highest number of haplotypes.

Southern Abaco had a low number of alleles and haplotypes, and also low unbiased genetic diversity (Table 3.10). All of its haplotypes (H5, H7 and H8) were shared with northern Abaco and Andros (Figure 3.8). Plastid markers did not detect any unique alleles or haplotypes for Mangrove Cay and northern Abaco (Table 3.10). This is in contrast to the nuclear data which have shown genetic similarities between these populations and genetic differences to all other islands. Mangrove Cay had, however, the highest genetic diversity (0.089). New Providence and North Caicos were the only two populations that contained unique haplotypes, H3 and H10, respectively. These two haplotypes showed the lowest observed frequencies (Figure 3.8).

3.3.3. Comparative genetic variability *ex-situ/in-situ* (TCI)

This analysis focused on the TCI populations only. The objective was to detect differences and similarities in genetic parameters and to evaluate representativeness of the sampled *ex-situ* collection.

Each population (Pine Cay, Middle Caicos and North Caicos) was divided into 'wild' (samples from field collection in natural forest areas), and '*ex-situ*' (samples from MCCC nursery on Middle Caicos). A total of six populations were then analysed: Middle Caicos wild (MCw), North Caicos wild (NCw), Pine Cay wild (PCw), Middle Caicos *ex-situ* (MCex), North Caicos *ex-situ* (NCex), and Pine Cay *ex-situ* (PCex). Individuals from the *ex-situ* collection were all seedlings, with a height of circa 15 to 50 cm, which were collected as seedlings or seed from the TCI natural forests (Figure 3.2).

Only samples with a complete data set for all loci were used in the analysis (Table 3.12). Selection of microsatellite loci and statistical analysis were the same used for the main dataset (this Chapter, section 3.2).

3.3.3.1. Nuclear microsatellites

Allelic diversity

All loci were polymorphic. The total number of alleles was higher in wild populations (27), when compared to *ex-situ* collection; however, the number of sampled individuals was also higher for wild populations (Table 3.12). The same was observed for allelic richness, with the lowest value for MCex population and the highest for MCw.

Private alleles were only present in wild populations and all loci, except PtTX2123, had at least one private allele. High numbers of unique genotypes were identified (159) and circa 11% of individuals shared genotypes (Table 3.12).

Genetic structure

AMOVA analysis showed higher variation between individuals (98.5%) than populations (1%) or groups (wild and *ex-situ*, 0.5%), a similar result to that obtained for the main dataset.

Wild and *ex-situ* populations had similar unbiased gene diversity (P=0.50) (Table 3.12). F_{ST} for wild populations was higher (0.018) than for the *ex-situ* collection (0.002); however, this difference was not statistically significant (P=0.67).

Pine Cay was the only island that showed significant genetic difference between wild and *ex-situ* populations (P<0.05) with an F_{ST} of 0.022. No significant genetic differences were observed between the *ex-situ* population pairs, apart from MCex and NCex (P=0.04) (Table 3.11).

Within-population inbreeding coefficient (F_{IS}) was negative for North and Middle Caicos wild populations, and positive for Pine Cay. On average, wild populations showed a random outcrossing mating system (negative F_{IS}), whereas the *ex-situ* collection was slightly, but not significantly, inbred. The only population that showed significant inbreeding was the Pine Cay *ex-situ* collection (P< 0.01) (Table 3.12).

Levels of heterozygosity were slightly lower for the *ex-situ* collection, but no departures from H-W equilibrium were observed (P>0.61 for all populations) (Table 3.12).

	M.Caicos wild	**M. Caicos *ex-situ***	**N.Caicos wild**	**N. Caicos *ex-situ***	**Pine Cay wild**	**Pine Cay *ex-situ***
M.Caicos wild		0.013	-0.006	-0.012	-0.018	0.578
M. Caicos *ex-situ*	0.003 (0.27)		0.003	0.001	0.023	0.816
N.Caicos wild	0.000 (0.38)	0.007 (0.17)		0.003	0.000	0.639
N. Caicos *ex-situ*	0.000 (0.66)	0.001 (0.04)*	0.006 (0.21)		-0.001	0.296
Pine Cay wild	0.031 (< 0.01)**	0.041 (< 0.01)**	0.023 (< 0.01)**	0.027 (0.02)*		0.528
Pine Cay *ex-situ*	0.003 (0.30)	0.004 (0.36)	0.015 (0.07)	0.000 (0.83)	0.022 (0.05)*	

Table 3.11. Matrix of pairwise genetic differences between pairs of populations in the TCI for nuclear microsatellites. F_{ST} (below diagonal) and plastid microsatellites linearized φ_{PT} (above diagonal). P values in brackets, according to null hypothesis of no genetic difference between populations (26082 permutations). *P<0.05, ** P<0.01.

	Population	**Middle Caicos**	**North Caicos**	**Pine Cay**	**Total**
N_{nu}	Wild	37	36	35	**108**
	ex-situ	28	18	24	**70**
A_{nu}	Wild	21	21	19	**27**
	ex-situ	15	18	15	**20**
A_{priv}	Wild	1	3	1	**5**
	ex-situ	0	0	0	**0**
A_R	Wild	3.77	3.75	3.33	**3.61**
	ex-situ	2.86	3.60	2.92	**3.13**
Gen_{unique}	Wild	36	34	34	**97**
	ex-situ	27	18	22	**62**
uHs	Wild	0.579	0.563	0.479	**0.526**
	ex-situ	0.537	0.546	0.512	**0.551**
$H\text{-}W_{DEV}$	Wild	0.042	0.004	-0.022	**0.002**
	ex-situ	-0.016	0.009	-0.093	**-0.035**
F_{IS}	Wild	-0.074	-0.007	0.047	**-0.016**
	ex-situ	0.030	-0.017	0.186**	**0.069**
N_P	Wild	45	40	41	**126**
	ex-situ	29	28	28	**85**
P	Wild	50	50	33.33	**44.4**
	ex-situ	0	50	16.67	**22.2**
A_p	Wild	9	9	9	**10**
	ex-situ	6	10	7	**10**
Hap_N	Wild	4	4	5	**6**
	ex-situ	1	4	2	**4**
Hap_{unique}	Wild	0	0	1	**4**
	ex-situ	0	2	0	**2**
uh	Wild	0.036	0.033	0.052	**0.040**
	ex-situ	0.000	0.075	0.086	**0.052**

Table 3.12. Comparative table of genetic diversity for nuclear and plastid microsatellites for wild and *ex-situ* populations in the TCI. N_{nu}-number of samples used for nuclear microsatellite analysis; N_p-number of samples used for plastid microsatellite analysis A_{nu}–total number of alleles for nuclear loci, A_p–total number of alleles for plastid loci A_{priv}-private alleles, A_R–allelic richness, Gen_{unique}–unique genotypes, *uHs*- unbiased gene diversity, P- percentage of polymorphic loci, H-W_{DEV}-deviation from Hardy-Weinberg equilibrium (observed heterozygosity minus expected heterozygosity), F_{IS}-within-population inbreeding coefficient, Hap_N-number of haplotypes, Hap_{unique}-number of unique haplotypes, *uh*- unbiased estimate of genetic diversity. Total values across all loci per class. **$P<0.01$, 10000 permutations within each population).

3.3.3.2. Plastid Microsatellites

Allelic diversity

One (Pt36480) out of the six microsatellite loci was monomorphic for the TCI. Private alleles were present in microsatellite loci Pt87268, Pt30204 and Pt9383.

Polymorphism at the population level varied from 0% (MCex) to 50%, which is half or less than the value found for nuclear microsatellites (Table 3.12). The total number of alleles varied from six in MCex to 10 in NCex.

Genetic structure

AMOVA showed variance among individuals of 85%, which was lower than observed for the nuclear dataset. Variance among populations was 14%, probably due to high genetic differentiation of the Pine Cay samples. Variance between groups (wild and *ex-situ*) was only 1%.

Eight different haplotypes were identified for this dataset, with higher haplotypic variation in wild populations (H1, H5, H7, H8, H11 and H12) than in the *ex-situ* collection (H4, H7, H10 and H11). The most common haplotype H7 was present in 80.3% of all sampled individuals and in all populations, similar to the results from the main dataset.

Unbiased estimates of genetic diversity (*uh*) varied from 0.000 for MCex to 0.086 for PCex (Table 3.12). Mean total value of genetic diversity for the *ex-situ* collection was slightly higher than that for wild populations; however, there were no significant statistical differences (15000 permutations, P>0.05).

A higher number of haplotypes was found in the wild populations of Middle Caicos and Pine Cay, in comparison to the *ex-situ* collection for these islands, including the unique haplotype H1 in PCw (Table 3.12). MCex had only the most common haplotype (H7), i.e. there were no allelic differences. North Caicos on the other hand, had the same number of haplotypes, four, for the wild samples and the *ex-situ* collection. However, NCex had two unique haplotypes (H4 and H10). The Pine Cay *ex-situ* collection was the only one to show high pairwise genetic differences φ_{PT} with all other populations (Table 3.11).

3.4. Discussion and Conclusions

Allelic Variance

All regions selected were amplified successfully for *P. caribaea var. bahamensis*. All nuclear loci were polymorphic, whereas plastid locus Pt36480 was monomorphic for all populations (Table 3.9). The lack of polymorphism at this locus could be associated with the low number of repeat units in the microsatellite region (Table 3.9), as microsatellite loci with a low number of repeats appear to have lower mutation rates (Schlötterer 2000, Dieringer and Schlötterer 2003). Nonetheless, all primers selected for this research have previously shown polymorphism in *P. pinaster (Vendramin et al. 1998, Bucci et al. 2007), P. resinosa* Aiton (Echt *et al.* 1998) and *P. elliottii* var. *densa* (Williams, D. A. *et al.* 2007). Barbará *et al.* (2007b) observed the occurrence of several levels of polymorphism and size of alleles in different species. In contrast to the results observed in this research, primers Pt30204 and Pt9383 lacked variation in *P. caribaea* in a recent study (Jardón-Barbolla *et al.* 2011). Samples of *P. caribaea* var. *bahamensis* in that study were only from North Andros and New Providence, with 14 and 12 individuals from each population, respectively. Possibly the low number of samples and the close genetic distance observed between these populations could have contributed to a failure in detecting allelic variation in those plastid regions.

Low allelic range and number of alleles were observed for plastid loci (Tables 3.4 and 3.9), similar to results observed for *P. elliottii* var. *densa* and *P. pinaster* (Bucci *et al.* 2007, Williams, D. A. *et al.* 2007). This is probably due to lower substitution rates and lower mutation rates in plastid than in nuclear DNA (Provan *et al.* 1999). Locus PtTX3034 had the highest number of allelic combinations and higher gene diversity and was the most informative nuclear locus.

Genetic structure and variability

Most of the genetic variability was within populations (≥83%) for both nuclear and plastid regions (Figure 3.3). Similar results have also been reported for several other pine species, including *P. elliottii* var. *densa* (Williams, D. A. *et al.* 2007), *P. pinaster* (Mariette *et al.* 2001) and *P. strobus* L. (Rajora *et al.* 1998).

The inbreeding coefficient at species level was low F_{IS}=0.033 and non-significant (*P*=0.13) (Table 3.4). Although observed heterozygosity was lower than expected (negative H-W_{DEV} values), there was no significant deviation from H-W equilibrium, indicating near to random mating (Table 3.5). Pine populations of *P. caribaea* var. *hondurensis* in Belize and Guatemala did not show significant inbreeding (F_{IS}= 0.011, *P*>0.05) (Dvorak *et al.* 2005), whereas the Mexican populations were inbred (F_{IS}= 0.097, *P*<0.05) (Delgado *et al.* 2011). In Mexico, populations have undergone a bottleneck possibly between 8,121 and 34,721 yr BP because of drought and flood events; resulting in the levels of fragmentation and inbreeding observed presently (Delgado *et al.* 2011). High historical gene flow and large areas of continuous pine forest could have contributed to the predominant outbreeding system observed in *P. caribaea* var. *bahamensis* during this research.

The fixation index F_{ST} at species level of 0.114 (Table 3.4) was close to the mean F_{ST} value of 0.166 obtained from five species of pines (*P. pinaster, P. resinosa, P. halepensis, P. brutia* Ten., *P. nelsonii* Shaw and *P. heldreichii* H.Christ) by Delgado *et al.* (2002). According to Wright (1965), random mating occurs in a species when F_{ST} (genetic variation in subpopulations relative to total variation) and F_{IT} (total genetic variation across all populations) values are equal, with F_{IT} decreasing with an increase in inbreeding. Close values for this variety of F_{ST} and F_{IT} (Table 3.4), in addition to low inbreeding and no deviations from H-W equilibrium confirm a predominantly random and outcrossing mating system.

Results, in general, have shown higher variability and genetic structure for nuclear than plastid microsatellites. Isolation by distance model had a higher genetic variability attributed to distance among populations with nuclear microsatellites than with plastid microsatellites (Figure 3.6), which can be partly correlated to short-distance seed dispersal and low gene flow due to geographical isolation. Seed dispersal distance is uncertain for this variety, but has been reported for other pine species as varying from 15-60m from the mother tree (Chapter 1 section 1.4.3). On the other hand, pollen can be dispersed over long distances (30-1000 km), although most of the pollen cloud (99%) falls closer to the source (Williams, C. G. 2008). Thus, it would be expected that there would be higher gene flow and less genetic differentiation revealed by the plastid than nuclear regions of the genome, as seen here. Wolfe e*t al.* (1987) observed that substitution rates in plastid genes correspond to circa half of that present in nuclear genes for the plant genome. Therefore, plastid regions are more conserved and slower to mutate than nuclear ones. Long-distance pollen dispersal and low mutation rates have contributed to the low differentiation at plastid regions observed in this research, indicating high and widespread gene flow in the past.

Bahaman archipelago, regional structure

Isolation by distance between the Bahamas and the TCI was evident, regarding nuclear regions, as seen in the population graph (Figure 3.4), PCoA (Figure 3.5) and regression analysis (Figure 3.6).

Genetic structure within each region for the Bahamas and the TCI was low (F_{ST} 0.028 and 0.009, respectively), when compared to the high observed genetic structure at variety level (F_{ST} 0.114). Genetic flux (*Nm*) was considerably lower between regions (0.84 to 1.70) than within regions (1.8 to >160.01) (Table 3.6). Therefore, it seems that distance between regions is hindering gene flow through migration. Similar results were obtained for populations of the endemic Mexican piñon (*P.pinceana*) by Ledig *et al.* (2001). Fragmentation and isolation by distance were observed. The northern fragment had low F_{ST} (0.056), whereas F_{ST} for the species (including all three fragments, northern, central and southern) was high (0.152). High unbiased genetic distance was observed between the northern populations and central and southern populations.

Distances >600 km between populations, like those between the Bahamas and the TCI, have shown the highest genetic distance values (F_{ST} 0.12 to 0.22 and $\hat{G}_{ST}$ 0.20 to 0.39, as in Tables 3.6, 3.7 and Figure 3.6). The lack of correlation between geographical and genetic distances among populations within regions indicates that such populations (<300 km) are exchanging more genes and are genetically interdependent, than those further apart (>600 km). Dvorak *et al.* (2005) found similar results for *P. caribaea* var. *hondurensis,* with more genetically similar populations <65 to 100 km apart.

The population graph shows a split between the Bahamas and the TCI populations on the basis of nuclear microsatellites (Figure 3.4). This sort of split occurs with low gene flow and high genetic drift, leading to allele fixation in the population (Dyer 2007). It seems that genetic drift and low gene flow between regions reduced genetic differentiation in the TCI, a smaller and more isolated population. However, there is still some level of shared genes at the nuclear level, indicated by an overlapping area in the PCoA graph (Figure 3.5).

No strong correlation between area and allelic richness was observed (R^2=0.078, P=0.43). More private alleles in nuclear regions were present in Bahamas (seven) than the TCI (one) and the former also had a higher number of haplotypes (11 in the Bahamas and eight in the TCI) and unique genotypes (Table 3.5). The Bahamas region has populations covering a larger area and thus more individuals, as observed in Chapter 2 section 2.3.2. They also show high genetic flux among populations and possibly lower genetic drift. Loss of rare alleles in the TCI could have been driven by isolation, smaller populations and/or genetic drift. The effects of genetic drift are more accentuated in smaller populations, tending to a loss of rare alleles and fixation of common alleles (Silvertown and Charlesworth 2001).

Plastid regions had low φ_{PT} values between Bahamas and TCI populations (Table 3.6), in contrast to nuclear regions. A weaker correlation between genetic and geographical distance and a tightly compacted topology were observed. Only 12% of the variance in genetic differentiation could be attributed to distance, a low value compared to 72% for nuclear microsatellites (Figure 3.6). There was also no separation in the topology, with all populations linked together (Figure 3.4). Thus, low genetic differentiation among strata and high pollen flow could be inferred. In addition, a low number of haplotypes, with the majority of individuals sharing the same haplotype H7 (Figure 3.8), also suggests widespread pollen dispersal throughout the distribution range for the variety. The low mutation rate for plastid DNA could have played a part in low differentiation among populations and the presence of an ancestral haplotype with high frequency. A study on *P. elliottii* var. *densa* in Florida also demonstrated low genetic differentiation, high pollen flow across populations and one common haplotype with a frequency of 70% in all populations (Williams, D. A. *et al.* 2007).

Wind and hurricanes could partly explain the high level of gene flow and low level of population structure within regions. Seed dispersal is mainly over short distances, usually from August to November (Greaves 1978). At this time moderate easterly and south-easterly trade winds prevail. As the TCI lies >300 km southeast of the Bahamas, effective migration between regions would require high speed winds from the northwest or southeast. The main seed dispersal is short-distance and within regions, as demonstrated by low F_{ST} values for populations <300 km apart in the regression analysis (Figure 3.6), which corresponds to the distance between populations within regions. Nonetheless, occasional long-distance dispersal could also occur. Hurricanes can play a part in the transport of seeds and cones further away than normal winds (Dvorak *et al.* 2005). There have been at least 17 major hurricanes affecting pine islands in the archipelago since 1926 (Chapter 2, Table 1.2). Increased wind speed and changeable direction could have contributed to long-distance seed dispersal. Northern Abaco and Mangrove Cay are 288.7 km apart but despite this showed more genetic similarities in the Bayesian analysis (Figure 3.7) and F_{ST} pairwise values (Table 3.6) than with other neighbouring islands. In this case, migration could have been driven by high-speed winds during hurricanes. Hurricanes were recorded during the late 1960s and 1970s in the Bahamas (Chapter 1 Table 1.2), when logging was still on-going and many areas were favourable for new colonisation.

Pollen dispersal usually occurs from January to March (Farjon and Styles 1997). Cold wind fronts, mainly originating in North America (northerly and north-easterly) are more common at this time of the year (Stone 1953, Sealey 1985, Whitaker and Smart 1997). Pine pollen is known to travel long distances, even 1000 km, in special circumstances of weather fronts, hurricanes and storms (Williams, C. G. 2010) due to its buoyancy and low weight, as previously mentioned (Chapter 1 section 1.4.3). Long-distance pollen dispersal associated with northerly winds could lead to dispersal throughout the whole archipelago, resulting in the low population structure observed for plastid regions in the graphical topology (Figure 3.4) and many shared haplotypes throughout the range for the variety (Figure 3.8). Regression analysis showed low genetic differentiation for all population pairs, but populations closer together (<300 km apart) seem to have more pollen flow between them (lower F_{ST} values) than those further apart (Figure 3.6).

Ancient migration patterns could also be inferred from the observed genetic structure. The presence of a high frequency haplotype common to all populations suggests a common origin for populations in both regions. A higher number of private alleles, fewer shared genotypes, lower total number of haplotypes and higher unbiased gene diversity in the Bahamas (Tables 3.5 and 3.10) suggest that this region could have been the founder population, with a later expansion into the TCI.

A hypothetical north-south migration route for the species can be inferred from previous studies. Links between the Caribbean and North American floras have been explored by Francisco-Ortega *et al.* (2008). Additionally, Adams and Jackson (1997) and Farjon

(1996) suggested a migration route for pines from North America to the Caribbean. The lower sea level present in southern Florida and the Bahamas during the Late Glacial period and Early Holocene extended land and reduced distances between islands (Figure 1.5), as described in Chapter 1 section 1.2.4. This could have facilitated pine migration between the continent and the islands and within the Bahaman archipelago. Fossil records show that pine pollen was present in the Bahamas at this time (Steadman *et al.* 2007). Therefore, pines could have migrated from North America to the Bahamas in this way.

Nevertheless, a study by Jardón-Barbolla *et al.* (2011) identified ancestral plastid haplotypes for *P. caribaea* in Central America and inferred a migration route from Central America to the Caribbean for the species, but with different lineages for *P. caribaea* var. *caribaea* in Cuba and *P. caribaea* var. *bahamensis* in the Bahamas (Chapter 1 sections 1.2.4 and 1.3). This would indicate a west to east migration route accross the Caribbean Sea due to long-dispersal events. Taking into account that hurricanes originate in the east (Atlantic Ocean) and travel west and north-west across the Caribbean Sea, distances between islands in the Bahaman archipelago and Central America are longer than those to North America, and the Bahamas have shown higher genetic diversity than the TCI, it seems unlikely that this would have been the dispersal route taken by ancestral pines.

The focus of this research is to reveal migration patterns and genetic structure in the Bahaman archipelago. In the cases of a north to south or west to east colonisation, the Bahamas would have been the closest land encountered by migrating pines. This concurs with the observations that the TCI populations are smaller in size and have less genetic diversity and higher linkage disequilibrium, indicating that these populations could have resulted from an expansion of the Bahamas populations. Fewer founding individuals would result in less genetic variation and a lack of rare alleles. A more recent colonization event would contribute to higher linkage disequilibrium in this smaller population. Thus, a migration route for pines from the Bahamas to the TCI is plausible.

High genetic flux (number of migrants) for most population pairs, gene flow, high pollen flow and a predominantly random outcrossing breeding system are all indications that the Bahamas and the TCI are still part of the same evolutionary unit. Nevertheless, some level of genetic differentiation is apparent, as demonstrated through analysis. The recent geological history of the islands with a great part of land built up only circa 130,000 yr BP (Chapter 1 section 1.2.4), in combination with the oldest fossil records of pine trees dating back to only circa 8,338 yr BP (Slayton 2010), suggests a recent pine colonisation of these islands.

TCI populations and comparative study

The TCI showed a low level of population structure (F_{ST} 0.009). All populations are supposedly primary forest, and there are no records of commercial exploitation for the TCI (Chapter 2 section 2.3.2.2).

All populations had a higher number of linked pairs of loci than the Bahamas populations (Table 3.5). Forest area and population size are considerably smaller in the TCI than in the Bahamas (Table 3.1, Chapter 2 Table 2.3). In addition, populations have been undergoing drastic reduction in the number of individuals since 2005, due to tree death caused by the invasive scale insect *Toumeyella parvicornis* and fire, as discussed in Chapter 1 section 1.5.4 and Chapter 2 section 2.4. Small populations and populations under bottleneck can show increased linkage disequilibrium, as fewer alleles are present in new generations (Flint-García *et al.* 2003).

