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Présentée par
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Biology and population genetics of the White-crowned Pigeon *Patagioenas leucocephala*, and the Scaly-naped Pigeon *P. squamosa*: implications for conservation

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« L'Homme semble privilégier la croyance au savoir, l'avoir à l'être, et l'image du bonheur au bonheur lui-même. Il se pense maître de tout, mais ne se maîtrise pas. Il est la seule espèce à avoir développé la capacité de détruire son propre environnement, sans avoir développé la sagesse de ne pas le faire. L'humain, si précoce, est encore immature, capable du meilleur comme du pire. Parviendra-t-il à l'âge de raison avant d'avoir brûlé sa propre maison ? Cette question est tout, sauf insignifiante. »

Translation attempt

"*Man seems to privilege belief over knowledge, having over being, and the image of happiness over happiness itself. He thinks he is the master of everything, but does not control himself. He is the only species to have developed the capacity to destroy his own environment, without having developed the wisdom not to do so. The human, so precocious, is still immature, capable of the best as well as the worst. Will he reach the age of reason before he burns his own house? This question is anything, but not insignificant*"

Franck Courchamp DR1 CNRS et Laurent Mizrahi
Une espèce à part, série documentaire diffusée sur ARTE

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INTRODUCTION

Biodiversity issues in the Caribbean region: between conservation and tradition

The rise of the Caribbean islands, its current location and climate

With a total area of 230,000 km² spreading across 4 million km² of sea, the Caribbean region is an archipelago consisting in a succession of close islands situated to the East of Central America, and between North and South America (Miloslavich et al. 2010; Figure 1). The region can be divided into three island groups. In the northern extremity of the archipelago, the Northern Caribbean region corresponds the Bahamas Bank islands (i.e., formerly The Bahamas and Turks and Caicos Islands). To the south, the Greater Antilles consist of Cuba, Hispaniola (*i.e.,* Haiti and the Dominican Republic), Jamaica, Puerto-Rico and the Virgin Islands. The southern extremity of the region, corresponds to the Lesser Antilles that includes all the islands between the Virgin Islands and the island of Tobago (Figure 1).

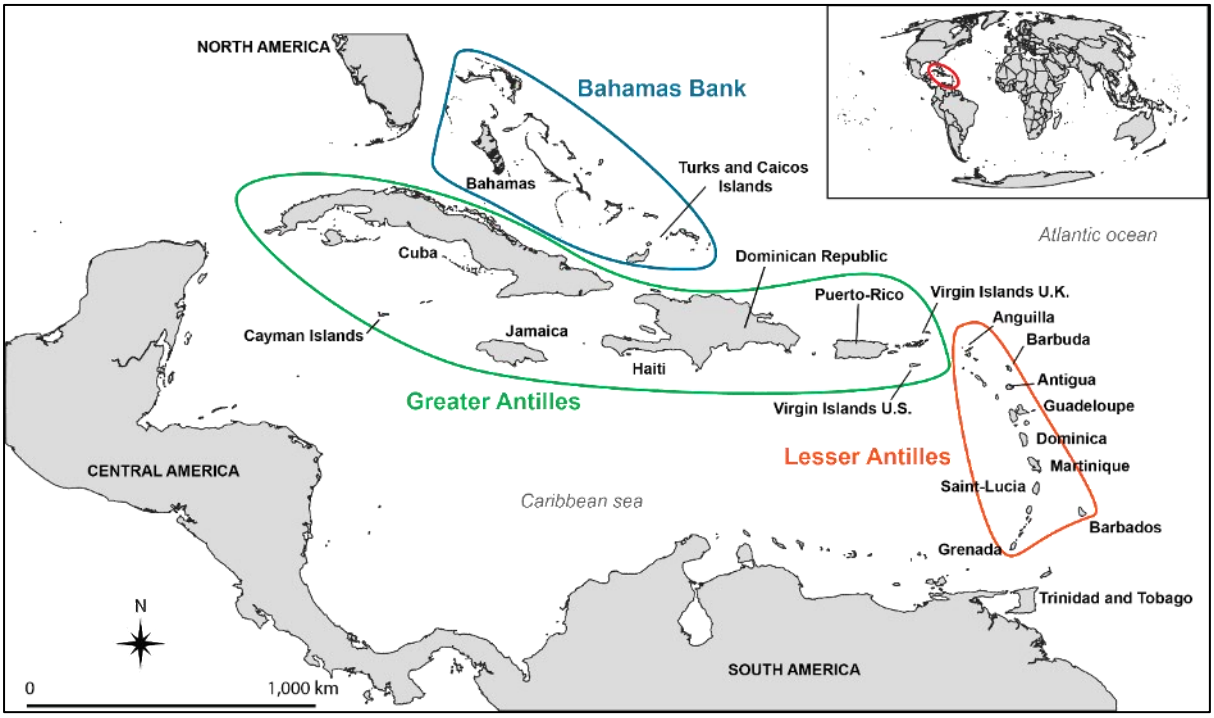


Figure 1 : Map of the Caribbean islands as defined by Higman (2010). In the framed map, at the top right, is a world map in which the Caribbean islands are located (red ellipse).

These islands have emerged from geological events, very active in the region in consequence of the interactions between the Caribbean Plate and the Atlantic Plate to the north, south and east, and between the Nazca and Cocos plates to the west (Jackson 2002; James 2005, 2009). These interactions have resulted in subduction zones and rifts that led to

the formation of contemporary Caribbean islands over time and geological events (e.g., volcanic activity and emergence of oceanic ground above sea level or accretionary prism; James 2005). Accordingly, this results in a region consisting of islands presenting different topographies, with flat and calcareous islands, such as The Bahamas or Barbados, and mountainous and volcanic islands, such as Hispaniola or Dominica.

Although the weather may vary among and within Caribbean islands, in relation to differences in topography and to island location within the Antillean arc, the Caribbean climate is globally defined as tropical and year-round warm to hot (Moron et al. 2016). For instance, temperature and humidity within and among islands vary according to an elevation gradient (e.g., Daly et al. 2003; Sobel et al. 2011). Overall, the region is exposed to two main seasons: the wet season extending from June to December, concentrating most of annual rainfall and coinciding with the hurricane period, and the dry seasons that occurs the other part of the year (Taylor et al. 2011). Recently, and due to global change, the Caribbean has experienced more frequently high-damaging climatic events such storms and hurricanes, and droughts and floods in relation to El Niño and El Niña events (Giannini et al. 2000; Pulwarty et al. 2010). Climate has already changed due to natural factors, such as natural climatic variation of the Earth, as occurred in the past (Hodell et al. 1991; Higuera-Gundy et al. 1999), or geological events, such volcanic eruptions, which can shortly influence global climate (Atwell 2001) or the rise of the Panamanian isthmus (Molnar 2008). However, climate is now rapidly changing due to human activities that results in the Caribbean islands in warmer surface temperatures (prediction up to +4°C in 2100, without human activities change), increased variability of rainfall intensity in relation to El Niño and El Niña events, higher prevalence and/or intensity of storms and hurricane or rising of sea level (Taylor et al. 2012; IPCC 2014; Douglass & Cooper 2020). In addition to the weather modification (Taylor et al. 2012), climate change will also geographically and ecologically impact the Caribbean islands