Nuclear markers revealed no genetic distance between Middle and North Caicos (F_{ST} and $\hat{G}_{ST}$ zero), low genetic differentiation for plastid regions (φ_{PT} -0.001) and high gene flow (Tables 3.6 and 3.7). This suggests high pollen and seed exchange between these two islands. Both of them had negative inbreeding coefficients (Table 3.5), indicating outcrossing and high gene flow (Wright 1965). The short geographical distance between them, only 13.1 km (Figure 3.2), could have facilitated migration and pollen exchange.

Some interesting results were observed from comparisons between wild populations and *ex-situ* collections from these two islands. *Ex-situ* collections showed an increase in inbreeding coefficient on Middle Caicos and a decrease on North Caicos (Table 3.12). Sampling of siblings could result in an increase in the inbreeding coefficient; however, in this case, the opposite was observed. The more restricted collection area (North Caicos) showed lower inbreeding than a more widespread collection area (Middle Caicos), as can be observed in Figure 3.2. This could have been influenced by a major stochastic event. The number of adult trees has been considerably reduced in the past six years due to pest attack, as previously mentioned, which could explain increased inbreeding due to the lower number of seed bearers, as is the case for Middle Caicos. On the other hand, more open areas for colonization and fewer obstacles to seed movement were created with die back of trees. Predominantly southeasterly and easterly winds could have led to long-distance seed dispersal from Middle Caicos to North Caicos. It has been shown that species with long-distance dispersal mechanisms can migrate more quickly through a fragmented landscape, but still depend on suitable habitat for establishment (Pearson and Dawson 2005). Providing that suitable open areas are present, seedlings from Middle Caicos could have been established in North Caicos pine forest, which would have potentially added new genes to the population and could have decreased inbreeding.

Pine Cay showed significant genetic difference and distance (F_{ST} and $\hat{G}_{ST}$) from all other islands; however, these differences were lower within region (Tables 3.6 and 3.7). Gene flow (*Nm*) was higher with North Caicos and φ_{PT} lower, indicating some level of genetic exchange between these islands. This is probably due to closer geographical distance to North Caicos (20 km) than Middle Caicos (33.17 km), which would make seed and pollen dispersal easier between them (Figure 3.2). Pine Cay also used to be the smallest population in the archipelago with a pine forest area of only 1.06 km^2 (Table 3.1); but this is no longer the case since the stochastic fire event in 2009 on North Caicos (described in Chapter 2, section 2.4). The highest inbreeding coefficient of all populations was observed for Pine Cay wild and *ex-situ* collection (Table 3.12). The same occurred in the main dataset, where Pine Cay showed significant inbreeding (Table 3.5). This island also showed the lowest allelic richness of all populations, absence of private alleles, high number of common alleles ($A_{0.95}$), inbreeding, low unbiased gene diversity, low polymorphism and reduced number of haplotypes and genotypes (Tables 3.5 and 3.10). These are signs of reduced gene flow with other islands, a small population with a low number of mature individuals and genetic drift.

Habitat is another factor to be considered in the differentiation of the Pine Cay population. Pine Cay pine forests are the only ones in the archipelago growing on sandy soils (Holocene marine calcareous sand); forests on all other islands grow on limestone rock with pockets of lateritic soil (Sealey 1985). Therefore, it seems that differentiation could have been driven by ecotypic variation. Failure in seedling establishment due to a unique environment could also have affected allele frequencies and survival of migrants. A phylogenetic study of *Araucaria* species from New Caledonia showed high genetic divergence of the Mont Panié population of *A. montana* Brongn. & Gris, possibly associated to speciation to local environmental conditions, as this is the only population of the species growing on acid soils, instead of ultramafic soils (Gaudeul *et al.* 2012).

The Pine Cay *ex-situ* collection was observed to have a four times larger F_{IS} and a lower allelic richness than the wild population (Table 3.12). This could be a result of the reduction in the number of seed and pollen bearing trees lost due to the scale insect infestation. Early effects of habitat degradation with loss of rare alleles and increased inbreeding for seedlings were reported for *Araucaria nemorosa* de Laub., by Kettle *et al.* (2007). Nonetheless, *ex-situ* collections for Pine Cay in this research were only made in the western part of the island (Figure 3.2), which could have led to a misrepresentation of the total genetic diversity, capture of fewer alleles and sampling of siblings.

Ex-situ collection in the TCI, in general, showed similar unbiased genetic diversity (*uh*) to wild populations; however, it had no private alleles, fewer total alleles, lower allelic richness, less polymorphism and fewer unique genotypes and haplotypes than those found in wild populations (Table 3.12). Seedlings also showed a higher inbreeding coefficient than wild populations. All these factors indicate the loss of genetic diversity at plastid and nuclear levels for the *ex-situ* collection. This loss could be the result of misrepresentation of the wild populations, due to collection of a small number of individuals and/or collections not covering the whole area of population occupancy, or they could represent an early response to forest reduction and fragmentation.

Bahamas populations

High pollen flow throughout the whole region was demonstrated by the population graph (Figure 3.4) and low φ_{PT} values (Table 3.6). Most of the Bahamas islands (Grand Bahama, New Providence, northern Andros, South Andros and southern Abaco) also showed high gene flow and low genetic distance for both nuclear and plastid regions. Population graphs show a tight topology for these populations, indicating genetic dependence and gene flow (Figure 3.4). Bayesian analysis also showed a clustering of these populations (Figure 3.7). All these islands are <300 km from each other. As discussed before, seed dispersal is mostly short-distance, but seeds and cones can be occasionally dispersed further by hurricanes and humans. Pollen can usually travel longer distances. Slatkin (1985) stated that low genetic differentiation could be derived from frequent short-distance dispersal and occasional long-distance dispersal.

The close genetic distance between Mangrove Cay and northern Abaco was demonstrated by non-significant pairwise genetic distances (P=0.11) (Tables 3.6 and 3.7) and Bayesian analysis (Figure 3.7). This could have been a case of occasional long-distance dispersal and a single colonization event. Mangrove Cay was genetically distant from all other islands based on nuclear regions (significant P values for pairwise genetic distance and F_{ST}) (Table 3.8). Small sample sizes, like the one for Mangrove Cay, can increase sampling error and affect estimates of F_{ST} (Nei and Chesser 1983). However, errors in unbiased genetic distance $\hat{G}_{ST}$ tend to be small, even for small sample sizes, when distance is large (Nei 1978). In this case, the large genetic distances observed are probably not an artefact of error due to small sampling.

Mangrove Cay was the last island to be logged in the Bahamas for pulpwood in the early 1970s (Henry 1974), but there are no records in the literature for intensity of logging, how many standing trees were left per site, and if any re-seeding took place. All trees observed and sampled during this research originated after logging, as mentioned in Chapter 2 section 2.4. Taking into account that inbreeding was low and allelic richness high (Table 3.5), one could assume that new gene combinations were introduced through gene flow. Slatkin (1987) suggested that gene flow through re-colonization or founding of new populations can lead to new allelic combination and speciation. An example of single or few founder events leading to genetic similarities between distant populations of *Phylica arborea* Thouars (Rhamnaceae) from Gough Island and New Amsterdam was reported by Richardson *et al*. (2003). A single colonization event from northern Abaco to Mangrove Cay could have occurred in the Bahamas, explaining the similarities between these two populations.

Northern Abaco showed significant genetic distance $\hat{G}_{ST}$ and F_{ST} from all other islands (Table 3.8), except Mangrove Cay as explained above. Genetic flux (Nm) with other populations was also low (Table 3.7). It also had the highest unbiased gene diversity, allelic richness and number of private alleles (Table 3.5). Northern Abaco and South Andros are the only islands in the Bahamas which still have areas of old-growth forest. These remaining areas of old-growth forest could account for some of the rare and private alleles and genetic diversity. However, unbiased genetic diversity was lower and near average in South Andros (Table 3.5). Maybe location, size of population and lower fragmentation could also have influenced genetic diversity in northern Abaco. This population is located in the most northeastern part of the archipelago, it covers a larger area and the forest area is not as fragmented as in South Andros (Table 3.1 and Figure 3.1). Considering that the predominant wind direction is easterly and southeasterly during seed dispersal, it is possible that gene flow is mainly among populations on the same island of Abaco instead of other populations located to the west and south-west. A positive, but non-significant, inbreeding coefficient F_{IS} (Table 3.5) could be a result of reduced gene flow and genetic drift. Intensive logging carried out from 1905 to 1967 (Chapter 1 Table 1.3) reduced the number of mature individuals temporarily, possibly reducing gene flow and increasing genetic drift. Another factor to be considered is the relative proximity of this island to North America. High gene diversity could be explained by a higher number of founders and higher initial genetic variability through an ancient pine migration route from North America to the Caribbean, as suggested before.

Bayesian analysis showed significant genetic difference between northern and southern Abaco, which were grouped in different clusters (Figure 3.7). The same was demonstrated through significant pairwise genetic distance $\hat{G}_{ST}$ and differences in F_{ST} (Table 3.8). Southern Abaco also had lower unbiased gene diversity, lower allelic richness and a lower number of private alleles (Table 3.5), in addition to a lower number of unique genotypes and haplotypes (Tables 3.5 and 3.10). It was, however, genetically similar to Grand Bahama, New Providence, northern Andros and South Andros.

Almost all area of the forest in southern Abaco was exploited from 1930 to 1944 and then from 1959 to 1967 (Chapter 1 Table 1.3), and no old-growth forest remains (Henry 1974, Myers, R. *et al.* 2004). Regeneration was good for northern Abaco, which was exploited during the same period, but not for southern Abaco, as described in Chapter 2 section 2.4 and shown in Figure 2.17. Most trees were killed by storm surge, pests or fires in that area (Henry 1974, Radabaugh 1974), seed crop was poor and existing cones were attacked by insects (Greaves 1978). A small re-seeding experiment was carried out by the Department of Lands and Survey in 1971 south of Crossing Rock, but size and outcomes of the experiment are unknown (Henry 1974). Seed provenance was probably from northern Andros, where logging and seed collecting were ongoing at the time (Greaves 1978). If this was the case, then it would explain the observed low genetic diversity and similarities to northern Andros and genetic distance to northern Abaco. A reduction in population size due to logging and posterior introduction of new individuals through re-seeding could have caused alterations in allele frequencies, reduction in number of rare and private alleles and introduction of new alleles. This could also explain why southern Abaco was also the only population in the Bahamas showing negative F_{IS} and outcrossing preference (Table 3.5). In this case, logging followed by stochastic events had a detrimental effect on regeneration and contributed to loss of genetic diversity in the population.

Andros has also shown some interesting results. Northern Andros and South Andros showed little and no significant genetic difference for plastid and nuclear regions, respectively (Table 3.8); however, high genetic distance to Mangrove Cay, which is situated between the two populations, was observed (Table 3.6 and Figure 3.1). South Andros had higher allelic richness and higher gene diversity than northern Andros, but no private alleles were detected. These results are in agreement with the higher genetic diversity observed in populations with old-growth forest (northern Abaco). The inbreeding coefficient was positive and higher than average (Table 3.5). Fragmentation of populations and reduced habitat could have played a role in decreasing heterozygosity and increasing inbreeding.

The pine population in South Andros is fragmented into small blocks (Figure 3.1) and its expansion have been restricted by wetlands since ancient times, as discussed in Chapter 2 section 2.4. Increased salinity of the fresh water lenses, competition and low rainfall could have affected survival rates and prevented expansion of pine forests on this island. Lack of private alleles could have been an artefact of sampling error, due to reduced sampling area; however, similar sampling area in Mangrove Cay did detect a private allele. Loss of rare alleles ($A_{0.95}$) was also observed (Table 3.5), suggesting genetic drift and natural selection in these small populations that are surviving in limited suitable habitat.

New Providence had the lowest gene diversity of all islands; allelic richness was slightly lower than average, with highest number of shared genotypes and significant inbreeding (Table 3.5). These findings indicate a loss of genetic diversity. New

Providence has the smallest forest area in the Bahamas region (Table 3.1) and no primary forest. It has also lost circa 63.6% of its pine forest in the last century due to development and exploitation for charcoal and wood (Chapter 2, section 2.3.2.1, d and Figure 3.1). A small population size and further exploitation and fragmentation could have led to a loss of genetic diversity through increased inbreeding, homozygous excess and genetic drift, as observed in other studies (Frankham 1996, Kramer *et al.* 2008). It has also been observed that it was the population with higher number of pairs of loci in linkage disequilibrium in the Bahamas region (Table 3.5). Small population size, inbreeding and genetic drift increase homozygosity and reduce recombination between different alleles, increasing linkage disequilibrium (Flint-García *et al.* 2003). New Providence did, however, have a private allele, a unique haplotype and an above-average genetic diversity for plastid microsatellites (Tables 3.5 and 3.10). This new unique haplotype could have been the result of introduced new alleles through pollen flow or mutation of an existing allele. Population graphs, both for plastid and nuclear regions have shown high gene flux between New Providence and other islands (Figure 3.4), which could have added to the genetic diversity through migration and pollen flow.

Grand Bahama still has private alleles, high gene diversity and allelic richness, despite forest reduction of 24.6% in the past century (Chapter 2 section 2.3.2.1, c). The population area is large (Table 3.1) and regeneration was good and fast after logging, as discussed in Chapter 2 section 2.4. Despite large forest loss in the western and northern parts of the island resulting from the storm surge after hurricane Wilma in 2005 (Chapter 2 section 2.4, Figures 2.29 and 2.32), a large area of forest and a high number of mature individuals still remain (Figure 3.1). Successful recruitment from the soil seed bank and high gene flow with neighbouring islands helped to maintain genetic diversity. It is also possible that founder genetic diversity was high due to a pine migration route from North America, as this is the closest island to Florida.

Main conclusions

- Do plastid and nuclear regions have similar levels of variability and dispersal patterns? No, plastid regions were more conserved with a lower number of alleles and fewer mutations than nuclear regions. However, the combination of nuclear and plastid microsatellite loci made possible the inference of pollen flow and seed dispersal. This proved useful, as these two parameters had different patterns of differentiation. Short- (seed) and long- (pollen) distance dispersal mechanisms were revealed, respectively, by nuclear and plastid regions, with the highest population structure related to seed dispersal. The markers chosen, apart from the monomorphic marker Pt36480, were informative and revealed genetic structure of the populations.

- Are loci in linkage equilibrium? No, many populations showed linkage disequilibrium between pairs of loci. The highest number of linked pairs of loci occurred in the TCI populations, suggesting a more recent colonisation and higher genetic drift in this region.

- What is the population structure for this variety? Higher genetic variability was found within populations than among populations or regions for both microsatellite types, showing a high individual genetic variation in agreement with other studies on pines. IBD analysis did show that most populations within regions (<300 km) were also genetically closer. Genetic flux was higher and genetic distance and population structure lower in plastid regions (pollen flow) than in nuclear regions (seed dispersal).

- Is mating system random and outcrossing in all populations? Most populations showed a random and outcrossing mating system, with the exception of New Providence and Pine Cay, which showed signs of inbreeding.

- Are pines in the Bahamas and the TCI genetically different? Significant genetic differences between these regions and isolation by distance were observed for nuclear loci. However, no clear pattern was identified for plastid regions, where genetic differentiation was low. Data indicate high historical gene flow in the past and a recent population expansion followed by isolation, with populations from both regions being part of the same evolutionary unit. The presence of an ancestral haplotype present in all populations and linkage disequilibrium between loci indicate a recent colonisation. Higher allelic richness in most of the Bahamas populations in comparison to TCI populations and lower linkage disequilibrium suggest that the Bahamas could have been the founder population in the archipelago. Low levels of migration between the regions, genetic drift and natural selection could lead eventually to speciation.

- Are populations genetically distinct from each other? Pine Cay was the only population genetically different from all the rest, including the TCI populations, probably due to small population size, high inbreeding, high genetic drift and natural selection. Mangrove Cay and northern Abaco were distinct from all other populations in the Bahamas region regarding seed dispersal (nuclear loci). The geographical location of northern Abaco in the extreme northeast part of the archipelago associated with remaining areas of old-growth forest could have favoured genetic drift and differentiation. A possible single colonization event from northern Abaco to Mangrove Cay could explain their genetic similarity and high genetic distance to other populations.

- Have historical fragmentation and reduction in population size affected genetic diversity? It seems that the effects of population size and fragmentation varied according to regeneration potential, habitat, initial population size and genetic diversity.

- Is the *ex-situ* collection representative of wild populations? The *ex-situ* collections showed lower genetic diversity than wild populations, failing to capture many of the private and common alleles present in the region. Higher homozygosity and inbreeding were also observed for this collection. These results could indicate that collections did not cover the whole area of forest or an early response to fragmentation and high mortality of mature trees due to pest attack. Further collections of individuals from all populations are advised for a good representation of the total gene pool.

Chapter 4 . Morphology and Ecology

4.1. Introduction

The analysis of morphological characters of a species can reveal changes in plant form and structure through time (Sattler and Rutishauser 1997). These changes can result in different phenotypes. Phenotypes can be created by genotypic variation or phenotypic plasticity of a genotype in response to gene expression and/or environmental conditions (Jong 2005), and individual variation can be linked to epigenetic processes, natural selection and stochastic events. Phenotypic variation can occur at species, population or individual levels and is not only expressed through changes in morphology, but also through physiological or biochemical changes (Silvertown and Charlesworth 2001, Ingrouille and Eddie 2006). Kemp (1973) observed high individual phenotypic variation in populations in natural stands of *P. caribaea* var. *hondurensis* in response to ecological and environmental pressures, e.g. competition, density, fires and rainfall. Desert and montane populations of *P. ponderosa* Douglas ex C.Lawson showed phenotypic plasticity in physiological traits, i.e. hydraulic conductance and biomass allocation, but low genetic differentiation (φ_{PT}) between populations and lack of geographic and genetic correlation (Maherali *et al.* 2002). *Pinus pinea* L. growing in different habitats has also shown high phenotypic plasticity and low genetic differentiation (Gordo *et al.* 2007, Vendramin *et al.* 2008). On the other hand, *P. hartwegii* Lindl. populations from low and high elevations showed genetic differentiation and phenotypic variation for height, phenology and resistance to frost (Viveros-Viveros *et al.* 2009). Studies of *P. pinaster* in Morocco demonstrated genetic, morphological and anatomical variation following a latitude and altitude cline (Wahid *et al.* 2006, Wahid *et al.* 2010).

Morphological characters are always used in species descriptions in taxonomy. The subdivision of *P. caribaea* in three varieties is based on the geographical distribution and morphological variation, i.e. height, diameter, number of needles per fascicle, sheath size at maturity, colour and leaf disposition of primary leaves in seedlings (Chapter 1, Table 1.1). Therefore, information on morphological variation at the individual, population and regional levels in association with the study of the genetic variation and ecological parameters can provide an insight into adaptation and speciation.

The effect of environmental conditions or genetic differences in adaptation and morphological changes can be better explained in experimental situations with controlled variables (Schlichting 1986). In natural populations, biotic and abiotic factors are present simultaneously, usually varying in a non-linear and interactive way, and linking cause to effect is not always straight forward (Valladares *et al.* 2007). Progeny and provenance trials are useful tools in the comparison of phenotypic and genotypic variation in different lineages and families and also the influence of environmental conditions in the expression of characters. A direct comparison of different phenotypes and genotypes can be carried out with known environmental parameters by planting trees of diverse origins in the same location. Although this approach was not feasible in the present research some insight can be obtained from the results of provenance and progeny studies of *P. caribaea* carried out in the past.

Results from trials of all three varieties of *P. caribaea* planted in experimental research plots in Zimbabwe (Rhodesia), in the late 1960s showed that *P. caribaea* var. *hondurensis* is a more vigorous variety, which can achieve larger diameter than varieties *caribaea* and *bahamensis* (Barnes *et al.* 1977). It also showed that most morphological variation was related to individual genetic variation and response to environmental conditions. Similar trials with *P. caribaea* var. *hondurensis* in Costa Rica, Puerto Rico, Venezuela, Jamaica and Trinidad in the 1980s identified differences in growth associated to soil type, elevation and climate in different localities (Liegel 1991). Moura and Dvorak (2001) reported trials with *P. caribaea* var. *hondurensis* in Planaltina, Brazil. Trees were sourced from provenances in Honduras and Guatemala which had different elevation and mean annual rainfall. Genetic inheritance of growth traits, mainly height, girth and volume, was higher at family level than individual or provenance levels. The comparison of results from this site with plantations of the same provenance sources in Venezuela and Colombia demonstrated an interaction between genetic and environmental factors in phenotypic plasticity. Low survival rate and volume growth seemed to be related to poor soils (sandy) and pronounced drought. In Mato Grosso do Sul, Brazil a study on the same variety showed a correlation between environmental parameters and phenotypic and genotypic variation (Tambarussi *et al.* 2010). Sebbenn *et al.* (2008) observed differences in growth (height and girth) between and within provenances from different islands in *P. caribaea* var. *bahamensis* plantations in São Paulo, Brazil.

These studies show different levels of phenotypic plasticity in *P. caribaea* and an association of morphological characters to genotypic variation and environmental conditions.

Previous studies have focused on some morphological and ecological aspects of natural forests of *P. caribaea* var. *bahamensis* in specific areas of the Bahamas and the TCI (Henry 1974, Myers, R. *et al.* 2004, Miller, A. C. 2007, Earle-Mundil 2010, Lloyd and Slater 2010, Green 2011), and a more comprehensive and standardised comparison across the whole archipelago and including all populations has yet to be carried out. A study of morphological and ecological parameters, in addition to species distribution and genetics, can reveal the potential of the species to respond to stochastic events and natural selection. Such a comparison is important for species management and planning future conservation efforts.

This chapter will focus on the comparison of some morphological and ecological parameters of *P. caribaea* var. *bahamensis* at species, regional and inter-population levels encompassing all known populations for this variety and its whole range of distribution.

4.2. Material and Methods

Collection of data was carried out during fieldwork in 2008-2010 in the Bahamas and in 2008-2009 in the TCI. Populations sampled and sampling strategy were the same as those adopted for the genetic data, as described in Chapter 3 section 3.2.1. Morphological and ecological data collected for each sampling quadrat of 10×10 m (100 m^2) are described in Table 4.1. Data was recorded using handheld computers with built-in GPS running ArcPad™ v.7 ($^{©}$2002-2006 ESRI Inc.) and then transferred to a database in Brahms software, as explained in Chapter 2 section 2.2.1.3. All quadrats were only recorded once, as no parameters required repeated measurements. The National Vegetation Classification standards (Rodwell 2006) recommends a quadrat size of 10×10m for the observation of the ground layer in tall woodland habitats and Hill *et al*. (2005) suggested an optimal quadrat size of 100 m^2 for frequency estimates in woodlands. The same quadrat size was used by García Quintana (2006) on his research for the collection of morphological data on *P. caribaea* var. *caribaea* in Pinar del Río, Cuba. The size of quadrat chosen in the present research has also taken into consideration the temporary nature of recording, forest density, size of mature trees, terrain and time constraints. Geographical references, e.g. island, village, road, and coordinates (latitude and longitude) for each quadrat were also recorded.

4.2.1. Morphological and ecological parameters

Several morphological parameters were recorded for the trees sampled for DNA (Table 4.1). Many of these are commonly used in the detection of morphological variation between and within species, in the estimation of growth rate and evaluation of provenance characters (Little Jr. and Dorman 1954, Farjon and Styles 1997, Chen *et al.* 2004, Sebbenn *et al.* 2008, Shayanmehr *et al.* 2009, Nicotra *et al.* 2010). A random sample of fascicles from older branches was used to measure the number of needles per fascicle, size of the shortest and longest needles in a fascicle and minimum and maximum size of the sheath. The number of needles per fascicles was recorded for each tree and divided in three classes: two needles only, mixed fascicles with two or three needles in the same tree and three needles only. Cone size and width of open cones, in addition to presence of strobili were recorded for each quadrat when present. Differences in size, shape and colour of the cones allowed visual distinction between stages of cone maturity. Cones were classified into two categories, old and new; old being brown cones already opened and new being first or second year cones before dispersal (Figure 4.1).

Height and dbh (diameter at breast height) were measured from the tree sampled for DNA and also from the tallest tree in the quadrat. Dbh was measured at 1.3 m from the ground using a dbh tape. Tree height could be estimated using a Suunto PM-5/360 PC clinometer and the following formula: % horizontal distance/ (100*20) + observer's height at eye level; where % of the horizontal distance was equivalent to slope angle in degrees between the observer and the tree top at a distance of 20 m from the tree. Measurements and observations were always taken by the same observer using the same instrument to minimize errors of interpretation. Dbh and height values were rounded to the nearest decimal value. The slenderness coefficient or slenderness ratio was also calculated by dividing the height of the tallest tree in the quadrat by its own dbh, both in the same scale, in this case meters (Dahle and Graboski 2009).

Ecological parameters were recorded for each quadrat and included pine tree density (number of mature individuals per quadrat), presence of seedlings (recruitment), signs of past or recent fire, percentage of ground cover, threats (observed or inferred) and presence of invasive species (Table 4.1). Seedlings with a height <30 cm were recorded as recruitment. Mature individuals were those with a height ≥2 m, as reproductive structures were observed in trees of this height during the research. Differentiation between recent and past fire damage could be assessed through observation of plant growth in the understory and level of charring in the vegetation. The parameter percentage of ground cover was divided into four classes (0-24%, 25-49%, 50-74% and 75-100%) and estimated through visual assessment of the proportion of vegetation and bare ground in each quadrat. Imminent threats to the pine forest in the location of the quadrat and adjacent areas and presence of invasive plant species were also recorded.

Parameters recorded	Description of parameters
unique id	Unique number given to each sampled tree
major area	Island name where sample was collected
gazetteer	Location name for quadrat, e.g. road name, area name, village
locality notes	Description of locality and reference points for quadrat
latitude	Latitude measured with a GPS
longitude	Longitude measured with a GPS
date	Date of collection
plant description	Description of tree sampled, e.g. tree shape and health
flower	Presence or absence of strobili
fruit	Presence or absence of cones
height tallest tree	Height of tallest tree in meters measured with a clinometer
dbh tallest tree	Diameter at breast height (dbh) in meters of the tallest tree
height of sampled tree	Height of tree sampled for DNA in meters measured with a clinometer
dbh of sampled tree	Diameter at breast height (dbh) in meters of tree sampled for DNA
number of needles	Number of needles per fascicle
min. length needle	Minimum length of needle from a random bunch of fascicles from end of sheath to end of needle in centimetres
max. length needle	Maximum length of needle from a random bunch of fascicles from end of sheath to end of needle in centimetres
min. length sheath	Minimum length of sheath from a random bunch of fascicles in centimetres
max. length sheath	Maximum length of sheath from a random bunch of fascicles in centimetres
length of cone	Length of cone in centimetres
width of cone	Width of cone in centimetres
cone maturity	Stage of cone maturity, i.e. first year, second year or old cones
tree density	Number of pine trees > 2m in height per quadrat
recruitment	Presence or absence of seedlings
past fire damage	Presence or absence of signs of past fire damage
recent fire damage	Presence or absence of signs of recent fire damage
notes	General notes on the site and threats
ground cover	Estimated percentage of the ground cover
threat	Main threat to the continuing existence of forest in the area, e.g. urban development, logging, pests and roads
invasives	Description of invasive species, if present
photo id	Unique number given to photograph of sampled tree
Parameters below only recorded in the TCI	
scale presence	Presence or absence of pine tortoise scale insect (*Toumeyella parvicornis*)
infestation level	Level of infestation in a scale of 0 to 5, with 0 being no visible scale insect and 5 being totally infested
canopy damage	Level of damage to pine trees in a scale from 0 to 5, with 0 being no visible damage and 5 being tree mortality
sooty mould	Estimated percentage of the total leaf area covered by sooty mould, including canopy and undergrowth

Table 4.1. Morphological and ecological parameters recorded in the field for the tree sampled for DNA, tallest tree in the quadrat or quadrat area. Parameters regarding infestation by the pine tortoise scale insect were recorded in the TCI only, as this insect was absent in the Bahamas.

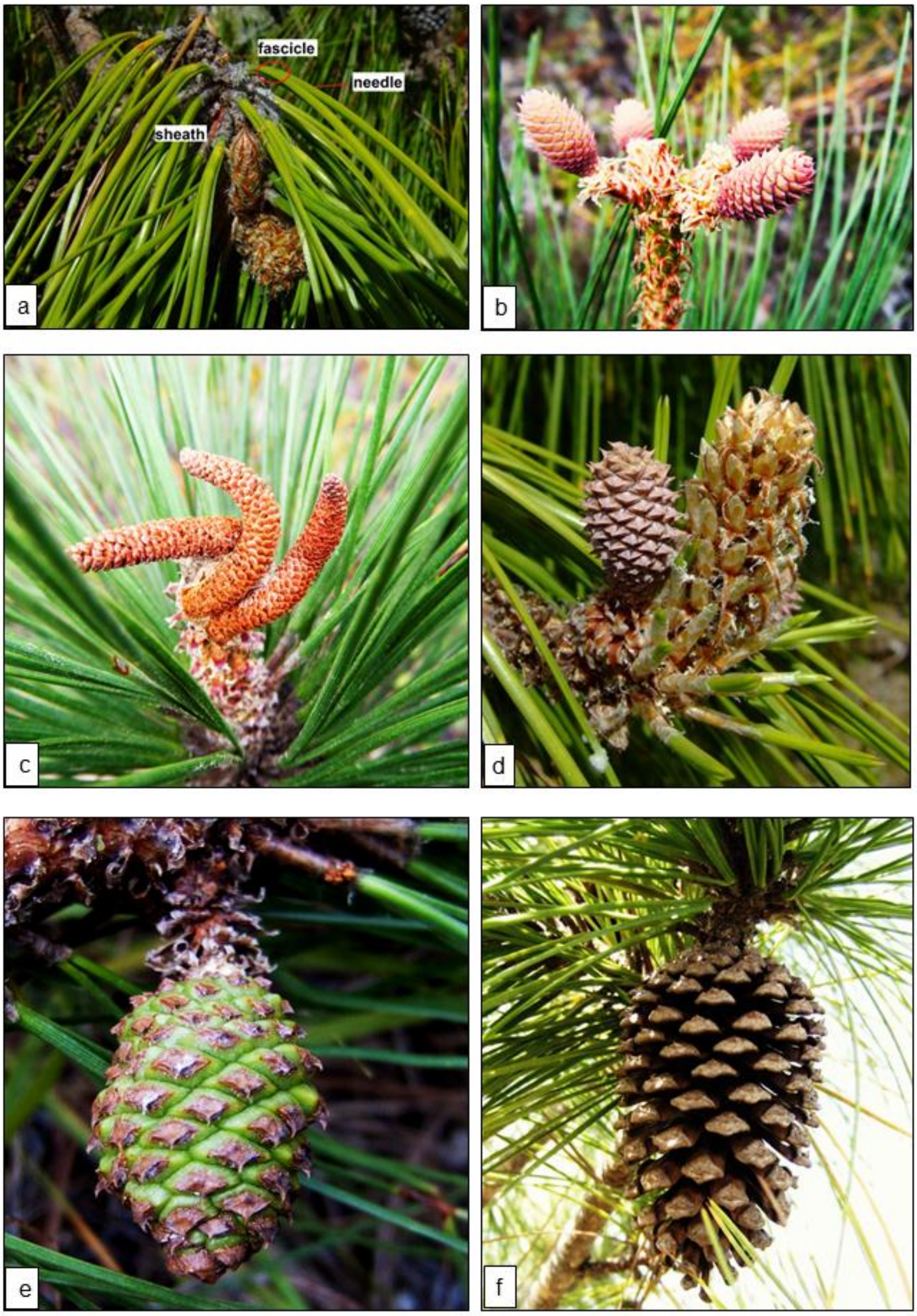


Figure 4.1. Branch features and reproductive structures in *P. caribaea* var. *bahamensis.* (a) branch showing needles, sheath and fascicle, (b) female strobili (c) male strobili (d) first year cone, (e) second year cone, and (f) old cone.

4.2.2. Statistical Analysis

Data was analysed with SPSS Statistics Standard v. 18.3 statistical package (©1993-2007 Polar Engineering and Consulting). Levene's Test of Equality of Error Variances was applied to check equality of variances for the variables and choose tests to be performed. Independent samples T-test or One-Way ANOVA was used to test the null hypothesis of no differences between regions for scale variables with homogeneity of variance. The Mann-Whitney U test (U) or Kruskal-Wallis One-Way ANOVA were used for scale variables without homogeneity of variance and ordinal variables. Univariate Analysis of Variance was used to test the null hypothesis of no difference across populations. When significant difference was observed, *post hoc* tests were applied to identify these differences. Student-Newman-Keuls or Fisher's Least Significant Difference tests were performed for variables with homogeneity of variance and Tamhane T2 test was used for variables with unequal variance. Spearman's correlation was used for height and dbh variables and also percentage of ground cover and fire presence. Variation of morphological characters at hierarchical levels, i.e. regions, populations within regions and trees within populations, was tested through nested ANOVA using Type III Sum of Squares in SPSS with a significance level of 0.05. Links between morphological, geographical and genetic data were tested through Pearson's product-moment correlations. Morphological distance was based on Euclidean distances between pairs of populations for ten morphological parameters (maximum height and dbh, mean height and dbh of tallest tree, minimum and maximum sheath length, minimum and maximum needle length, cone length and diameter) standardised by Z-scores. Genetic distance corresponded to Nei unbiased genetic distance between pairs of populations ($\hat{G}_{ST}$). Geographical distance was equivalent to mean distance between populations in km, as calculated for isolation by distance model (Figure 3.6).

4.3. Results

Results were analysed at three hierarchical levels: species (*P. caribaea* var. *bahamensis*), regional (Bahamas and TCI) and inter-population. At the inter-population level the individuals were grouped in ten populations, following the same structure used for the genetic analysis (Chapter 3, section 3.2.1), in order to facilitate comparison and allow correlations between genetic and morphological data.

4.3.1. Taxon (*P. caribaea* var. *bahamensis*)

Maximum tree height and dbh observed for the taxon were 23.7 m and 34.3 cm, respectively. However, most trees in the quadrats studied had lower height and dbh, with an average of 10.1 to 11.1 m in height and 12.4 to 13.8 cm in dbh (95% C.I.) (Table 4.2). Slenderness coefficient varied from 35.9 to 181.7 (95% C.I.) with greater variance between individuals within populations within regions (Table 4.3). A positive and strong correlation between height and dbh of the tallest tree per quadrat was observed (Spearman's rho= 0.77, $P<0.01$), as shown in Figure 4.3. Number of needles per fascicle varied from two to three (Figure 4.5) and needle size varied from 7.5 to 30.0 cm (Table 4.2). Sheath length and cone size had the lowest variance of all parameters (Table 4.3). However, a small number of cones were sampled during fieldwork, which could have led to a failure to detect variation. Average cone length and cone width varied, respectively, from 6.2 to 7.0 cm and 4.2 to 4.8 cm (95% C.I.) Sheath length was quite uniform across samples, with an average of 0.8 to 0.9 cm. Variance for all morphological characters was higher at the individual than at regional or inter-population levels (Table 4.3).

Percentage of ground cover varied among quadrats, with the majority of them having <50% ground cover. Only 7.3% of the quadrats had > 75% ground cover (Table 4.4). As expected, a significant negative correlation between percentage of ground cover and evidence of fire was observed (Spearman's rho -0.13, $P=0.04$). Evidence of fire (past and recent) was observed in 52% of the quadrats; however, only 5% of the quadrats had signs of recent fires. A great majority of the quadrats with evidence of recent fires (75%) had < 25% ground cover. Most common tree density observed was <six mature trees/100 m^2.

Most quadrats (57%) had presence of seedlings and circa 80% of all quadrats observed had no cones or old cones only (Table 4.4). Strobili were present in only 14% of the quadrats.

Parameter	Location	N	Mean (95% C.I.)	Min - Max	*P*-value (test)
Height tallest tree per quadrat (m)	Bahamas	214	11.1 (+- 0.6)	2.7 - 23.7	*P* <0.01 ** (U)
	TCI	53	8.9 (+- 0.7)	3.5 - 15.7	
	Archipelago	**267**	**10.6 (+- 0.5)**	**2.7 - 23.7**	
dbh tallest tree per quadrat (cm)	Bahamas	210	13.5 (+- 0.8)	3.6 - 34.3	*P* =0.02 * (U)
	TCI	55	11.3 (+- 1.3)	2.0 - 26.5	
	Archipelago	**265**	**13.1 (+- 0.7)**	**2.0 - 34.3**	
Slenderness coefficient (height/dbh)	Bahamas	210	84.6 (+- 2.7)	39.4 - 136.3	
	TCI	53	84.0 (+- 8.0)	35.9- 181.7	*P* =0.27 (U)
	Archipelago	**263**	**84.5 (+- 2.7)**	**35.9 - 181.7**	
Minimum needle length (cm)	Bahamas	258	17.1 (+- 0.3)	11.0 - 25.0	*P* <0.01 ** (t)
	TCI	76	14.6 (+- 0.7)	7.5 - 21.0	
	Archipelago	**334**	**16.6 (+- 0.3)**	**7.5 - 25.0**	
Maximum needle length (cm)	Bahamas	257	21.0 (+- 0.3)	13.5 - 30.0	*P* <0.01 ** (U)
	TCI	76	17.3 (+- 0.8)	8.5 - 24.0	
	Archipelago	**333**	**20.2 (+- 0.4)**	**8.5 - 30.0**	
Minimum sheath length (cm)	Bahamas	258	0.8 (+- 0.0)	0.3 - 1.5	*P* =0.44 (t)
	TCI	76	0.8 (+- 0.0)	0.3 - 1.3	
	Archipelago	**334**	**0.8 (+- 0.0)**	**0.3 - 1.5**	
Maximum sheath length (cm)	Bahamas	257	1.0 (+- 0.0)	0.5 - 1.7	*P* =0.16 (t)
	TCI	76	0.9 (+- 0.0)	0.6 - 1.5	
	Archipelago	**333**	**0.9 (+- 0.0)**	**0.5 - 1.7**	
Length of mature cone (cm)	Bahamas	23	6.3 (+- 0.5)	4.5 - 8.5	*P* =0.14 (t)
	TCI	15	7.0 (+- 1.0)	4.5 - 12.0	
	Archipelago	**38**	**6.6 (+-0.4)**	**4.5 - 12.0**	
Width of mature cone (cm)	Bahamas	21	4.4 (+- 0.6)	2.5 - 8.0	*P* =0.43 (t)
	TCI	15	4.7 (+-0.5)	3.5 - 7.0	
	Archipelago	**36**	**4.5 (+- 0.3)**	**2.5 - 8.0**	

Table 4.2. Comparative table of morphological characters in *P. caribaea* var. *bahamensis*. N- number of individuals sampled, Min- smallest observed value and Max-largest observed value. Mean value for each parameter was calculated per region and archipelago. The null hypothesis of no differences in the mean value between regions was tested using T test for independent samples (t) or Mann-Whitney U test (U) in SPSS. Significant differences are indicated by * *P* <0.05 or ** *P* <0.01.

Parameter	Source of variation	*SS*	d*f*	MS	F	Sig.
Height tallest tree per quadrat	Regions	237.99	1	237.99	15.49	<0.01
	Populations within regions	594.07	8	74.26	4.83	<0.01
	Trees in populations	3949.34	257	15.37		
dbh tallest tree per quadrat	Regions	360.26	1	360.26	11.91	<0.01
	Populations within regions	1036.20	8	129.52	4.28	<0.01
	Trees in populations	7710.19	255	30.24		
Slenderness coefficient	Regions	14.51	1	14.51	0.03	0.86
	Populations within regions	2249.84	2	1124.92	2.32	0.10
	Trees in populations	125475.67	259	484.46		
Minimum needle length	Regions	409.92	1	409.92	54.35	<0.01
	Populations within regions	177.52	8	22.19	2.94	<0.01
	Trees in populations	2428.41	322	7.54		
Maximum needle length	Regions	795.77	1	795.77	96.81	<0.01
	Populations within regions	162.52	8	20.31	2.47	0.01
	Trees in populations	2654.97	323	8.22		
Minimum sheath length	Regions	0.03	1	0.03	0.69	0.41
	Populations within regions	0.83	8	0.10	2.80	<0.01
	Trees in populations	12.05	324	0.04		
Maximum sheath length	Regions	0.10	1	0.10	2.78	0.10
	Populations within regions	0.78	8	0.10	2.81	<0.01
	Trees in populations	11.17	323	0.03		
Cone shape	Regions	0.033	1	0.033	0.309	0.589
	Populations within regions	0.901	7	0.129	1.961	0.098
	Trees in populations	1.773	27	0.066		

Table 4.3. Comparison of variation in morphological characters at regional, inter-population and individual levels. Parameter cone shape corresponds to mature cone length/cone width. SS- Type III Sum of squares, d*f*- degrees of freedom, MS- Mean square (=SS/d*f*), F- ratio of variation within to between regions or populations, Sig.- *P*-value at 95% confidence level. Nested ANOVA analysis performed in SPSS. Great variation was observed at regional and inter-population levels for vegetative characters, such as height, dbh and needle length, while reproductive character, i.e. cone shape, was not so variable across the archipelago.

Parameter	Nq	Classes	Occurrence (%)
Number of mature trees per plot	315	0-5	59.4
		6-10	27.3
		11-15	9.5
		16-20	2.6
		21-25	0.6
		26-30	0.6
Strobili	270	Present	14.1
		Absent	85.9
Cones	375	Old only	51.8
		None	27.7
		New and old	14.4
		New only	6.1
Recruitment	377	Present	57.3
		Absent	42.7
Past fire	329	Present	48.3
		Absent	51.7
Recent fire	339	Present	5.0
		Absent	95.0
Ground cover	313	0-24 %	29.1
		25-49%	36.4
		50-74%	27.2
		75-100 %	7.3
Invasive Plants	314	None	95.2
		Dominating	2.9
		Encroaching	1.6
		Overtaking	0.3

Table 4.4. Ecological parameters for *P. caribaea* var. *bahamensis* in the Bahaman archipelago. Quadrat size was 100 m^2. Nq- number of quadrats and Occurrence (%)- percentage of quadrats observed for each category. The majority of the quadrats had low tree density (<10trees) and low ground cover (<50%).

Threats to pine forest survival inside the quadrat and adjacent areas were identified in circa 58% of all the quadrats studied. The major threat observed consisted of insect pests, followed by urban development. Removal of trees by logging and opening of new roads only accounted for <10% of the observed threats (Figure 4.2). Invasive plants were present in <5% of the quadrats and overtaking the native vegetation in only a few of them (Table 4.4). The main invasive species observed were *Casuarina equisetifolia* and *Leucaena leucocephala*.

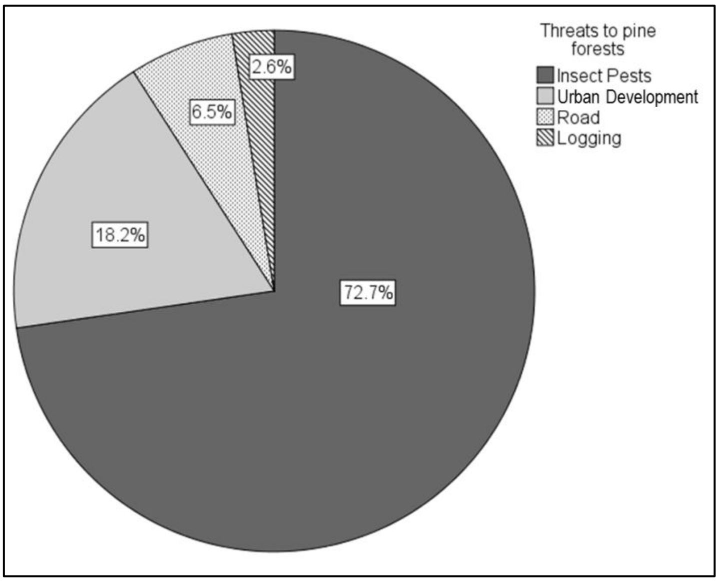


Figure 4.2. Main threats observed in the pine forests of the Bahaman archipelago. Threats recorded for 221 quadrats. Insect pests, mainly the pine tortoise scale in the TCI, are the major modern threat to these ecosystems, followed by urban development.

4.3.2. Regional

Sheath length, cone size and cone width were not significantly different between the two regions (Bahamas and TCI) and variance was low (Table 4.3). However, significant differences were observed for height and dbh of the tallest tree and needle size (Table 4.2). All these parameters showed significant variation between regions and between populations within regions (Table 4.3). Trees in the Bahamas were higher and had greater girth (dbh) than those in the TCI (Table 4.2, Figures 4.3 and 4.5), although differences in average height of the tallest tree per plot were more prominent than differences in the dbh. Slenderness coefficient was not significantly different between regions and showed low variation (Tables 4.2, 4.3 and Figure 4.5). Needles were also significantly longer in the pine trees of the Bahamas than the TCI ($P<0.01$) (Table 4.2 and Figure 4.6). Most individuals in each region (54%) had two or three needles per fascicle; however, the percentage of individuals with only two needles per fascicle was higher in the Bahamas (14%) than in the TCI (4%) (Figure 4.5). A negative correlation between needle length and infestation by insects was observed in the TCI (Spearman's rho= -0.40, $P<0.01$), where trees with signs of infestation or die back of branches had smaller needles than healthy trees (Figure 4.4). However, trees in the Bahamas did not show the same pattern.

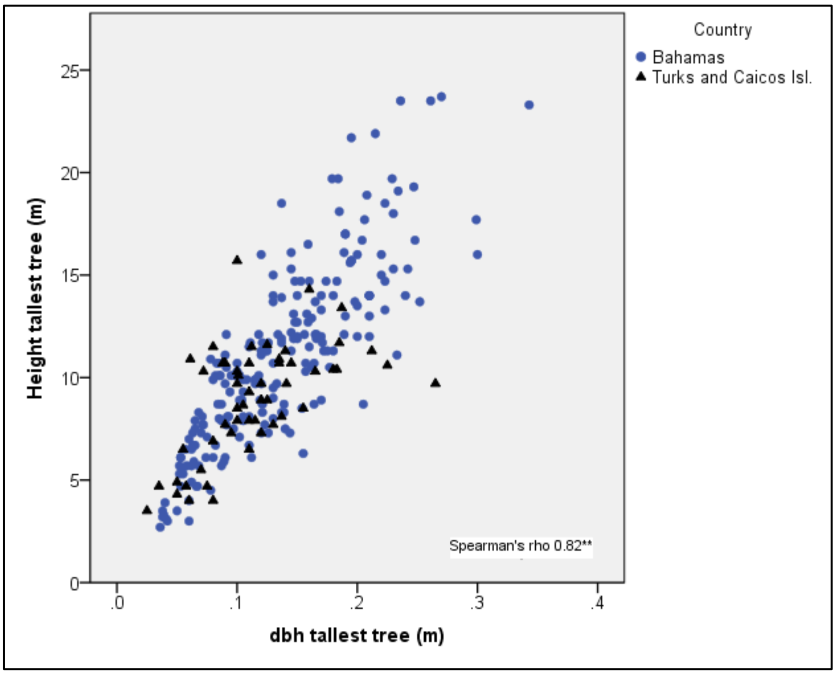


Figure 4.3. Correlation of height and dbh of the tallest tree per quadrat per region. A strong positive correlation was observed (Spearman's rho=0.82, ***P*<0.01). Number of individuals sampled was 263. Highest height and dbh were observed in the Bahamas.

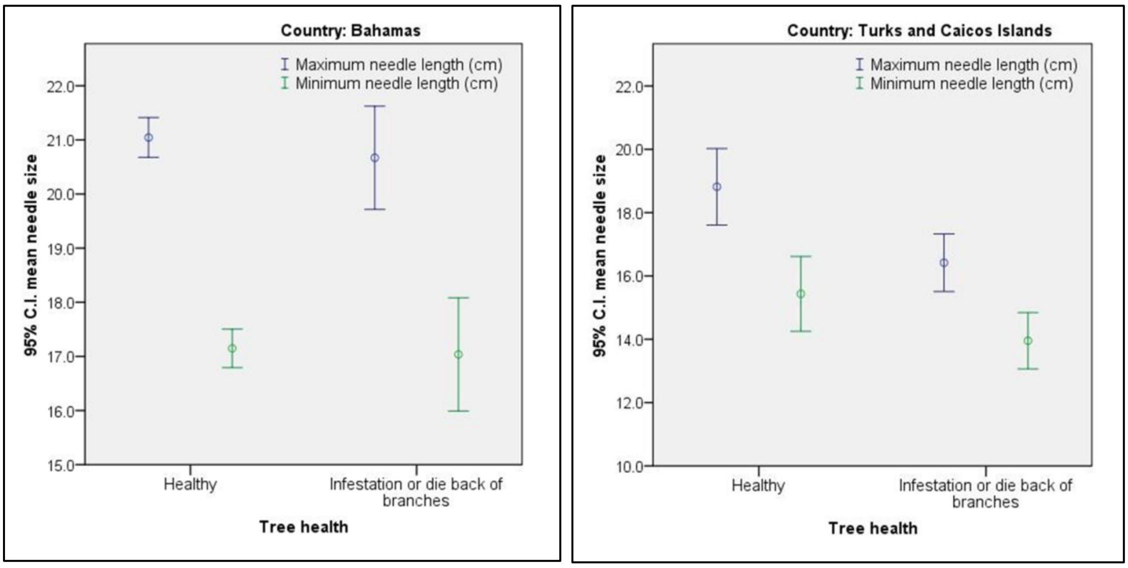


Figure 4.4. Comparison of needle size and tree health in the Bahamas and the TCI. Error bars show mean needle size (circle) and 95% confidence interval (C.I.) of the mean (whiskers). Number of individuals sampled was 256 in the Bahamas and 75 in the TCI. TCI trees show significantly larger needles in healthy trees (minimum needle size *P*<0.05 and maximum needle size *P*<0.01), while no differences were observed in the Bahamas (*P*>0.49).

The majority of the quadrats in the Bahamas and in the TCI had <75% ground cover and there were no significant differences between the distribution of these classes in the two regions (*P*=0.41). Evidence of fire was significantly higher (*P*<0.01) in the Bahamas than in the TCI and 66.2% and 28.8% of the observed quadrats had signs of past or recent fires, respectively (Figure 4.8). However, this difference was even more accentuated regarding recent fires, which account for 7.5% of the quadrats in the Bahamas and only 0.8% of the quadrats in the TCI.

There were no differences between regions (*P*>0.47) in number of quadrats with new seedlings (recruitment) and presence of strobili. However, TCI quadrats had significantly (*P*<0.01) lower percentage (6%) of new cones than the quadrats in the Bahamas (29%) (Figure 4.10). Pine tree density was also significantly different in the two regions (*P*<0.01). All studied areas in the TCI had a tree density <21 mature trees/100 m^2 and circa 72% of them had <six mature trees/100 m^2, whereas in the Bahamas categories were more spread out and 5% of the observed areas had between 16 to 30 mature trees/100 m^2 (Figure 4.9).

The only threat recorded for the TCI pine forests was insect pests, whereas threats in the Bahamas were variable. Threats were identified for circa 35% of all Bahamas quadrats: 44.1% due to urban development, 35.3% new roads, 10.8 % logging and 9.8% insect pests.

Presence of insect pests was remarkably higher in the TCI (92%) than in the Bahamas (10%). Pine tortoise scale insect *Toumeyella parvicornis* was the most common and widespread insect pest in the TCI, with only a few other genera observed on Middle Caicos, i.e. *Ceroplastes cirripediformis* Comstock (barnacle scale) and *Coccus hesperidium* L. (brown soft scale) (Malumphy 2010b). None of the studied areas in the Bahamas had pine tortoise scale insect, but a few individuals of *Toumeyella* sp. probably from another species were observed and collected in northern Andros and New Providence. Other insect pests spotted in the Bahamas included *Aspidiotus sp.* (scale) in northern Andros and South Andros, *Chionaspis heterophyllae* Cooley (pine needle scale) in northern Abaco and on Grand Bahama and three mites, *Trisetacus ehmannii* Keifer in northern Andros, *Proiectus* sp. on New Providence and *Setoptus* sp. in northern Andros (Malumphy 2010a).

Damage to the pine forests of the TCI by the pine tortoise scale insect was also recorded during fieldwork and is shown in Table 4.5. Tree mortality was observed in 40.5% of the quadrats (damage to the canopy class 5) and severe loss of needles and die back of branches in another 35.9% of the quadrats (class 4). Infestation level varied among the quadrats, with most of them showing low infestation (0 to 2). The same was observed for percentage of vegetation covered by sooty mould, including canopy and undergrowth.

Parameter	Location	Occurrence (%)
Insect pests	Present	90.5
	Absent	9.5
Level of infestation	0	9.5
	1	38.7
	2	12.8
	3	19.5
	4	19.5
	5	0.0
Damage to canopy	0	0.0
	1	3.8
	2	9.1
	3	10.7
	4	35.9
	5	40.5
Sooty mould	0%	38.3
	5%	45.1
	10%	6.8
	15%	6.8
	20%	0.7
	40%	1.5
	50%	0.8

Table 4.5. Level of infestation and damage caused by pine tortoise scale insect on the pine forests of the TCI. Number of quadrats observed was 126. Level of infestation was from 0 to 5, where 0 means no visible scale insect and 5 all trees infested. Damage to canopy scale varied from 0 corresponding to no visible damage to trees and 5 to tree mortality. Sooty mould coverage on foliage was measured as a percentage of the total leaf area covered by mould including all vegetation in the plot area. The scale insect is still present in the great majority of quadrats observed, causing high damage to the canopy and the undergrowth.

4.3.3. Inter-population

No significant patterns of variation in cone shape were observed (Table 4.3). However, number of samples per population was very small when compared to other parameters and could have failed to detect variation. Height and dbh of the tallest tree per quadrat and needle length showed significant variation at regional and population levels. Southern Abaco had the highest mean dbh value (21.1 cm), although maximum tree height did not exceed 16 m (Figure 4.5). Height of the tallest tree varied considerably among populations within regions and between regions (Figure 4.5 and Table 4.3). Middle Caicos and Pine Cay had shorter trees than North Caicos in the TCI ($P<0.01$) and all other populations in the Bahamas, with the exception of Mangrove Cay. Mangrove Cay had significantly ($P<0.01$) shorter trees than the other two populations on Andros (northern Andros and South Andros) and southern Abaco. South Andros, northern Abaco and northern Andros had the tallest trees recorded, respectively 23.7 m, 21.9 m and 21.7 m. Dbh was similar for most populations ($P>0.14$), except for southern Abaco which had higher mean and median dbh than all the other populations (Figure 4.5). Maximum dbh was recorded in South Andros (34.3 cm), followed by southern Abaco (30.3 cm) and northern Abaco (29.9 cm). Slenderness coefficient did not show significant variation at regional or population levels (Table 4.3), apart from southern Abaco population which was quite distinct from most populations ($P<0.05$), had the lowest mean and median values and the lowest amplitude of variation (Figure 4.5).

Most populations had trees with two and/or three needles per fascicle on the same tree (Figure 4.5). Grand Bahama had the highest percentage of trees with only two needles per fascicle, i.e. circa 29%, whereas this category was completely absent in Mangrove Cay and North Caicos. These two latter populations had 65% and 50% of observed trees with only three needles per fascicle, respectively.

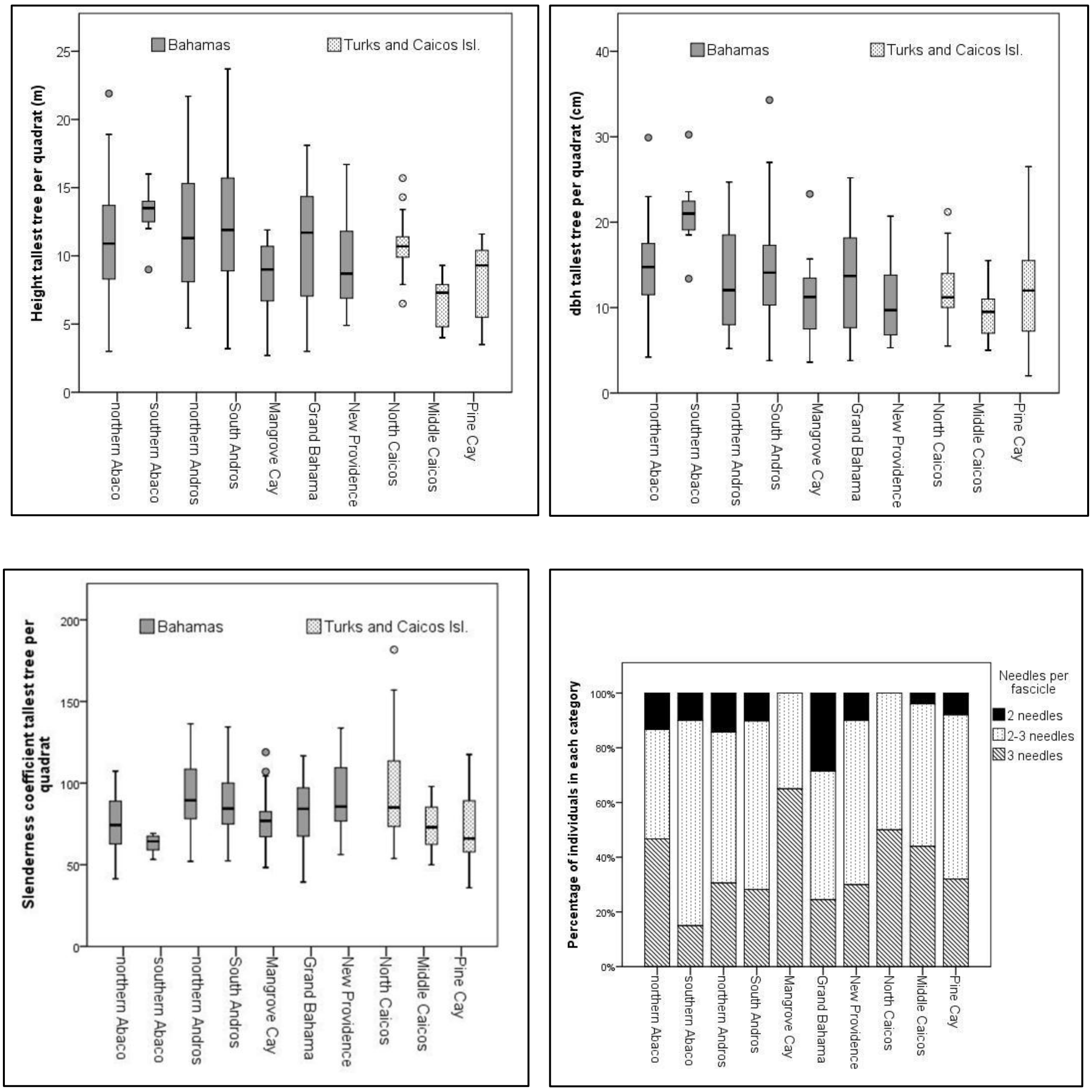


Figure 4.5. Comparison of height, dbh, slenderness coefficient (height/dbh) and number of needles per fascicle per region and per population. Number of quadrats observed in each population and used to calculate median, quartiles and outliers in SPSS were 8 for southern Abaco, 14-15 for Middle Caicos and Pine Cay, 20 for Mangrove Cay, 24-25 for North Caicos, 30 for northern Abaco, 35 for New Providence, 39 for Grand Bahama, 40 for South Andros, and 38-42 for northern Andros. Number of individuals sampled in each population for number of needles per fascicle was the same as for other morphological parameters shown in Figure 4.6.

Sheath and needle lengths varied significantly across populations (Table 4.3 and Figure 4.6), but maximum sheath length was similar within the TCI region (*P*=0.22). Largest needle size was recorded in Grand Bahama (30 cm). Pine Cay and Middle Caicos had slightly smaller needles on average (13.1-16.8 cm) than North Caicos (15.9-18.7 cm) (Figure 4.6), the latter having a mean needle size more similar to the mean for the Bahamas region (17.1-21.0 cm).

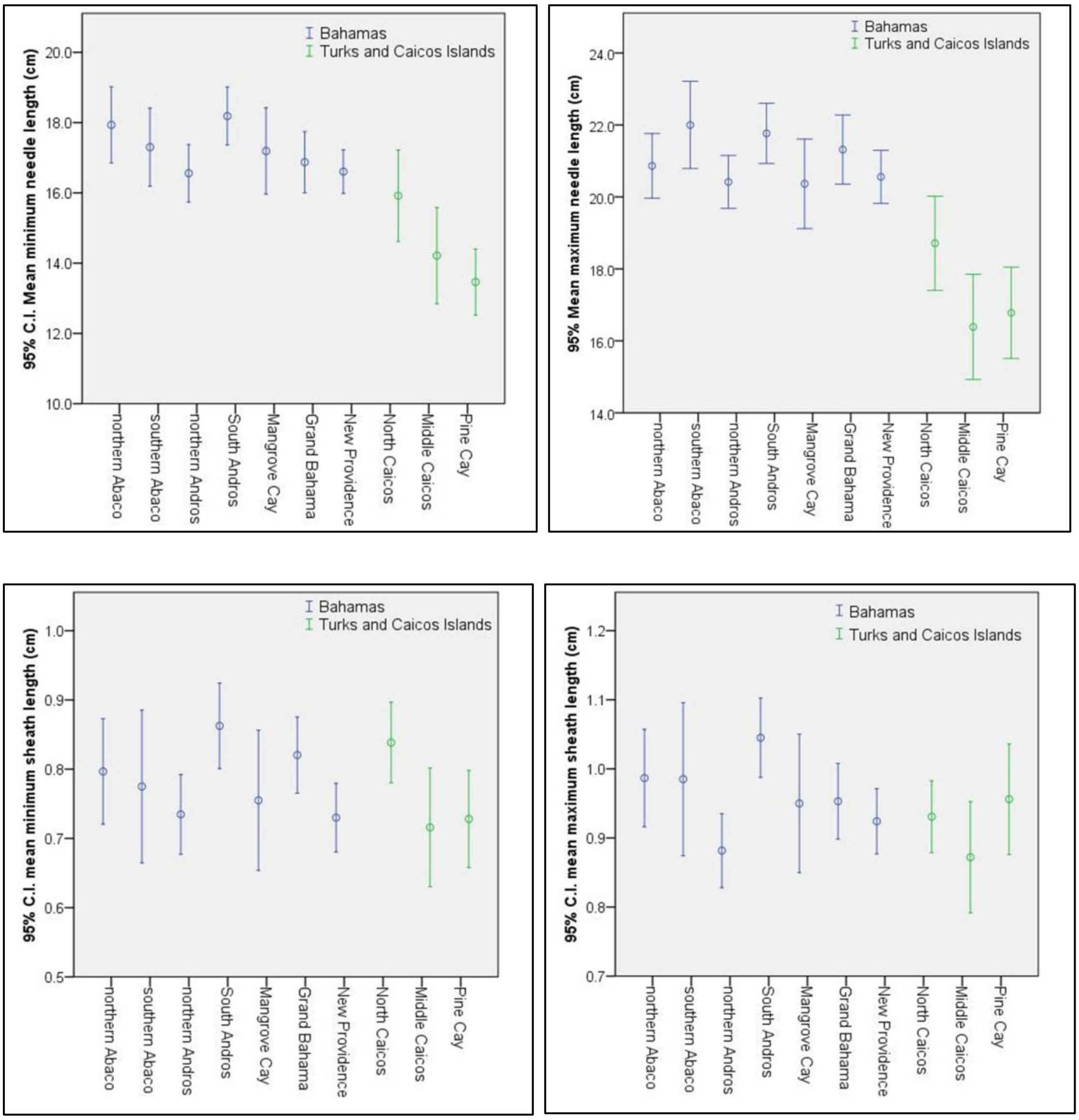
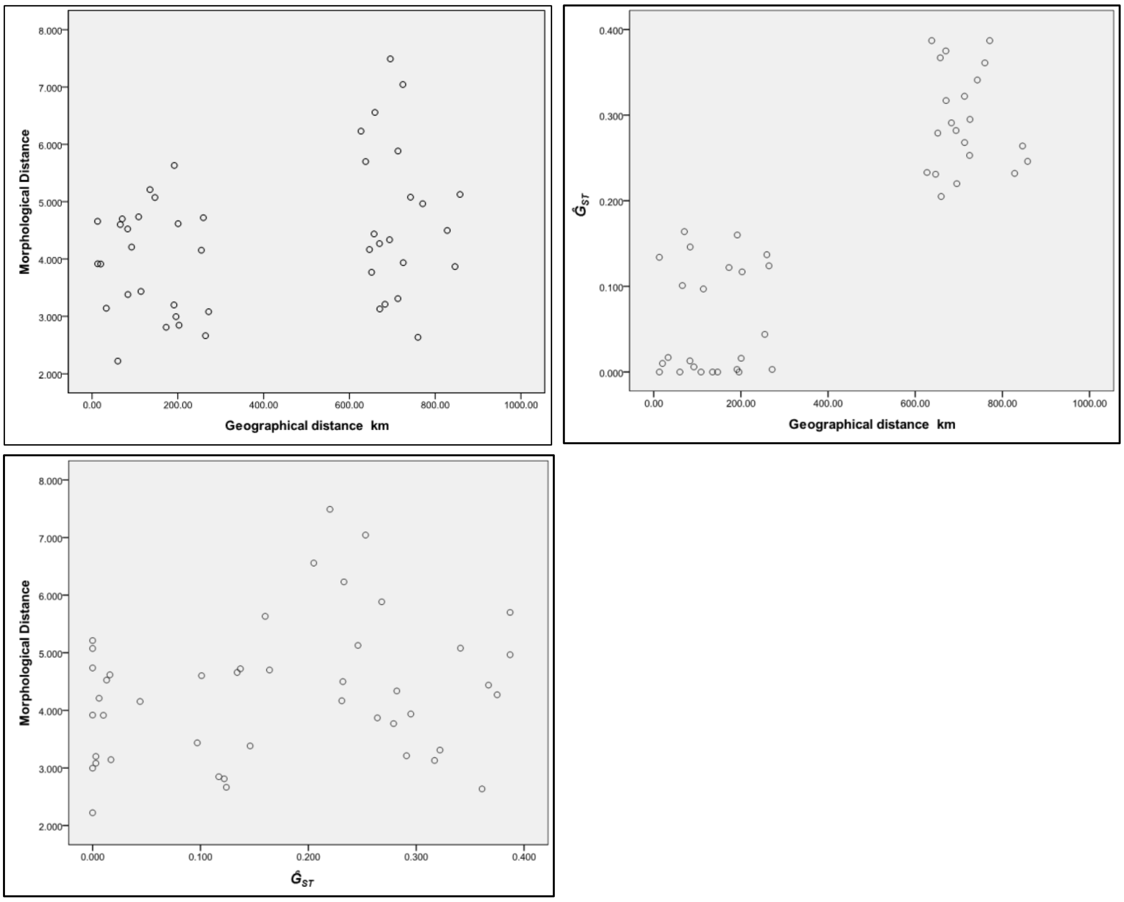


Figure 4.6. Comparison of needle and sheath sizes per region and per population. Error bars show mean (circle) and 95% confidence interval (C.I.) of the mean (whiskers). Number of individuals sampled was 20 for southern Abaco and Mangrove Cay, 25-30 for TCI populations and northern Abaco, 40 for South Andros and 49-50 for northern Andros, Grand Bahama and New Providence.

Pearson's correlation was used to test links between geographical, genetic and morphological distances (Figure 4.7). Despite observation of significant correlation between geographical and genetic (r=0.865 , P<0.01) or morphological parameters (r=0.303, P<0.05), no significant correlation was found between genetic and morphological parameters (r=0.23 , P>0.05).



Pearson Correlation Coefficient (Bootstrap 95% confidence interval) sig. 2-tailed * <0.05 ** <0.01	**Geographical distance**	**Morphological distance**
Nei unbiased genetic distance ($\hat{G}_{ST}$)	0.865** (0.813-0.913)	0.23 (0.009-0.457)
Geographical distance		0.303* (0.069-0.514)

Figure 4.7. Analysis of correlation between genetic, morphological and geographical distances observed in the pine populations of the Bahaman archipelago. Significant correlations were observed between geographical and genetic or morphological distance, but not between the latter two parameters, indicating genetic and morphological differences between the Bahamas and TCI populations and also possible phenotypic plasticity as a response to distinct regional environmental conditions.

Fire presence was higher in Mangrove Cay (83%) and ground cover lower; however, only one plot had signs of recent fire. New Providence and Abaco (northern and southern) also had high incidence of fire, respectively 77% and 70-75% (Figure 4.8). Highest percentages of recent fire were observed in northern Andros (17%), northern Abaco (13%) and Grand Bahama (8%), where the majority of the quadrats had < 50% ground cover. No recent fires were observed on New Providence. In the TCI, North Caicos was the only population with signs of recent fire and similar percentages of fire presence to South Andros (50%). However, observations were recorded before the large accidental fire of 2009, which burned most of North Caicos pine forest (Chapter 2, section 2.3.2.2, Figure 2.39). Middle Caicos and Pine Cay had the lowest fire incidence. All populations had low ground cover (<50%) in the majority of the quadrats, apart from Pine Cay and southern Abaco. The latter did not have any quadrats with < 25% ground cover (Figure 4.8).

The majority of the quadrats in all populations had < 10 mature pine trees (Figure 4.9), with the exception of southern Abaco where most quadrats had 11-15 trees. Northern Andros and Grand Bahama were the only populations with quadrats containing high tree density (>21 trees). South Andros had a similar structure to Mangrove Cay, but no quadrats had >15 mature trees. Middle Caicos and northern Abaco had the lowest observed tree density with >10 mature trees per quadrat.

Strobili were observed in circa 25% or less of the quadrats in all the populations. All the populations had new cones forming. Most quadrats had old cones only or no cones, except for South Andros and Mangrove Cay (Figure 4.10). South Andros and Mangrove Cay had the highest percentage of quadrats containing new cones (60%). In the TCI, a high percentage of the quadrats (80%) had only old cones. Seedlings were present in all the populations in the majority of the quadrats, except in northern Abaco, which had recruitment in only 26.7% of the quadrats. Southern Abaco and Mangrove Cay had the highest percentage of quadrats with recruitment circa 86% and 90%, respectively.

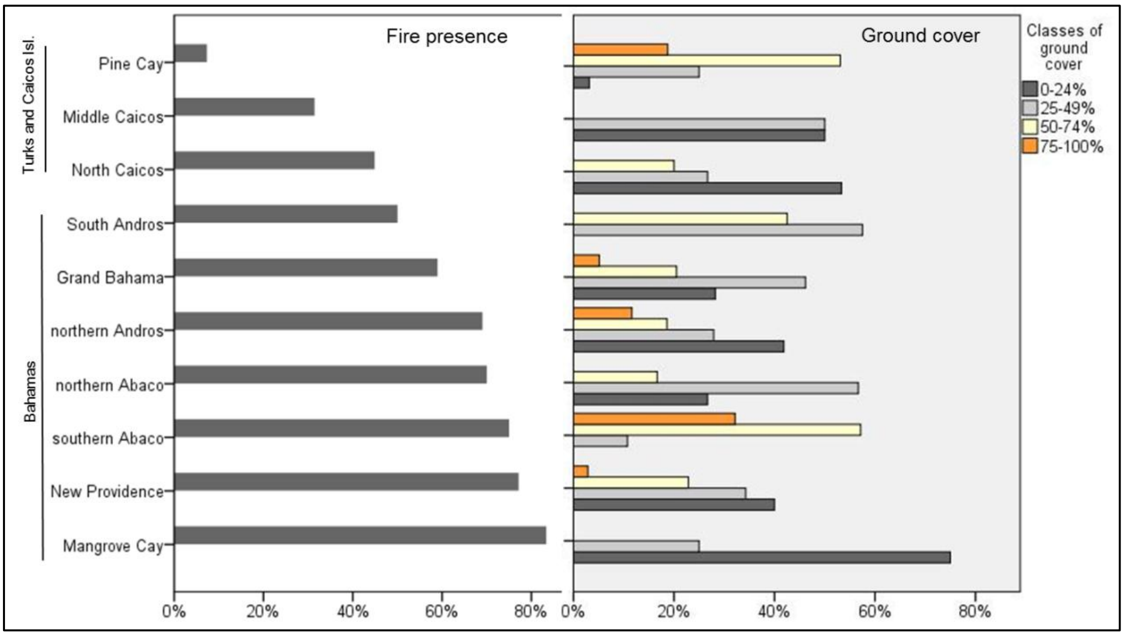


Figure 4.8. Fire presence and ground cover in the quadrats per region and per population. All quadrats had less than 85% ground cover, most with <50%. Highest percentage of ground cover was in southern Abaco and Pine Cay, where fire has been suppressed in the recent past. Low fire incidence was observed in the TCI and high fire incidence in the Bahamas.

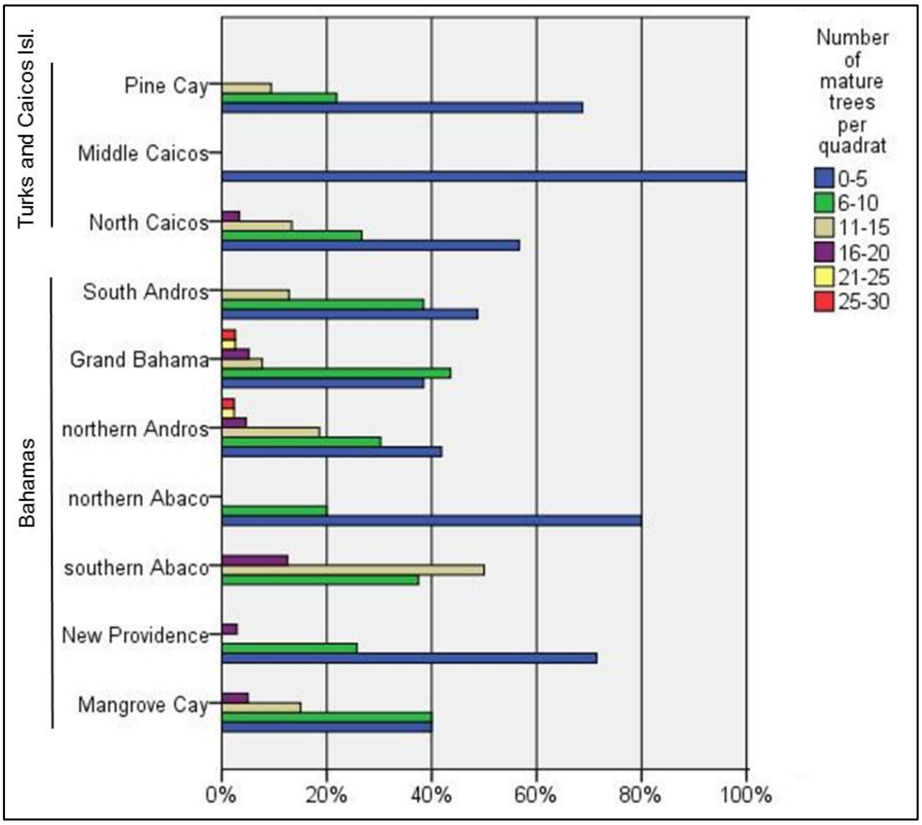


Figure 4.9. Pine tree density in the quadrats per region and per population. Quadrats were 100 m^2 in size. The majority of quadrats had <11 mature trees. Grand Bahama and northern Andros had the highest tree densities observed >21 trees, whereas the lowest and most uniform tree density was found on Middle Caicos.

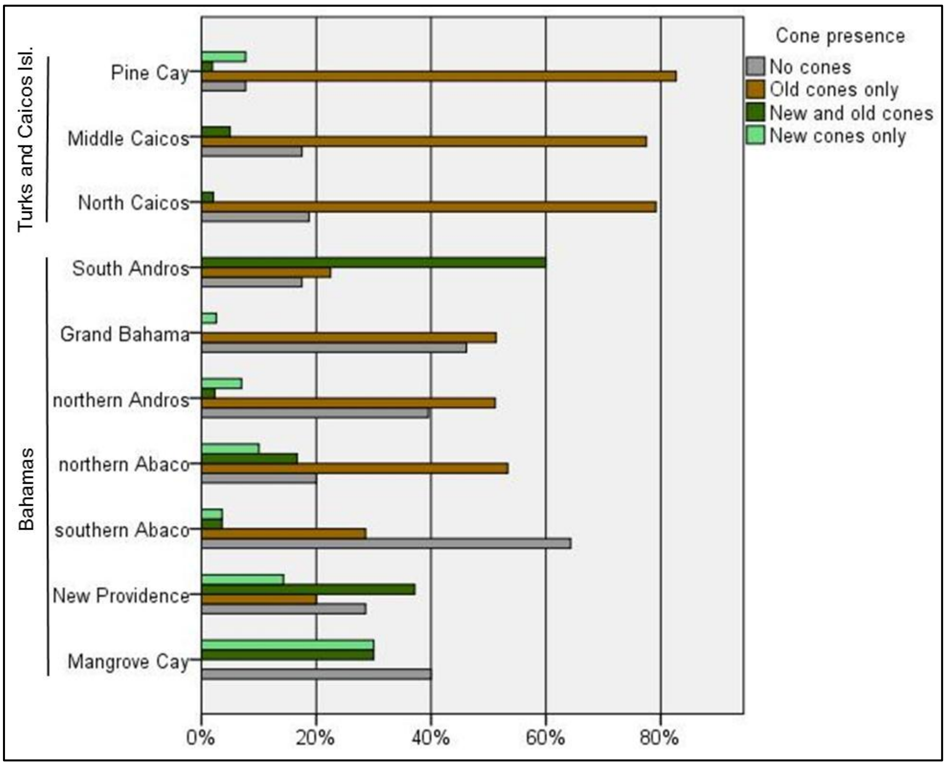


Figure 4.10. Presence of cones in the quadrats per region and per population. All populations had new cones forming. The majority of the quadrats in the TCI had no cones or old cones only. South Andros showed the highest percentage of trees with continuous reproduction, as observed by the presence of new and old cones in the same tree.

4.4. Discussion and conclusions

Morphology and ecology of the variety

Morphological characters observed during this research (Table 4.2) were similar to those described previously for *P. caribaea* var. *bahamensis* by Barrett and Golfari (1962) and Farjon and Styles (1997) (Chapter 1 Table 1.1). Variation in tree height and dbh was observed across populations (Figure 4.5 and Table 4.3). Most individuals recorded were 7 to 13 m tall and had 8 to 16 cm dbh (Figure 4.3), on the lower side of values previously reported in Table 1.1. Maximum height observed (23.7 m) was in accordance with the observation by Henry (1974) of a 24 m tall pine tree on Andros and a little above the value of 20 m reported by others (Farjon and Styles 1997, Miller, A. C. 2007). Observed maximum dbh of 34.4 cm is smaller than previously reported 50 cm by

Barrett and Golfari (1962) and Farjon and Styles (1997) (Table 1.1). The presence of few old trees in the Bahamas after intensive logging in the 1900s, successive stages of regeneration and the lack of previous survey in the TCI, where trees tend to be smaller than in the Bahamas, probably contributed to the lower girth and average height observed during this research.

Cone size, i.e. from 4.5 to 12.0 cm in length and an average of 6.6 cm long by 4.5 cm wide, was also similar to previous observations (Table 1.1). Sheath size was long in young shoots and reduced to 0.8 to 0.9 cm at maturity. Sheath length and cone size were more conserved characters without major variations between regions, while needle size showed significant variation across populations and regions (Table 4.3). Fascicles had two and/or three needles measuring 7.5 to 30.0 cm in length, but average values were 14.6 to 20.2 cm. The number of needles found concurs with that previously reported (Table 1.1). Although average needle length was within limits previously observed by Farjon and Styles (1997) for *P. caribaea* var. *bahamensis* (13-26 cm), maximum needle length recorded (30 cm) surpassed previous observations. Garden experiments with *P. elliottii* showed that sheath length was a more conserved character between clones than needle length, suggesting higher heritability in the former and some environmental influence in the latter (Sorensen 1963). In the same study, number of needles per fascicle was highly variable in the same and also in different trees, indicating high phenotypic plasticity. Anatomy of the needles was not studied in this research, but a previous study showed that variation in the number of needles per fascicle did not influence needle anatomy in *P. caribaea* var. *bahamensis,* as all needles had three to eight resin ducts and three to five hypoderm layers (Little Jr. and Dorman 1954).

Slenderness coefficient varied from 35.9 to 181.7 in different trees, but no significant variation was detected at regional or inter-population levels (Tables 4.2 and 4.3). It was observed that trees of *P. sylvestris* L. with a slenderness coefficient >109.1 were more prone to wind damage than those with a lower coefficient (Jelonek *et al.* 2011). Average value of slenderness coefficient observed (84.5) was below this threshold and similar to that observed for other pine species, i.e. *P. contorta* var. *latifolia* Engelm. (76.8 to 130.2) and *P. sylvestris* (89.0 to 109.1) (Rudnicki *et al.* 2003, Jelonek *et al.* 2011). Tree stability and resistance to snapping is an advantage in a place subjected to high winds and hurricanes, such as the Bahaman archipelago. Gardiner *et al*. (2005) observed that forest stands with gaps and different canopy heights are less subjected to wind damage during high speed wind conditions. Most forest stands observed during this research had low tree density (<10 trees/100 m^2) (Table 4.4) and variation in tree height (Figure 4.5). Therefore, ecological and morphological characteristics of these pine forests are important to their survival during high wind and hurricane events.

Most morphological characters showed high variation across populations (Table 4.3), but most genetic variation in nuclear regions was found within populations (Figure 3.3). This was reflected in the lack of significant correlation between morphological and genetic distances, despite regional differences at morphological and genetic levels observed through the positive correlations in Figure 4.7. Provenance tests on the three varieties of *P. caribaea* showed that differences in growth and wood density were related to individual genetic variability and phenotypic plasticity (Barnes *et al.* 1977). A similar experiments on *P. caribaea* var. *bahamensis* by Sebben *et al.* (2008) also showed high individual variation in height, dbh and tree volume related to genetic and environmental factors. Therefore, it seems that phenotypic plasticity could be playing an important role in the adaptation of these pine trees to different environmental conditions.

Pinus caribaea var. *bahamensis* forests are fire-climax ecosystems, as previously discussed in Chapter 1 section 1.4.2. Most quadrats (51.7%) had some evidence of past or recent fires. Fires reduce competition, increasing gaps in the vegetation and light levels in the ground promoting the germination of pine seedlings and the maintenance of this ecosystem. Percentage of ground cover was low in most areas, mostly <50%, due to fires and limestone rock patches, and seedlings were observed in 57.3% of the quadrats (Table 4.4). Old cones were also abundant, but cones in *P. caribaea* var. *bahamensis* remain on the tree after seed dispersal, so observed old cones are a sum of previous reproductive events. Strobili and new cones give a better representation of annual reproduction and seed dispersal. Strobili and new cones were only observed in a small percentage of the quadrats (Table 4.4), suggesting that recruitment is not only dependent on seed dispersal but also on the soil seed bank. López (2003) observed germination in 43.3% of the plots in a natural forest of *P. caribaea* var. *caribaea* in Cuba at a rate of 1.86 seedlings/m^2 in the first year after dispersal. However, germination dropped to 0.5 and 0.23 seedlings/m^2 in the subsequent years. He also estimated that after 2.2 years after dispersal only 10% of the seeds in the soil seed bank would be viable. Thus, the soil seed bank would not have the potential of forest maintenance alone without reproduction and seed dispersal by live trees. No studies have been carried out in the Bahaman archipelago to estimate seed viability and quantity of seeds in the soil seed bank, but it seems that in good conditions forests have the potential to regenerate well (Chapter 2 section 2.4).

Nonetheless, most pine forest areas studied during this research were under some sort of threat. The major threat to this variety was insect pests (Figure 4.2). It seems that insect pests are present in most pine forest islands, including rural and inaccessible areas of old-growth forest, e.g South Andros, but level of damage to the trees varies according to tree health and environmental conditions. A few specimens collected during field work in northern Andros and New Providence were identified as *Toumeyella* sp. (Malumphy 2010a). This could be of major concern, as *Toumeyella parvicornis*, the pine tortoise scale insect, has been reducing the pine forests of the TCI, as discussed in Chapter 1 section 1.5.4. However, in a subsequent visit to the areas no signs of live insects or further damage were observed. It seems that the infestation was localised and only damaged already weakened pine trees, one of them parasitized by *Cassytha filiformis* L. Unfortunately, no material could be obtained for further identification of this insect to species level, but it seems that it is distinct from *T. parvicornis* (Malumphy 2010a). Another five species of scale insects and three mites were observed feeding on pine trees in the archipelago, but no major damage to these trees was observed (Malumphy 2010a, b).

Regional differences

Genetic, morphological and environmental differences between the Bahamas and the TCI were observed in this research. Dbh and height of the tallest tree per quadrat and needle length showed significant variation at the regional level (Tables 4.2 and 4.3). Trees in the TCI had lower maximum height and dbh and smaller needle size than those in the Bahamas. There was also significant genetic differentiation between these two regions (Chapter 3, section 3.3.1.2). Natural selection and drift could have contributed to phenotypic variation and adaptations of these populations to low rainfall, high temperatures and prolonged drought. Little Jr. and Dorman (1954) observed that the hypoderm was thicker and stomata were usually sunken in needles from trees of *P. caribaea* growing in drier conditions. Adaptations to withstand high exposure to light and drought were also reported for *P. eldarica* Medw. in Iran (Shayanmehr *et al.* 2009).

The conical-shaped pine morphotype had shorter needles, fewer and more sparse stomata and also a thicker cuticle.

Considering that height and dbh were strongly correlated to each other and slenderness coefficient did not vary significantly across the archipelago (Table 4.2, Figures 4.3 and 4.5) growing patterns should be similar across the regions. However, trees in the Bahamas were able to reach higher height and dbh than those in the TCI. Difference in maximum tree height was more pronounced than in dbh, with trees in the TCI having a maximum height of 15.7 m (Table 4.2). Tree growth, in height and girth, is highly responsive to water availability and drought. Drought during bud formation can have a negative effect on shoot elongation, leaf surface area and number of needles, not only in the year of drought but also in years to come (Breda *et al.* 2006). Callaway *et al.* (1994) observed that trees of *P. ponderosa* from desert populations had lower height and higher allocation of biomass to sapwood than those from montane populations, a possible adaptation to increase storage of water and carbohydrates during droughts. A study on the response of shoot elongation and growth rate to temperature in *P. contorta* Douglas ex Loudon and *P. monticola* Douglas ex D.Don showed adaptations of these trees to different environments (Chuine *et al.* 2006). Tree height and growth were influenced by the number of internodes produced during the growing season and not the temperature itself. Thus, trees growing in harsher conditions, i.e. drought and frost, had a shorter growing season, producing less internodes and consequently showed reduced growth and height. Another study on the wood anatomy of *P. caribaea* by Little Jr. and Dorman (1954) showed that growth was not continuous throughout the year, but there was a dormant period during the dry season. The TCI have a longer dry season and lower annual rainfall than the Bahamas, what could result in reduced growing season.

Statistical analysis of morphological parameters showed that some reproductive characters, e.g cone size, did not vary greatly between regions and are likely to be the least environmentally influenced (Table 4.2). Vegetative features, e.g. height, dbh and needle length, seemed to be more influenced by edaphic/ecological factors, showing differences between regions and among populations within regions. Despite significant genetic distance between the Bahamas and the TCI populations (P<0.01) (Chapter 3 Table 3.6), no significant correlation was observed between morphological and genetic distance (Figure 4.7), suggesting the possibility of adaption to environmental pressures through phenotypic plasticity.

Needle size was one of the most variable characters, as expected. Smaller needles were found in the TCI populations and a negative correlation between needle size and tree health was observed (Table 4.2, Figures 4.4 and 4.6). Although insect pests were recorded in almost all the Bahamas populations, these were in low numbers and in localised areas (this Chapter section 4.3.2). Trees responded well to infestation in normal conditions and no correlation between needle size and tree health was observed for this region. More severe infestation problems only occurred after storm surge events which reduced fresh water availability (Chapter 2 section 2.4). However, in the TCI an intense and widespread infestation and high pine tree mortality were observed (Table 4.5). Insects are present in most of the quadrats (90.5%) and trees have lost branches and needles or died completely. Pine forests in the TCI are located at the southern and eastern limit of distribution for *P. caribaea* var. *bahamensis*. Water stress due to low rainfall, high temperature and a prolonged drought period could be related to reduction in needle size and high susceptibility to pest attacks. A study by Grulke (2010) showed that trees of *P. jeffreyi* A.Murray growing in drier environments had lower needle elongation and higher susceptibility to pest attacks by pine needle scale (*Matsucoccus bisetosus* Morrison) and bark beetle (*Dendroctonus jeffreyi* Hopkins) than trees with longer needles in areas with higher rainfall. Wahid *et al.* (2006) observed a cline in needle length and number of stomata in *P. pinaster* populations in Morocco. A population from higher altitude and a more intense drought regime in Tadiwine in the Rif Mountain had smaller needles and fewer stomata than those at lower altitudes with higher rainfall. This same population also showed genetic differences to other populations in the region (Wahid *et al.* 2010). Rouault *et al*. (2006) reported an increase in the populations of woodborers and some broadleaf defoliators in the temperate forests of Western Europe, after high temperatures and severe drought of 2003. Therefore, as expected, more severe infestations occurred in the hotter and drier parts of the archipelago (TCI) or after storm surges (Bahamas).

Not many old trees were observed in the Bahamas, since most forests were logged in the last century (Chapter 1 Table 1.3) and many different successional stages were present in the areas studied. Trees of different ages and sizes were observed (Figure 4.5) and tree density was high in several areas (Figure 4.9). This indicates good general regeneration, as previously discussed in Chapter 2 section 2.4. They also showed low percentage of ground cover in the majority of the quadrats. Forests in the TCI had lower tree density and variation in tree height than those in the Bahamas (Figures 4.3, 4.5 and 4.9), maybe because of lower fire incidence and their old-growth status (Figure 4.8). Recruitment was similar in both regions, suggesting good regeneration in both areas. However, there are already signs of challenges to the maintenance of the pine forest ecosystem in the TCI.

High tree mortality in the pine forests of the TCI caused by the pine tortoise scale insect (Table 4.5) is reducing the number of mature individuals dramatically and the number of new cones produced, as reported by Malumphy *et al*. (2012). Green (2011) showed a total decline in number of pine trees of 57.5% in the TCI pine forests and a ratio of immature to mature trees of circa 68:1. The high number of immature individuals concurs with the high rate of recruitment observed in this work, where seedlings were present in 60-70% of all the quadrats. However, neither Green (2011) nor this research has included data from North Caicos after the fire, which could have reduced recruitment rate for the island (Malumphy *et al.* 2012). Nonetheless, future regeneration of these forests is in peril due to the reduced number of mature individuals, low number of new cones, high levels of infestation and increased competition. As mature trees die, the scale insects move to seedlings and immature trees; resulting in high infestation levels even in the absence or reduction in number of mature live trees (Table 4.5). The possible adaptation of this insect to multivoltism (Malumphy *et al.* 2012), the lack of natural predators, the isolation of most of these areas, the abundance of pine trees and the non-optimal conditions for pine growth were probably the causes of this explosion in insect numbers.

A change in species composition of the undergrowth has also been observed in the pine forests of the TCI. Increased light and gaps in the vegetation became more common due to the die back of pine trees and the reduction of canopy. Green (2011) has noticed an increase in the number of *Sabal palmetto* in the TCI forests, following the decrease in mature pine trees. Williams *et al*. (2003) also showed an increase in *Sabal palmetto* numbers in the coastal forests of Florida after an increased mortality of *Juniperus virginiana* var. *silicicola* due to storm surge and drought. *Sabal palmetto* is a fire-tolerant species which recovers well after fire events, but their leaves are highly flammable (O'Brien *et al.* 2010). Therefore, an increase in the number of palms could also increase fuel load and fire intensity. Additionally, the high load of dead wood now present in the TCI due to the high number of dead pine trees could lead to high intensity fires, which can kill immature individuals and seedlings, e.g the dry-season, high intensity fire on North Caicos in 2009 (Chapter 2 Figure 2.39).

Threats to the pine forests of the Bahamas were not so evident and were only identified in a small percentage of the areas studied. Urban development has caused the loss of 63.6% of the pine forests of New Providence (Chapter 2 section 2.4) and is still the major threat to the pine forest ecosystem in the Bahamas combined with the opening of new roads (79.4%). New roads are usually followed by agriculture or new urban centres, increased hunting activities and higher frequency of accidental non-seasonal fires. It is not surprising that fire evidence in the Bahamas was much higher than in the TCI, since population and urban areas are much larger in the former.

Needle thickness, number of stomata per needle, gross photosynthesis and respiration rates are other traits that have been previously reported as variable and related to climatic conditions, e.g. temperature, water stress and light levels (García Quintana *et al.* 2009, Grulke 2010, Hou *et al.* 2011). The study of these parameters in *P. caribaea* var. *bahamensis* in the future could shed more light on the regional adaptations of these pine trees to the lower rainfall and prolonged droughts occurring in the TCI.

Inter-population variation

Morphological variations in tree size and number of needles per fascicle were observed between and within populations. A negative correlation between longitude and the number of needles per fascicle was reported by García Quintana (2006) in the natural forests of trees of *P. caribaea* var. *caribaea* in Pinar del Río, Cuba. However, no clear pattern of variation was observed in the present research for this parameter in *P. caribaea* var. *bahamensis*, despite some differences in the proportion of needles per fascicle in the populations (Figure 4.5). Cone size and sheath length were more conserved parameters with low variation (Table 4.3), suggesting higher genetic influence on these characters. However, the number of cones sampled was small and a more extensive sampling should be carried out to confirm lack of significant variation in this character. Inter-population variation in fire regime, ground cover, reproduction and regeneration were also observed. The slenderness coefficient, which is associated with tree stability and wind resistance, did not vary greatly between populations, except for southern Abaco. It is expected that trees growing in the hurricane belt would be adapted to wind resistance and increased stability, as observed here.

Trees on southern Abaco were more uniform in size and showed low variation in height, dbh and number of needles per fascicle (Figure 4.5). Most trees had two to three needles per fascicle, with only a few individuals containing one or the other, which was quite unusual across the populations. Tree height did not exceed 16 m, but mean dbh (21.1 cm) was higher than in any other population (9.3 to 14.9 cm) and slenderness coefficient was lower. These results indicate an even-age stand. March (1949) recorded a dbh of 15 cm in a 33-year-old tree in the Bahamas, so most of the trees observed in the southern Abaco stand during this research are probably between 30 and 40 years-old. The theory that southern Abaco was re-seeded in the 1970s following hurricane and fire damage during the late 1960s (Chapter 2 section 2.4 and Chapter 3 section 3.4) fits the observed data. Greaves (1978) reported low seed set in this area in 1966 and also high damage to the seeds by insects, implying low natural regeneration. He also reported that tree density was 2.88 trees/100 m^2 (=288 trees/ha) in an unexploited mature forest in southern Abaco. Nonetheless, tree density observed in this work in the majority of the quadrats (circa 88%) was between six and 15 trees/100 m^2 and no areas had < six trees/100 m^2 as observed in the other populations (Figure 4.9). Tree densities in plantations of *P. caribaea* usually vary from eight to 13 trees/100 m^2 after thinning (Liegel 1991, Wadsworth 2008), in order to reduce competition for nutrients and light and maximize growth. Thus, low density, even-age stands and high dbh together with genetic differentiation from the northern Abaco population (Chapter 3 section 3.4) corroborate to the theory of a managed forest that has been re-seeded in the 1970s. A correlation between tree spacing and dbh was observed in plantations of *P. caribaea* var. *hondurensis* in Puerto Rico, with an increase in growth rate after thinning (Wadsworth 2008). This could explain the high dbh achieved by trees on southern Abaco in a short period of time.

Other interesting observations from this area regarded fire regime and reproduction. Most of southern Abaco is part of the Abaco National Park, which was created in 1994. It is also the home for the Bahama parrot *Amazona leucocephala* subsp. *bahamensis*, which is a ground nesting bird (Wiley 1991, Myers, R. *et al.* 2004). Although there was evidence of past fire in a great part of the plots, no recent fires have been observed and ground cover for most quadrats was high (>50%) (Figure 4.8). Fire suppression in the park area seems to have favoured growth of herbaceous and woody plants, thus increasing ground cover. Nonetheless, recruitment was higher (85.7%) than in northern

Abaco (26.7%), where ground cover was sparser. The majority of the plots on Abaco had no cones or opened old cones only and low percentages of new cones and strobili. Mast seeding could have occurred in the past in response of predation of the seeds by the Bahama parrot (Stahala 2005), followed by small reproduction rates in the subsequent years.

Mast seeding events are more common in wind-pollinated species than animal-pollinated species. They are coordinated events, when individuals synchronize their reproductive efforts in order to maximise recruitment, minimise detrimental effects of predation on seeds and increase outcrossing rate (Kelly and Sork 2002, Koenig *et al.* 2003). Such events can exhaust trees in areas of poor resources, so trees need time to recover and build up energy for the next reproduction event (Kelly and Sork 2002). This could explain the high recruitment in southern Abaco, even in the absence of recent fires and with a great part of the ground covered by vegetation. However, there is not enough evidence that such phenomenon happened. Old cones are persistent, remaining on the trees after dispersal, making it difficult to estimate past mast seeding events. Nonetheless, the low presence of strobili (25% of the quadrats) and new cones (7.2%) could indicate a drop in reproduction following mast years.

Fires in northern Abaco and northern Andros were observed on several occasions during fieldwork, along main roads and near settlements. In more remote areas evidence of hunting was observed (fired shotgun shells). The use of fire to force animals out of the forest and clear vegetation is a common practice in the Caribbean (Robbins *et al.* 2010). The frequent accidental and non-seasonal fires recorded in these islands were also reported by Myers *et al*. (2004), Miller (2007) and Robbins *et al*. (2010). However, Abaco has almost twice the population of Andros (Chapter 1 section 1.2.1) in an area one quarter the size (Chapter 2 Table 2.3). Pine forests are also more accessible than on Andros. Even though fire incidence observed was similar in both islands, fire frequency was higher in northern Abaco due to the smaller area and increased pressures. Northern Andros had more variation in tree density and recruitment of circa 51%, whereas in northern Abaco most areas had <six trees/100 m^2 and recruitment was only 26.7%. Nonetheless, cone presence was higher in northern Abaco than southern Abaco or northern Andros (Figure 4.10). It was expected then that recruitment would be higher in northern Abaco due to higher number of cones, sparser vegetation and more gaps for seed germination. Mature pine trees are fairly resistant to fire, but seedlings are not. The

frequent fires observed in northern Abaco seem to be affecting forest regeneration and seedling survival, as previously reported by Myers *et al.* (2004).

Mangrove Cay showed the highest percentage of plots with signs of past fire and no signs of recent fires within the plots (Figure 4.8). There was evidence of both an old and a recent fire that had run along the road that cuts through the pine forest; however, none of the sampling plots was within the recent fire area. The location of the sampling quadrats along the road and the low number of observations (20) could have biased results, increased observations of past fire and omitted recent fire records. Evidence of a lightning strike was visible on a utility pole along the road running through the pine forest. Charring was visible around the pole and recent fire radiated out from this location, suggesting a natural fire. There were also many felled trees and some cleared areas, indicating human activity in this area. Mangrove Cay also had the lowest percentage of ground cover in the Bahamas, i.e. all plots had <50% ground cover. Fire presence, gaps in the vegetation and signs of continuous reproduction in most of the quadrats (Figure 4.10) could explain the high recruitment observed (90% of the quadrats).

Mangrove Cay was the last island to be commercially logged in the Bahamas during the 1970s (Chapter 1 Table 1.3), according to Henry (1974). The fact that trees on this island had reduced maximum height (11.9 m) and low dbh (Figure 4.5) could be the result of such logging activities. Only one tree had a dbh of 23.3 cm, but tree height was only 11.1m. The absence of taller trees suggests that most of the area must have been clear cut in the past. However, it seems that natural regeneration took place instead of re-seeding. There are no records of any re-seeding programme in this area. Additionally, variation in height and dbh was greater than that observed in a managed forest, i.e. southern Abaco (Figure 4.5). Tree density was variable across the area and resembled that of other forests that have undergone natural regeneration, i.e. northern Andros and South Andros (Figure 4.9). However, this was the only population in the Bahamas without any two-needled fascicles. Southern Abaco has also shown unusual distribution for this character, which could have resulted from lower genetic variability. The genetic similarity of this population to northern Abaco and the possibility of a single colonisation event from northern Abaco to Mangrove Cay, as suggested in Chapter 3 section 3.4, could have limited genetic variation on this character and also helped with forest regeneration after logging.

Grand Bahama and northern Andros showed similar mean height, dbh and slenderness coefficient (Figure 4.5). However, Grand Bahama had a higher percentage of two-needled trees, as previously reported by Farjon and Styles (1997). Ecological parameters were also similar in these populations, i.e. classes of ground cover, fire incidence, cone presence and recruitment (Figures 4.8 and 4.10). Both areas have large tracts of pine forests, which have been logged in the past (Chapter 1 Table 1.3). However, forest regeneration was good after logging, mainly due to uncut seed-bearing trees or forest tracts on ridges and the soil seed bank (Henry 1974, Greaves 1978). Genetic similarities and low genetic distances were also observed between these populations, as previously discussed in Chapter 3 section 3.4. Therefore, phenotypic similarities observed in pine trees on Grand Bahama and northern Andros seems to be related to low genetic distance and similar forest ecology.

Although the New Providence population was genetically close to Grand Bahama and northern Andros populations (Chapter 3 section 3.4), morphological and ecological differences were observed. New Providence trees showed lower height and dbh than those in the other two populations, but similar slenderness coefficient (Figure 4.5). Ground cover was similar, but fire incidence was higher and tree density lower (Figures 4.8 and 4.9). The New Providence pine forest is small in size and the surrounding area is highly urbanised (Chapter 2, Figure 2.38). Since most fires in the Bahamas are human-ignited and non-seasonal (Chapter 1 section 1.5.2), it is likely that accidental fires have occurred in the past. The fact that there were no signs of recent fire and ground cover was varied throughout the area suggests that a fire event happened in the past and natural regeneration took place afterwards. The presence of old and new cones in most of the plots suggests good regeneration potential (Figure 4.10), but the low number of trees per quadrat indicates otherwise. High fire frequency or selective logging at small scale could have reduced tree density. Greaves (1978) noticed that in 1966 most trees in the stand were circa 7 to 15m tall, as a result of previous exploitation. Lack of genetic diversity through inbreeding (Chapter 3 section 3.4) and selective felling of older and taller trees could have resulted in the presence of less vigorous genotypes for growth in the modern forest and the observed reduction in mean height and dbh.

The observations of old-growth forest on South Andros from this research concur with those reported by Lloyd and Slater (2010). Quadrats had a varied composition and structure, with ground cover varying from 25 to 74% and tree density spread among the three lowest categories (<16 trees/100 m^2) (Figures 4.8 and 4.9). There was a variation from areas with a sparser and more open understory to a more dense and shrub-dominated understory. Stands were not even, with number of mature trees varying according to disturbances and undergrowth. Levels of regeneration and reproduction were also good throughout the area (Figure 4.10). Maximum values of tree height and dbh for *P. caribaea* var. *bahamensis* were observed in this area, as it was expected due to its old-growth forest status. Average height and dbh were similar to those for Grand Bahama even though the latter is a secondary forest (Figure 4.5). This suggests that growth is slower on South Andros, even though these two populations are genetically similar (Chapter 3 section 3.4). However, mean annual rainfall is lower on South Andros than on Grand Bahama. Barnes *et al*. (1977) observed an increase in growth in *P. caribaea* with higher rainfall in plantations in Zimbabwe, suggesting the influence of environmental conditions in tree growth. It is possible then that Grand Bahama trees have faster growth rate than those on South Andros, since there are more fresh water resources available in the former.

Tree density was similar for most old-growth forests, i.e. South Andros, North Caicos and Pine Cay. Middle Caicos, however, had the lowest tree density of all populations (<six trees/100 m^2) and also low ground cover (Figures 4.8 and 4.9). Observations by Earle-Mundil (2010) of the Caicos Pine Recovery Project permanent plots located in the three pine forests in the TCI (Middle Caicos, North Caicos and Pine Cay) showed that the lowest percentage of ground cover and canopy density is found in the Middle Caicos plots, possibly due to the occurrence of a fire in the past decade and early stages of succession in the area. Although Middle Caicos has the largest area of pine forest in the TCI (Chapter 2 Table 2.3), tree growth seems to be limited by environmental factors. Reduced growth and low tree density on Middle Caicos could be associated to the fact that this forest is located at the extreme boundary for this variety (lowest longitude and latitude) under sub-optimal growing conditions, i.e. low water availability and drought.

Maximum tree height, needle length and proportion of three-needled fascicles were higher on North Caicos than on Middle Caicos or Pine Cay (Figures 4.5 and 4.6). The fact that North Caicos receive more rainfall than the other two islands (Sears and Sullivan 1978) could explain why some of the growth characters in this forest are more similar to the Bahamas populations than to the other two TCI populations. Breda *et al.*

(2006) observed that severe drought in temperate forests can reduce shoot elongation, number of needles and leaf surface in trees. However, pines on Pine Cay can be taller than those on Middle Caicos and maximum dbh can be similar to those found in the Bahamas. Pine Cay is the only island with sandy soils in the whole archipelago that supports a pine forest and has shown high genetic differentiation and distance to all other populations (Chapter 3 section 3.4). Pine trees growing in sandy substrate can grow deeper roots, reducing the effects of drought (Hacke *et al.* 2000) and being able to increase girth (Gordo *et al.* 2007). Experimental trials with *P. caribaea* across the Caribbean have demonstrated that higher yields were obtained in sites with good drainage and enough moisture (Liegel 1991). Therefore, it seems that there is a combination of genetic and environmental factors contributing to optimal fitness of the pine trees on Pine Cay. This is also reflected in the reproductive success in this area. Pine Cay had the highest percentage of new cones in the TCI and a little higher recruitment, even in the presence of few gaps in the vegetation, low fire incidence and high competition (Figures 4.8 and 4.9). Green (2011) has also observed that the Pine Cay forest had the highest ratio of survival and regeneration since the pine tortoise scale insect infestation. Thus, natural selection could have improved fitness of this population and contributed to its adaptation to local environmental conditions.

Previous research by Grivet *et al.* (2009) demonstrated polymorphism and natural selection for drought-response using plastid microsatellites in *P. halepensis*. Tambarussi *et al.* (2010) observed phenotypic variation in *P. caribaea* var. *hondurensis* trial plots in Brazil, using quantitative traits and nuclear microsatellites. Variation in height, dbh and volume was found between and within families and a correlation to genetic and environmental factors was inferred. Genetic and morphological differences were observed in *P. caribaea* var. *bahamensis* in the Bahaman archipelago in the present research, but no significant correlation between morphological and genetic distances was observed. This could indicate that phenotypic plasticity is mainly responsible for adaptation to environmental conditions or perhaps microsatellites used in this research are not associated with genes regulating phenotypic expression. Further research is necessary to investigate the sources of adaptation and heritability of characters in these natural populations.

Main conclusions

- Significant variation in morphological characters was observed at regional, inter-population and individual levels. The most variable characters were height, dbh, and needle length, while sheath length and cone shape were more conserved.
- Maximum needle length (30 cm) observed was higher than previously reported for this variety in natural forests.
- Phenotypic variation was observed between the Bahamas and the TCI regions. Trees in the Bahamas reached a larger maximum height and dbh and had longer needles.
- A positive and significant correlation between needle size and tree health was observed in the TCI trees, possibly resulting from non-optimal growing conditions of low rainfall and prolonged droughts.
- Decline of the TCI pine forests due to infestations by the pine tortoise scale insect, *Toumeyella parvicornis*, is serious and on-going. High insect presence, infestation of trees and seedlings, high tree death and low number of new cones observed indicate low forest regeneration and continued forest decline in the future.
- The major threats to the pine forest ecosystems were the pine tortoise scale insect in the TCI and urbanisation in the Bahamas.
- Most islands in the Bahamas showed good pine forest regeneration, but northern Abaco is showing signs of reduced regeneration due to increased frequency and intensity of non-seasonal fires.
- Tree density in most quadrats observed was lower in the TCI (<six mature trees/100 m^2) and higher in the Bahamas (<16 mature trees/100 m^2). Most areas in both regions had <75% ground cover, but fire incidence in the Bahamas was double of that in the TCI.
- Differences in tree growth in the TCI populations are possibly linked to fresh water resources, genotypic variation and or phenotypic plasticity.
- The southern Abaco population showed morphological and ecological characteristics of a managed secondary forest, which concurs with previous genetic and biogeographic observations in Chapters 2 and 3.
- Further research is needed to determine if phenotypic variation observed in *P. caribaea* var. *bahamensis* is linked to phenotypic plasticity and or genetic variation.

Chapter 5 . General discussion and future research

The multi-disciplinary approach adopted during this research on *P. caribaea* var. *bahamensis* proved effective in identifying historical and current gene flow in the archipelago, past, present and future threats to the pine forest ecosystem, species plasticity and response to stochasticity. Besides furthering knowledge of the genetics, biogeography, morphology and ecology of this variety, this research can be a valuable tool for future conservation and management of the pine forests in the Bahamas and the TCI.

Pinus caribaea var. *bahamensis* showed a slight total expansion in area (5.3%) since the 1960s, mainly due to an increase in the frequency of dry-season fires on the larger islands, i.e. Abaco and Andros. However, reductions in forest area and effective population size due to stochastic events were observed on the rest of the islands and some significant inbreeding was also reported for some small populations, i.e. New Providence and Pine Cay. Genetic and morphological parameters showed differences between regions and across populations. Pine plasticity and forest regeneration seemed to be closely linked to environmental and ecological parameters.

Regional differences in P. caribaea var. bahamensis

Genetic differentiation through isolation by distance was observed in *P. caribaea* var. *bahamensis* in the Bahaman archipelago. Populations within a 300 km range had higher genetic similarity than those further apart, resulting in significant genetic distance between the Bahamas and TCI populations ($\hat{G}_{ST}$>0.205, P<0.01). Therefore, current levels of seed dispersal and pollen flow are limited by the long distance separating these two regions (>600 km). No pine forests are found in the intermediate islands between regions, probably due to lack of suitable habitat and competition with broadleaf and salt-tolerant species. Nonetheless, this isolation is probably recent. Data from plastid microsatellites revealed a history of high pollen flow and low differentiation (φ_{PT}<0.06) between all populations.

The presence of an ancestral haplotype at high frequency (80.3%) in all populations and presence of linkage disequilibrium between nuclear loci in many populations in both regions indicate that this expansion occurred in the recent past. Sea level during the Pleistocene was lower than present, as shown in Figure 1.5. Data for a sea level in the

Caribbean region of circa 100 m below present level was inferred by Tushingham and Peltier (1991), and this would have enhanced island area significantly. It is therefore possible that pine forest area at that time was larger than present. Pines are pioneer species with high reproductive output, they show high plasticity and individual genetic variation, they are adapted to survive in stressful environmental conditions, they can also migrate long distances and self-pollinate if necessary making them successful colonists in new habitats, especially those that are open with high light levels (Ledig 1998). Suitable climatic conditions for pine forest expansion and newly emergent land could have driven seed dispersal and colonisation of new environments in the intermediate islands, reducing distance between forests in the different regions and increasing gene flow. A contraction of forest area and extirpation from some islands could have followed the increase in sea level, submergence of land and climate changes during the transition period to the Holocene, resulting in isolation between the Bahamas and the TCI populations. Dvorak *et al.* (2005) and Delgado *et al.* (2011) suggested a more widespread past population of *P. caribaea* var. *hondurensis* in Mexico, Guatemala and Belize based on evidence of high historical gene flow followed by bottleneck events and isolation. Jardón-Barbolla *et al.* (2011) demonstrated a sudden population expansion in *P. caribaea* var. *hondurensis* and *P. caribaea* var. *bahamensis* circa 331,100 yr BP and 97,900 yr. BP, respectively. Therefore, expansion and contractions were possibly present during the glacial and interglacial periods.

The common history of populations in Bahamas and TCI and recent isolation indicate that they are part of the same evolutionary unit. Smaller population size, lower number of private alleles, lower allelic richness and higher linkage disequilibrium between loci in the TCI populations indicate that they could be derived from an expansion of the Bahamas populations southwards. The TCI populations are located at the lowest latitude and longitude limit of the distribution range for this variety. Suitable habitat and local climatic conditions have probably limited forest size and distribution in this region. Pine forests exist on only three of the islands and occupy only circa 3.5% of the total land area of the TCI (2.6% since 2009), whereas in the Bahamas they are present on seven of the islands and cover circa 28.4% of the total land area. It is possible that low rainfall and prolonged drought periods were limiting factors for tree growth and needle length in the TCI populations. Phenotypic variation between the Bahamas and TCI populations was observed for these characters ($P<0.02$). Despite observed morphological and ecological limitations, levels of reproduction and recruitment were similar in both regions, indicating some plasticity or adaptation of local populations to such conditions. Kemp (1973) also reported high individual variation in morphological and physiological traits in the natural forests of *P. caribaea* var. *hondurensis*. He suggested that such variation in traits such as total height, straightness of the stem and branch persistence could be associated to local environmental and ecological differences. It is uncertain if phenotypic plasticity or genotypic variation is responsible for the observed regional morphological variation in *P. caribaea* var. *bahamensis* during this research. Loci under selection with favourable mutations can change patterns of polymorphism in linked neutral loci (genetic hitchhiking), whereas selection against loci carrying deleterious mutations can cause loss of variation in linked neutral loci (background selection) (Fu 1997). Therefore, genetic variation detected in supposedly neutral microsatellite loci could be under the influence of adaptive genes. Nonetheless, further evidence is necessary for such assumptions regarding phenotypic variation and adaptation of *P. caribaea* var. *bahamensis* in the Bahaman archipelago.

Several methods have been suggested in the investigation of genetic×environmental interaction, such as common garden and translocation experiments or the study of heritable variation in adaptive traits (Kawecki and Ebert 2004). A common garden experiment on *P. caribaea* var. *hondurensis* in Brazil used neutral microsatellite loci to infer relatedness and heritability of traits among and within families from different sources (Tambarussi *et al.* 2010). It showed that height, dbh and volume traits were under strong genetic control and genetic correlations between traits were higher than phenotypic correlations. Common garden experiments or translocations are used to compare genotypes from different populations under the same environmental conditions, allowing the identification of traits under selection and specific environmental factors driving such changes. The study of candidate loci linked to local adaptation or quantitative trait loci (QTL), i.e. chromosomal regions or loci involved in the genetic variance observed in quantitative traits, can indicate local adaptation (Kawecki and Ebert 2004, Guevara *et al.* 2005). Several studies in the past two decades have identified QTLs in pines related to growth, water-use efficiency, hardiness, height and diameter and wood density among other traits (Guevara *et al.* 2005). Markussen *et al.* (2003) detected ten putative QTLs for growth traits in *P. pinaster*, which explained circa 22% of the total variance in growth and 48% in the variance in dbh. A study of neutral loci and candidate genes in *P. halepensis* showed a reduction in neutral and functional genetic diversity through natural selection of best adapted genotypes to local conditions, following expansion events. It also identified candidate genes possibly linked to drought tolerance, i.e. Aqua-MIP, dhn-1, erd3, lp 3-1 (Grivet *et al.* 2009). A study on white spruce *Picea glauca* (Moench) Voss in a common garden experiment identified QTLs for bud flush, bud set and height growth, revealing that QTLs explained a great part of the total variance for these traits (≥59%) (Pelgas *et al.* 2011).

In the future some of these techniques could be used to identify genetic×environmental interactions in *P. caribaea var. bahamensis* and elucidate if morphological and genetic differences observed in this research are due to phenotypic plasticity or ecotypic variation. The study of physiological traits could also be interesting in detecting differences in physiological response in *P. caribaea* var. *bahamensis* from different regions to the local climate and xeric conditions. Plasticity in ecophysiological traits was observed in *Abies concolor* (Gordon) Lindl. ex Hildebr. in southern California through adaptation to a dry and hot climate, increased stomatal response to drought, minimisation of water loss and enhanced water conservation (Grulke 2010).

An indication that the TCI pine populations are growing in sub-optimal conditions is their reduced potential to overcome pest attacks. This was highlighted in this research by the high level of infestation by pine tortoise scale insect (90.5% of all quadrats) and high mortality observed in the TCI pine forests (dead pine trees in 40.5% of all quadrats). Although exotic pests have been observed feeding on pine trees in both regions, most pine forests in the Bahamas are able to overcome the attack and regenerate in the absence of stochastic events. The strong negative correlation observed between needle length and tree infestation by insects in TCI trees (Spearman's rho -0.40, $P<0.01$) was possibly related to the fact that drought can reduce production of metabolites and increase susceptibility to pest attacks (Breda *et al.* 2006, Grulke 2010). Research on the chemical response of pine trees to insect attack is currently being carried out in both regions by Dr. Paul Green from Jodrell Laboratory Kew in collaboration with Kew's UKOTs Programme, the Department of Environment and Coastal Resources (DECR) of the TCI and the Bahamas National Trust (BNT). Chemical data for individual trees will also be compared to genetic data obtained during this research, in order to check for genetic variation correlated to chemical response and resistance to pests.

The symbiosis of mycorrhizal fungi to *Pinus* roots is key to the survival of pine trees in extreme environments (Read 1998). It is known that mycorrhizal symbiotic associations maximise the absorption of nutrients and water by the host tree, increasing survivability in xeric environments and poor soils (Read 1998, Allen 2007), but little research has been carried out on ectomycorrhizal fungi symbiosis with *P. caribaea* var. *bahamensis* (Redhead 1982). Sampling and taxonomic identification of mycorrhizal fungi present in the pine forests of the TCI and Bahamas is currently being undertaken by Dr. Martyn Ainsworth from the Mycology Department at Kew in collaboration with Kew's UKOTs Programme, DECR and BNT. Such study could contribute to the understanding of the plasticity observed in the pine trees in the TCI and genetic differentiation of the Pine Cay population. This population showed higher girth and higher percentage of new cones and recruitment than any other population in the same region, despite distinct edaphic conditions, i.e. sandy soil, and small population size. This indicates good fitness of *P. caribaea var. bahamensis* on this island, which could be linked to adaptation or specificity of ectomycorrhizal fungi symbiosis.

The effects of exploitation, urban development and stochastic events on the pine forests of the Bahaman archipelago

Mapping of the historical and current pine forest distribution in the Bahaman archipelago provided valuable data on forest decline, expansion and fragmentation. In association with genetic and ecological data it also allowed the identification of historical, modern and future threats to the pine forest ecosystem and the response of *P. caribaea* var. *bahamensis* to such threats.

Species with a soil seed bank, e.g. *P. caribaea*, usually show better recovery from timber exploitation and deforestation than other wind-dispersed species without a soil seed bank, e.g. *Araucaria nemorosa*. Regeneration in the latter is limited by the existence of mature trees able to produce seed and short seed dispersal distance (<100 m from the source), making them more susceptible to fragmentation and over harvesting (Kettle *et al.* 2011). In fact, *A. nemorosa* is showing signs of increased inbreeding and selfing as a result of the habitat degradation and fragmentation experienced in the past 150 years in New Caledonia (Kettle *et al.* 2007). Nonetheless, López (2003) observed that only 10% of the seeds of *P. caribaea* var. *caribaea* in the soil seed bank of natural pine forest stands were still viable after 2.2 years of dispersal. Logging and fragmentation did not seem to have impaired regeneration in *P. caribaea* var. *bahamensis* in normal circumstances, as shown by the good natural regeneration of most previously logged areas in the Bahamas (Henry 1974, Greaves 1978), but the lack of mature live trees after stochastic events showed to hinder regeneration. No populations of *P. caribaea* var. *bahamensis* showed genetic depauperation and levels of gene diversity are still high in all populations (uH_S>0.487, H_T=0.614). Species with an overall nuclear diversity H_T<0.05 are considered genetically depauperate (Vendramin *et al.* 2008). The value previously reported in an allozyme study of natural populations of *P. caribaea* var. *hondurensis* in Belize and Guatemala was H_T=0.255 (Dvorak *et al.* 2005), which is lower than that found in this research but well above the threshold for genetic depauperation.

Several facts might have contributed to this recovery and present levels of gene diversity in *P. caribaea* var. *bahamensis*. Areas commercially logged in the Bahamas in the past were large tracts of continuous forest, i.e. >126 km^2, there was an established minimum dbh of 0.15 m for tree felling and enough mature trees were left intact as seed sources (Henry 1974). Despite a reduction in the effective population number, forest area was maintained through the presence of scattered mature individuals, intact forest patches and recruitment. In addition, long-distance pollen flow, soil seed bank, wind-dispersed seeds and possibly cleared areas with reduced competition could all have contributed to the recovery of these forests. Pine forests are now growing in most of the areas previously logged and fragmentation is low in the majority of the islands, as observed through mapping, corroborating this idea. The highest variance at neutral loci and morphological traits observed during this research at the individual level, high gene diversity, presence of private and rare alleles and the lack of signs of previous bottleneck events all indicate good forest regeneration.

Species that have undergone bottlenecks, e.g. *P. pinea*, experience loss of diversity, genetic drift and low polymorphism (Vendramin *et al.* 2008). Loss of alleles, significant inbreeding and reduction in effective population size were observed in Mexican populations of *P. caribaea* var. *hondurensis* after a bottleneck event (Delgado *et al.* 2011). In the present research a predominantly outcrossing and random mating system with no significant deviations from Hardy-Weinberg equilibrium was observed, in addition to extensive historic gene flow and high tree density (1-10 mature individuals/100 m^2 in most areas). All these factors contributed to the maintenance of good levels of heterozygosity (He=0.603, Ho=0.525) and allelic richness ($A_{R=}$3.19). Observed and expected heterozygosity were higher than those previously reported for natural populations of *P. caribaea* var. *hondurensis* in Belize/Guatemala (He=0.231, Ho=0.227) and Mexico (He=0.465, Ho=0.422) (Dvorak *et al.* 2005, Delgado *et al.* 2011). Although Grand Bahama lost circa 25% of its forest since the 1960s, as estimated by mapping, regeneration was good and high allelic richness and gene diversity was maintained (A_R=3.33, uH_S=0.558). Good regeneration and maintenance of genetic diversity after logging was also observed in *P. strobus* L., another outcrossing wind-pollinated and wind-dispersed species (Marquardt and Epperson 2004). Comparisons between old growth and secondary growth after logging showed similar levels of allelic richness, respectively 6.7 and 7.3, and expected heterozygosity, 0.48 and 0.49. Unfortunately, no DNA samples or genetic data are available for the period before logging for *P. caribaea* var. *bahamensis* in the Bahamas to allow such comparisons, but it seems that most areas were able to maintain gene diversity or minimize its loss through gene flow and soil seed bank.

Despite good regeneration for most sites (Henry 1974, Greaves 1978, Snyder *et al.* 2007), stochastic events, e.g. hurricanes and non-seasonal fires, did have a major impact on some recovering sites. The occurrence of hurricanes and frequent fires following logging had a major impact in southern Abaco, as observed in the historical maps as previously discussed. Regeneration was impaired by the lack of seed source and death of seedlings and saplings (Henry 1974, Radabaugh 1974, Greaves 1978). Although forest is now restored, a genetic discontinuity and loss of gene diversity and allelic richness was observed in this part of the island, possibly explained by the high death rate of pine trees and seedlings in the past and the introduction of individuals from Andros island during re-seeding (Henry 1974). The southern Abaco population is the only one in the Bahamas showing a tendency to outbreeding (F_{IS}= -0.063). The extensive gene flow between the Bahamas populations and similar environmental conditions between northern Andros (supposed seed source) and Abaco probably facilitated adaptation or plasticity of introduced genotypes and could have contributed to maintenance of fitness. Nonetheless, the current forest is now formed of even-aged stands with low genetic diversity, which could increase chances of stochasticity in the future. There are predictions of an increase in hurricane intensity in the future due to climate change (Geng and Sugi 2003, Bengtsson *et al.* 2007). The increased effect of strong winds on even-aged stands and the explosion in numbers of insect pests after hurricane events have been reported by other authors (Henry 1974, Tanner *et al.* 1991, Gardner *et al.* 1992, Everham III and Brokaw 1996) . These factors, in association with the lack of vegetation gaps and tall undergrowth observed in southern Abaco forest could impair future forest regeneration in the area.

The accidental introduction of the exotic pine tortoise scale insect in the TCI in 2004-2005 (Hamilton 2007) can be considered one of the major stochastic events to present. High pine mortality levels have been observed and forest regeneration is severely impaired, as reported here and from previous research (Green 2011, Malumphy *et al.* 2012). Death of mature trees has reduced seed source throughout the pine forests and surviving seedlings and saplings are under constant attack by the pine tortoise scale insects. Additionally, this drastic reduction in effective population size was followed by a non-seasonal fire on North Caicos Island causing a reduction in forest area of 24.4% in the TCI, as observed through mapping. Consequently, no regeneration followed in the burnt forest of North Caicos (Malumphy *et al.* 2012). The lack of seed source due to death of mature trees, seeds in the soil seed bank, seedlings and saplings is preventing any regeneration and will continue to do so into the future. The effective population size on Middle Caicos, North Caicos and Pine Cay is unknown. However, Green (2011) has estimated a ratio of 68:1 immature to mature live trees and reported a decline in mature pine trees of 96.9% on Middle Caicos and 100% on North Caicos; however, these observations had a restricted distribution and did not cover the whole population area. A future analysis of recent satellite imagery or aerial photography of the area, if available, would help in the estimation of current effective population size and make possible the mapping of live individuals for future seed and seedling collections to assist recovery efforts.

Although seedlings sampled in the *ex-situ* collection of the TCI showed a reduction in allelic richness, loss of private alleles and increased inbreeding (except for North Caicos), it is not possible to attribute these results to the reduction in effective population size due to the recent nature of this stochastic event, the presence of a soil seed bank and the sampling strategy used in this research. A future study of fine-scale genetic structure in these populations sampling adults and seedling cohorts in the field could be valuable in revealing early signs of inbreeding and loss of genetic diversity. Nonetheless, a reduction in effective population size and seedling mortality, associated with possible reduction in area due to climate change, high number of pests, increased inbreeding and reduced fitness could lead to the extinction of TCI populations (Brook 2008).

Additionally, a recent trend in shift in species composition in the pine forests of the TCI was reported by Green (2011). Several plant species, including *Evolvulus arbuscula* Poir., *Coccoloba krugii* Lindau, *Gochnatia paucifloscula* (C.Wright ex Hitchc.) Jervis ex Cabrera and *Scleria lithosperma* (L.) Sw., showed a decrease in abundance with decrease in the number of pine trees as palms started replacing pines in the pine forest ecosystem. Despite the fact that the scale insect is pine-specific and does not attack any of these species, the loss of the overstorey, i.e. pine trees, is altering the ecosystem, possibly due to alterations in light and moisture levels and increased competition by other opportunistic species.

Other frequent stochastic events in the archipelago are hurricanes. Hurricanes Jeanne and Wilma in 2004 and 2005 had a major impact on the pine forests of Grand Bahama. A reduction of 32.7% in pine forest area, corresponding to 49.1 km^2, was observed through mapping and field observations. In this case, the many natural creeks and waterways that cut through the island and low-lying salt marshes on its northern side possibly contributed to an increase in salinity in the area and the death of mature trees, seedlings and soil seed bank impairing regeneration. A change to more halophytic vegetation, i.e. mangroves and sawgrass, was observed in areas with no regeneration. The fact that such observations were carried out 3-5 years after the storm surge and a shift of vegetation was observed suggest that pines may not return to these areas. Loss of pine forest area in the Florida Keys due to climate change has already been reported by Ross *et al.* (2009). The small and isolated fragments of live pine forest that remain in the western tip of Grand Bahama (Figure 2.28) may also not be able to recover in the long term. Small population size and restricted gene flow could lead to inbreeding and loss of allelic richness, as observed in *A. nemorosa* by Kettle *et al.* (2007). Future genetic studies of mature trees and seedling cohorts in these fragmented populations on Grand Bahama could reveal such tendencies and guide management.

The increase in population size, urban areas and agriculture has also affected several islands in the Bahaman archipelago since the 1960s, as observed through comparisons of historical and modern maps. Existing agricultural plots inside pine forest areas are probably not extensive enough to impact gene flow and number of individuals in the population, but they can contribute to the spread of pests and invasive plants to native habitats. Also, the agricultural practice of ‘slash and burn’ poses a serious threat to the pine forest ecosystem. The loss of 91.5% of the pine forest on North Caicos, as previously discussed, shows the potential harm resulting from an escaped non-seasonal fire from an agricultural plot. Despite the fact that ecosystem maintenance and regeneration of the pine forests of the Bahaman archipelago are driven by fire, non-seasonal and frequent fires can have a negative impact on regeneration and pine survival (Myers, R. *et al.* 2004, Miller, A. C. 2007, O'Brien *et al.* 2008, O'Brien *et al.* 2010). Some effects of high frequency dry-season fires are starting to show in northern Abaco, where the lowest tree density for the Bahamas pine forests was observed, i.e. <six mature trees/100 m^2 in 80% of the quadrats and no quadrats with >10 mature trees, and also the lowest overall recruitment, i.e. 26.7% of the quadrats. The high frequency of accidental and dry-season fires reported for this island (Myers, R. *et al.* 2004, Miller, A. C. 2007, Robbins *et al.* 2010) could have resulted in the observed death of seedlings and saplings and reduced tree density (Myers, R. *et al.* 2004, O'Brien *et al.* 2008). Therefore, dry-season fires can impact pine forest ecology, structure and regeneration (Sealey-Sullivan *et al.* 2002, Myers, R. *et al.* 2004, O'Brien *et al.* 2008).

Urbanisation and deforestation had a major impact on the island of New Providence. The pine forest of New Providence was almost clear cut from 1923-1926 (Miller, W. 1920, Kellogg 1951) and further exploitation at a small, local scale for timber and charcoal persisted during the following decades (Henry 1974, Greaves 1978). Further deforestation followed reducing the pine forest area by 63.6% in just 48 years (1961-2009), due to urbanisation and population expansion (Bounds 1981b). Nowadays, just the western block of forest remains occupying an area of 24.8 km^2. Tree density is low (<six mature trees/100 m^2) for the majority of the plots. Trees previously cut for timber in the Bahamas were circa 30 years old, according to Henry (1974). Therefore, pine stands on New Providence could have been harvested several times since the 1900s and have possibly been exploited at a local scale since pre-history (Blick 2007). Significant inbreeding (F_{IS}=0.09, P=0.04), the lowest gene diversity overall (uH_S=0.487) and one of the lowest allelic richness values (A_R=2.76) were observed in the pine population on this island. They are probably the result of small initial population size with low genetic diversity, followed by continuous reduction in the effective population size, which led

to further losses of gene diversity and allelic richness through genetic drift and inbreeding (Brook 2008). Delgado *et al.* (2011) reported loss of genetic diversity due to genetic drift, small effective population size and inbreeding (F_{IS} 0.097 and 0.163) in *P. caribaea* var. *hondurensis* in Mexico. They suggested that a reduction in area and size resulting in only two Mexican populations, Ejido de Caobas and Ejido Pioneros del Río Nuevo Centro Poblacional, with <1000 and 14 mature individuals, respectively, was related to periods of abundant rainfall and prolonged floods.

To sum up, it seems that the effects of logging, deforestation, hurricanes and non-seasonal fires varied according to regeneration potential, habitat, initial population size, genetic diversity and gene flow. However, the additive effect of several stochastic events in a short period of time had major consequences at the population level, as is the case for southern Abaco and North Caicos, where such events had a major impact on population size, eliminating seed sources and regeneration and reducing population area. Properly managed exploitation of forests had less impact on genetic diversity and regeneration of *P. caribaea* var. *bahamensis* forests in Bahamas than did stochastic events. However, the exploitation of small forest areas, e.g. on New Providence, seemed to lead to loss of gene diversity and inbreeding which could have negative consequences on this population in the future. It is also not possible to compare genetic diversity and allelic richness before and after exploitation due to lack of data. Nonetheless, genetic data of modern forests indicate that most populations have a random and outcrossing mating system with high gene flow and high gene diversity. No data on loss of biodiversity in the pine forest ecosystem were recorded during this research. However, evidence shows that many parrot species found in the Caribbean have suffered population loss or even gone extinct due to loss of habitat (Wiley 1991). Green (2011) also showed a decline in species abundance linked to loss of pine trees in the TCI, as discussed above. Thus, managed forest exploitation should take into account environmental, ecological and stochastic factors and have contingency measures put in place to ensure future regeneration and maintenance of a functional ecosystem. Previous knowledge of existing species in the ecosystem and their interaction and biology is valuable information in management plans, as is seed collection and banking of species before exploitation.

Promoting forest health, regeneration and biodiversity

The effects of insect pests on pine forest health can be devastating, as it has been observed in the TCI. Insect pests were recorded in all pine populations observed during this research, with the exception of southern Abaco and Mangrove Cay (Chapter 4, section 4.3.2). Additionally, many other insect pests not previously recorded were collected in the pine forests of the Bahamas and the TCI during a recent visit by Dr. Chris Malumphy (M.A.Hamilton 2012, pers.comm., June 10). Although no major damage was observed in the Bahamas pine forests, such pests could become a major problem in the future. A trend to sea level rise, increased hurricane intensity, increased temperatures and changes in rainfall, as predicted by many authors (Angeles *et al.* 2006, Burkett *et al.* 2008, Elsner and Jagger 2010, Nicholls *et al.* 2011), will probably have an impact on water availability and pine tree susceptibility to pests and diseases in the Bahaman archipelago. Tree mortality has previously been related to increased temperatures, prolonged droughts and pest attacks (Rouault *et al.* 2006, Grulke 2010). Logan *et al*. (2003) predicted an intensification of pest outbreaks with global warming and Ross *et al.* (2009) showed a reduction in forest area due to sea level rise in the Florida Keys. Therefore, it seems that the pressure on natural island environments is increasing; hurricanes may become more intense and fresh water less available due to droughts and salinization. Pest outbreaks will also tend to become more frequent. Therefore, legislation, inspection and quarantine of imported plant material to the Caribbean islands will be even more important in the near future in order to avoid the introduction of exotic pests (Malumphy *et al.* 2012). Pest monitoring and control in natural areas will also be of the utmost importance in preventing and controlling pest outbreaks, avoiding demographic stochasticity of native plants, loss of biodiversity, loss of genetic diversity and decay of ecosystems.

The promotion of fire management programs and suppression of dry-season fires could also help maintain biodiversity and regeneration of the pine forest ecosystem. Evidence of fires and pine recruitment was observed in all pine populations during this research. Natural fires during the wet season and prescribed fires promote pine regeneration and the maintenance of the pine forest ecosystem, as previously discussed in Chapter 1 section 1.4.2. Prescribed fires are frequently used to reduce fuel load, minimize fire damage and increase fire control without affecting pine and undergrowth regeneration (Ne'eman and Izhaki 1999). Guldin (2007) suggested the use of several cycles of prescribed fires a couple of years before forest exploitation to reduce fuel load and quick start regeneration before reduction in number of the effective population as a strategy to maximize genetic diversity as a seedling bank. A study of a prescribed fire carried out during the wet season on the island of Abaco in 2004 showed that all trees and saplings survived the low intensity fire, and regeneration of the pine forest followed, proving the adaptation of this ecosystem to low intensity fires during the wet season (O'Brien *et al.* 2008). A relation between plant species diversity and fire in the pine forest ecosystem has been previously reported by De las Heras *et al.* (2005) and Van Lear *et al.* (2005), showing a decrease in species diversity with fire suppression or frequent high intensity fires and an increase with seasonal fires.

Myers et al. (2004) reported that most pine forests on Abaco and Andros are even-aged as a result of previous logging and regeneration seemed to be impaired by high tree density or frequent fires. Nonetheless, pine forest area expanded in these two islands according to the present research, mainly near settlements, resulting in an increase of circa 5% in the total pine forest area in the Bahaman archipelago. Such expansions of pine forest area driven by dry-season fires are known to cause a reduction in the area of broadleaf forest and associated species (Myers, R. *et al.* 2004). When such dry-season fires are too frequent they can also impair pine and shrub regeneration and promote the spread of grasses and ferns (Sealey-Sullivan *et al.* 2002, O'Brien *et al.* 2008), as it was observed in northern Abaco. Frequent dry-season fires during and after logging in the pine forests of Nicaragua and Guatemala resulted in sparse or no regeneration (Kemp 1973). They can also have an effect on local and migratory birds that depend on these forests for their survival (Dvorak *et al.* 2005). It seems that the occurrence of dry-season fires may become more frequent with urbanisation and higher demand for agricultural land. Despite existing legislation regulating illegal fires in the Bahamas, i.e. Forest Fires Act 1985 and Forestry Act 2010, fire incidence in that country is still high (Myers, R. *et al.* 2004, Miller, A. C. 2007, Robbins *et al.* 2010). Signs of changes to the pine forest ecology and species composition are starting to show as a result of such fires, as discussed above. In the TCI, on the other hand, dry-season fires are not so common, but there is no legislation in place against illegal burning of forests and uncontrolled fires during slash and burn practices. Nonetheless, the effect of dry-season fire in the current TCI pine forest was catastrophic and future occurrence should be avoided at all costs to prevent possible extirpation of the TCI pine populations.

Understanding local fire ecology and using such knowledge for fire management and awareness seems to be the most useful approach to the improvement and maintenance of pine forest areas (Rodríguez-Trejo and Fulé 2003, Myers, R. *et al.* 2004, Myers, R. 2006). The new Forestry Act (The Commonwealth of the Bahamas 2010) regulates prescribed fires in the Bahamas, until then illegal, opening the possibility of fire management in the pine forests of that country. At present, trials with prescribed forest fires and subsequent monitoring are being carried out in the TCI to reduce fuel load, prevent future high intensity fires and better understand the effects of prescribed fires in this decaying pine forest (M.A.Hamilton 2012, pers.comm., June 10).

Conservation and protection of **P. caribaea** *var.* **bahamensis** *native forests*

The Caribbean region is considered one of the world's biodiversity hotspots due to its high level of endemism and large loss of habitat, with only 11.3% of its original vegetation remaining (Myers, N. *et al.* 2000). Recent work by Anadón-Irizarry *et al.* (2012) identified 36 key biodiversity areas, i.e. areas with presence of globally threatened species, in the Bahaman archipelago and only two of those were under some protection in 2010. The preservation of the pine forests of the endemic *P. caribaea* var. *bahamensis* in the Bahaman archipelago has many implications at the social, economic and ecological levels. This species is a keystone species in the pine forest ecosystem which many other plants and animals depend upon for survival, it is an important timber species and it grows over the main fresh water reserves for these islands. The Bahamas Forestry Act (The Commonwealth of the Bahamas 2010) recognised three classes of forest use to be declared in any Crown land: forest reserves, protected forests and conservation forests. Forest reserves are managed natural or planted forests for sustained exploitation, which could be used as parks and recreational areas or for the conservation of natural resources, fauna and flora. Conservation forests are areas containing significant natural resources or biological diversity or being of ecological importance, natural or scientific interest. Protected forests have similar uses to forest reserves, but they can be designated for other land uses or development if necessary. No areas have yet been designated under this act, but it is indeed an important tool for forestry management, including fire management, sustainable timber exploitation and conservation of biodiversity and genetic diversity. Data on the genetic structure, genetic variability, gene flow and population sizes in *P. caribaea* var. *bahamensis* produced during this research could be a valuable tool for the future conservation and management of this variety. Frankham (2010) highlighted the importance of defining evolutionary units, understanding species biology, genetics, environmental and demographic variables for species conservation.

The ability of plants to survive threats and unfavourable conditions through phenotypic plasticity, migration or adaptation is essential for their long-term existence (Kramer and Havens 2009). Thus, the conservation of the genetic diversity and phenotypic plasticity of a species are important for their future survival in face of predicted environmental changes and stochasticity (Frankham *et al.* 2002, Chambel *et al.* 2005). Plasticity in slow-growing and long-lived species plays a major role in their response to extreme climatic conditions, habitat fragmentation and species competition for favourable habitats (Valladares *et al.* 2007). Plants need ways to adapt to changing conditions in their original habitat until they can migrate by seed dispersal to more favourable areas and even after migration they still depend on plasticity to adapt to new environments and compete with other species. Predictions for the response of *P. patula* Schiede ex Schltdl. & Cham. and *P. tecunumanii* to climate change and field trials indicate that both species have genetic diversity and plasticity to adapt to new environmental conditions, but active protection of forest areas and genetic resources must be implemented to avoid loss of such variability through short-term threats (Van Zonneveld *et al.* 2009). Shea and Furnier (2002) recognised the importance of preserving genetic variability across the species range of balsam fir (*Abies balsamea* (L.) Mill.), including marginal populations in order to preserve the evolutionary potential of the species and adaptability potential for future conditions. Frankham *et al.* (2002) mentioned the need to preserve species as dynamic entities which can adapt to environmental change, thus stressing the importance of conserving the species allelic richness, genetically different populations and ecotypes in order to preserve its maximum genetic diversity. Caballero and Toro (2002) suggested the use of expected heterozygosity and allelic richness of populations and subpopulations to select areas and individuals for long-term conservation programmes. Dvorak *et al.* (2005) highlighted the need for conservation of the pine forests of *P. caribaea* var. *hondurensis* in the swamps of the Yucatán Peninsula due to their high gene diversity and presence of private alleles and also their local ecological value. Parmesan (2006) also considered that species at lower latitudes will be subjected to more extreme conditions due to global warming and climate change and genetic variability at species level will be important for adaptation and survival. Thus, the conservation of the genetic variability and plasticity of *P. caribaea* var. *bahamensis* seem to be of utmost importance for its adaptation to harsher conditions and possible colonisation of new habitats in the Bahaman archipelago.

As mentioned before, more extreme climate and increased intensity of stochastic events are predicted for the future (Angeles *et al.* 2006, Bengtsson *et al.* 2007, Valladares *et al.* 2007, Burkett *et al.* 2008). Changes in the migration patterns of forest trees are expected as a result of climate change; thus, seed dispersal distance, habitat availability in transition zones and forest fragmentation will also influence the capacity of migration and establishment in new areas (Schwartz *et al.* 2001). Endemic island taxa, such as *P. caribaea* var. *bahamensis*, which have probably been isolated since the late Pleistocene (Jardón-Barbolla *et al.* 2011), will possibly have their range and population size reduced due to lack of island habitat for colonisation and distance to other islands and mainland. Many pine islands in the archipelago face risks of inundation in the coastal areas with current predictions of sea level rise (Chapter 2, Figure 2.41), which will tend to reduce land area, shift coastal populations inland, increase salinity near coastal areas favouring salt-tolerant species, reduce fresh water availability, increase pressure on existing forest areas and possibly reduce the size of pine forest populations across the archipelago (Burkett *et al.* 2008). In 2005, the Bahamas national policy for the adaptation to climate change recognised the threats associated to changes in temperature and sea level in the archipelago, including salinization of soils, rising water tables and soil erosion (The Commonwealth of the Bahamas 2005b). This resulted in the recommendation to develop a forestry management plan and increase protection of existing forests.

In-situ conservation through a system or network of effectively managed protected areas containing the species genetic diversity enables the occurrence of evolutionary changes in the species and populations to be preserved. However, economical and managerial limitations usually limit the size of such protected areas. (Heywood and Iriondo 2003). Ledig, in an article on the conservation of genetic diversity of forest trees (1988), recognised *in-situ* conservation in protected and managed natural forests the most effective means of preserving the species genetic pool. He also suggested the establishment of large genetic resource management units (GRMU) as a way of preserving and protecting the genetic diversity of a species or community. Such units would be large enough to allow gene flow and outbreeding, could support a range of different species and could also be used for sustainable timber harvesting, if this activity is controlled and does not deplete genetic diversity. In 1980, Soulé discussed the concept of conservation biology and stated that larger areas are more likely to survive stochastic events in the long term, maintaining genetic diversity of the species and the ecological function of the habitats; however, a network of several small areas can also have the same effect if linked by gene flow. Moritz (1994) defined management units (MUs) as populations with a current divergence of allele frequencies at nuclear or mitochondrial regions. Therefore, MUs are relevant for short-term management taking into consideration modern population structure and gene flow (Frankham *et al.* 2002). The consideration of the Bahamas populations and the TCI populations as different MUs in this case makes sense in the genetic, ecological and political context. Following Moritz's definition, further smaller MUs could be considered for each country due to its particular genetic frequencies, plasticity or adaptation and ecology, i.e. Abaco in the Bahamas and Pine Cay in the TCI. Crandall *et al*. (2000) suggested that even in the presence of only one evolutionary significant unit (ESU), as in the case of *P. caribaea* var. *bahamensis*, distinct populations should be preserved in order to maintain the adaptive diversity and evolutionary potential of the species. Therefore, the loss of the Bahamas or the TCI biogeographic population would result in lower diversity and reduced adaptation potential for the species in the long term.

Farjon and Page (2003) also recognised the urgent need for *in-situ* conservation of pine forests worldwide through protected areas. The combination of genetic, demographic and ecological parameters applied to targeted species management could enhance management efficiency and chances of species survival (Noël *et al.* 2010). Existing conservation areas in the Bahamas do not cover all the genetic variation present in the populations. The ecoregional plan for the Bahaman archipelago by Sealey-Sullivan *et al.* (2002) recognised the importance of preserving large tracts of pine forest on Grand Bahama, Abaco, Andros and North Caicos and smaller preserves on New Providence and Pine Cay in order to maintain a system of heterogeneous and functional ecosystems and preserve biodiversity in these islands.

The only pine forest conservation area on Abaco is South Abaco National Park, which is located in southern Abaco and is the second largest protected pine forest in the archipelago. Unfortunately, the pine population present in this area was devastated in the late 1960s and then possibly re-introduced with seed from another island (Chapter 2, section 2.4). Consequently, the current population does not represent the genetic diversity found in other parts of Abaco, being genetically similar to Grand Bahama, New Providence, northern Andros and South Andros. Also, gene diversity at plastid and nuclear levels in southern Abaco is one of the lowest in the archipelago. Forest structure is also similar to that found in secondary managed forests. Nonetheless, the area shows good regeneration and is ecologically important, being a major nesting site for the IUCN red listed Bahamas parrot (Stahala 2005, BirdLife International 2008a). As seen in this research, northern Abaco (including the northern part of Great Abaco and Little Abaco) has no protected areas of pine forest, but it is genetically distinct from all other Bahaman populations, with the exception of Mangrove Cay, showing the highest allelic richness and gene diversity of all populations (A_R 3.94, uH_S 0.643). Additionally, Little Abaco is one of only two remaining areas of old-growth forests in the Bahamas. The cryptic genetic similarity of Mangrove Cay to northern Abaco (F_{ST} 0.027, P=0.11) and the lack of detailed data on past logging activities and forest regeneration in this area suggest the occurrence of a single colonisation event from Abaco to Mangrove Cay (Chapter 3, section 3.4). Despite the low number of samples analysed and restricted collection area, this population showed one of the highest levels of allelic richness and gene diversity in the archipelago (A_R 3.60, uH_S 0.604) and lack of trees with fascicles containing only two needles as was observed in other Bahaman islands. Further genetic and morphological study of this population covering a larger sampling area and a higher number of trees could reveal if the same pattern is present throughout the whole area or only in previously logged parts of the island and evaluate the need for protection or conservation of these resources.

Mangrove Cay, South Andros and the western part of Central Andros pine forests have recently been included in the West Side National Park and a small part of the northern Andros pine population is included in the Blue Holes National Park (The Bahamas National Trust 2012), creating the largest area of protected pine forest in the archipelago. Despite the genetic similarity of northern and South Andros populations (F_{ST} 0.000, P=0.13), the latter is at present the largest old-growth forest remaining in the Bahamas. This area contains a varied forest structure and rich wildlife. It is possibly also one of the only areas without anthropogenic influence, because of its remoteness and limited access until recently; thus it is a unique resource for research on a native and practically untouched Bahaman pine forest. The rapid ecological assessment carried out by Lloyd and Slater (2010) in South Andros pine forests also showed a richer mosaic of tree ages and undergrowth composition, in addition to a greater abundance and higher frequency of endemic and near-endemic birds than that observed in the secondary pine stands in northern Andros. Old-growth forests can also be used as a model for the management of secondary forests, based on the observed community composition, processes and structures found in these old natural areas (Foster *et al.* 1996). Andros pine forests are also home to the Andros Island rock iguana *Cyclura cychlura* ssp. *cychlura*, which is an IUCN red listed species (Knapp, C. R. and Buckner 2004, Knapp, C. R. and Owens 2005). Therefore, the protection of the pine populations of Mangrove Cay and South Andros were an important step in preserving genetic diversity, plasticity and biodiversity of this ecosystem. Current levels of disturbance to the northern Andros pine forests include agriculture, high frequency dry-season fires and high volume of fresh water extraction for local use and exportation to New Providence. When fresh water extraction from the water lens exceeds recharging through rainfall, the saline layers rise and the water turns brackish. This happened in the Blue Hills well fields on New Providence, which took 30 years to return to its original state (Sealey 1985). Therefore, it is possible that the pine forests in northern Andros well fields will soon start showing signs of water stress. Demand for agricultural land, exploitation and sea level rise could be part of future threats to the pine population in northern Andros. However, its genetic similarity to Grand Bahama and South Andros could minimise losses in the species genetic pool even in the event of reduction of population size in this part of the island, considering that the other two populations are protected.

Grand Bahama has two protected areas, Rand Nature Centre in Freeport and Lucayan National Park in the central part of the island near the coast, totalling 0.56 km^2. Both areas are important centres for research, education and awareness in the Bahamas, but too small for *in-situ* conservation of the genetic diversity of the pine forest on the island, which comprises 456.1 km^2. Although this pine population was genetically similar to northern Andros and New Providence, it did have the highest number of alleles for plastid and nuclear regions (Tables 3.5 and 3.10) and some morphological variation in tree height and number of needles per fascicle (Chapter 4, section 4.4). Even though this population lost circa 33% of its forest area due to storm surges in the past decade and increasing salinity of the fresh water lenses due to artificial canals and sea level rise, large areas of forest still survive (Figures 2.28 and 2.31). Taking into consideration the predicted increase in sea level and urbanisation in the future and the resilience of surviving individuals on this island, this pine population could be a valuable source of adaptive genetic diversity for the survival of the species to coming changes. Sustainable forest management of the pine forest of Grand Bahama could be the key for the protection of such genetic resources in an economically viable way, as high genetic diversity is still present in this area after previous timber exploitation. Nonetheless, the creation of an *ex-situ* collection representative of such diversity in a seed bank before any exploitation takes place would be a safeguard measure against genetic erosion in the case of future stochastic events.

Pine populations with little or no legal protection are found on New Providence and the TCI. New Providence lost most of its pine forest area in the past 50 years; most of the remaining forest is not protected and there is a growing pressure for urbanisation and development. The pine population also shows signs of inbreeding and loss of genetic diversity. Further reduction in forest size would probably enhance inbreeding, reduce genetic diversity and reduce fitness of the existing pine population. Frankham (1996, 2005) showed that loss of genetic variation and reduced ability to adapt to changing environmental conditions are linked to the reduction in population size and that inbreeding depression can have an immediate impact on extinction; thus further reductions in forest area and number of effective populations could result in total forest loss on this island in the future. Kettle *et al.* (2007) also discussed the importance of management plans and reduction of habitat loss for the future survival of the inbred, small and fragmented populations of *Araucaria nemorosa* in New Caledonia. The close genetic distance of New Providence pine population to populations of Grand Bahama and Andros could be an advantage in forest management in the case of genetic reinforcement with seeds from these provenances to reduce inbreeding. Even though it showed lower genetic diversity, small size and similarities to other Bahaman populations, a high diversity of fauna and flora were observed in the remaining pine forests of New Providence during this research. Bounds (1981b) suggested the establishment of parks and wilderness areas on New Providence as a way of creating habitat for animals and plants, improving quality of life and protecting existing fauna and flora. Therefore, with protection and management New Providence pine forest areas could become a valuable resource for educational and scientific purposes, in addition to their own ecological value for local biodiversity.

In the TCI, no pine forests are legally protected and total pine forest area is reduced to only 13 km^2. It is unknown if these forests have ever occupied other parts of the islands, but current favourable habitat is extremely reduced with most forest growing inland in limestone rock areas with higher fresh water availability and protection from wind and salt spray by existing ridges. Despite the previously discussed pest outbreak and severe die back in these forests, such areas are vital for a successful recovery program and future re-introduction of the pine trees. The protection and management of existing pine forests *in-situ* are vital to ensure their survival, as are *ex-situ* collections. The Caicos Pine Recovery Project in the TCI, a partnership between Kew and the TCI Government, has been active in the study of forest biology, structure and regeneration, monitoring pest levels and trialling methods for pest control. They have also established an *ex-situ* collection of *P. caribaea* var. *bahamensis* on North Caicos, where seeds and seedlings rescued from the wild populations are grown for future re-introduction (Salamanca *et al.* 2010, Green 2011, Malumphy *et al.* 2012). The concept of re-introduction includes the introduction of new individuals within the range of a species in order to restore species diversity (Moritz 1999, Vergeer *et al.* 2008). The protection of the present forest area in this country is important for the success of future forest recovery and maintenance, because of limited favourable habitat for pine growth in these islands, on-going research, seedling rescue, seed banking and future re-introduction. It is also crucial to the maintenance and survival of the pine forest ecosystem and associated fauna and flora.

The comparative study of wild populations and *ex-situ* collection in the TCI showed that the *ex-situ* collection failed to capture all alleles present in the wild, but it does contain a good representation of the gene pool in the TCI (Chapter 3, section 3.3.3). It is unclear if this resulted from the restricted seedling collection area or if it is a response to early signs of genetic loss due to drastic reduction in the effective population size. Future study of mature trees and seedling cohorts in the wild could help to elucidate this matter. Meanwhile, it would be advisable to increase the number of individuals in the *ex-situ* collection and extend the collection area, if viable. In this way, future re-introductions would be less susceptible to founder effect and genetic bottleneck (Vergeer *et al.* 2008). The establishment of a nursery stock from wild collected seedlings has also been suggested by Kettle *et al*. (2007) for the conservation of *Araucaria nemorosa* forests, as seedlings supposedly contain similar genetic variation to the adult population and have already been subjected to natural selection.

No genetic differences were observed between North and Middle Caicos populations during this research (F_{ST} 0.000, P=0.53), but morphological differences were observed. It seems that lower rainfall and reduced water availability on Middle Caicos are limiting pine growth in that population. However, the Pine Cay population showed significant genetic distance (P<0.05) to all other populations in the Bahamas and the TCI and also good fitness, which could be an indication of some adaptation to local conditions, i.e. sandy soils and good fresh water availability. Common garden or translocation experiments could help identify adaptation and growing potential of seedlings from different provenances. However, such experiments can be costly and time consuming. Therefore, the observation of local provenance material during re-introductions in the TCI in the future, mainly on Pine Cay, could maximise rate of success.

The risks of introducing material from a genetically different and non-adapted source to a local population were discussed by Kramer and Havens (2009), suggesting that they could cause immediate or long-term survival failure due to lack of phenotypic plasticity of newly introduced material to adapt to new conditions. Crémieux *et al. (2010)* observed a decrease in fitness and dilution of local adaptation in local populations of *Plantago lanceolata* L. when plants from geographically or ecologically different sources were translocated to the area and crossed with native stock. Vergeer *et al.* (2008) discussed in detail the risks and benefits of introductions of nonlocal sources to small inbred populations, including heterosis, outbreeding depression and increased genetic diversity. However, a recent study by Frankham *et al.* (2011) showed that risks of outbreeding depression are considerably lower than previously estimated, being more likely to occur in crosses between populations with different karyotypes which have been separated >500 years and from different environments. Therefore, translocations and introductions of new source material should be based on knowledge of the species genetics and biology in order to balance risks and avoid further genetic depauperation or extinctions. The translocation of material to help plants adapt faster to climate change has been suggested by Etterson (2008) after garden experiment studies of phenotypic plasticity and adaptation in *Chamaecrista fasciculata* (Michx.) Greene. She found that aridity will increase in the next 25 to 35 years in northern areas of the species distribution in Minnesota and local plants will not have enough time to adapt to such changes. However, aridity adapted plants from the southern population of Oklahoma could be used in the future to increase speed of adaptation and fitness to new conditions in Minnesota.

Ex-situ collections in seed banks are a safe and economical way to preserve the genetic diversity of a species, but its success will depend on extent and representativeness of seed collection, seed storage behaviour and viability (Ledig 1988). Seed collection and banking from genetically distinct populations and throughout the species range can be a valuable way of preserving a great part of the gene diversity of a species for future use (Farjon and Page 2003). Williams *et al*. (2007) suggested seed collections of *P. elliottii* var. *densa* from several different forest fragments in Florida, observing seed collection distance of 15m between trees, in order to preserve genetic diversity for future restoration work. A collaborative program in North America has started collecting seeds from high elevation white pine species, including *P. albicaulis* Engelm., *P. aristata* Engelm., *P. balfouriana* Grev. & Balf., *P. flexilis* James, *P. longaeva* and *P. strobiformis* Engelm. since 2008 with the objective of conserving the genetic variation throughout the range of the species for future use and undertaking scientific research (Sniezko *et al.* 2011). According to Robbins (1983, re-issued 1994), seeds of *P. caribaea* are orthodox and can be stored for five to ten years at 6-8 % seed moisture content and 3-4 °C without significant loss in viability. Therefore, seeds could be probably stored for several decades in controlled humidity at lower temperatures, but eventually a new collection will have to be carried out from the existing populations or from material grown from stored seed. The genetic and biogeographic data produced during this research could help in planning representative seed collections. Considering all populations are represented in a seed collection, another limitation consists of the limited number of individuals that can be sampled in large populations with high individual variability, such as *P. caribaea* var. *bahamensis* (Ledig 1988, Heywood and Iriondo 2003). Nonetheless, seed collection and banking from remaining live mature individuals in the TCI pine forests is an important safeguard for the future survival of these small populations, taking into account the rapid decline of the TCI pine forests, high mortality of mature individuals and reduced resources for an extensive nursery collection of live plants. Although *ex-situ* collections of a species fail to preserve associated biodiversity and ecosystem services linked to that species; they are still valuable tools for the future conservation of species facing habitat degradation, climate change and stochasticity.

So it seems that the integrated *in-situ* and *ex-situ* conservation is still the best available option for preserving *P. caribaea* var. *bahamensis* forests and gene pool. The Global Strategy for Plant Conservation (GSPC) recognised the importance of *in-situ* and *ex-situ* conservation of plant species, in addition to plant research, sustainable use of plant resources and education and awareness of plant diversity (Secretariat of the Convention on Biological Diversity 2009). Heywood and Iriondo (2003) highlighted the importance of combining genetic, ecological and environmental data in species conservation. García Quintana (2006) used such data obtained from his research on *P. caribaea* var. *caribaea* forests in Pinar del Río in Cuba and other sources to suggest guidelines for a species management plan, including *in-situ* and *ex-situ* strategies for species conservation. Therefore, an integrated management plan including *in-situ* and *ex-situ* conservation strategies based on genetic, ecological and demographic data could maximise the maintenance of the genetic diversity and potentiality of adaptation of *P. caribaea* var. *bahamensis* to new threats and environmental changes, e.g. climate change, sea level rise, pest outbreaks and demand for land use.

The combination of genetic, morphological, ecological and biogeographical data obtained during this research allowed the observation of forest decline and expansion, patterns of historic and modern gene flow and genetic structure, levels and patterns of morphological and genetic variability, historic and current threats to the pine forest ecosystem and forest response to environmental and demographic stochasticity. Besides furthering scientific knowledge on the focus species, this work is also valuable in planning future species management and conservation work.

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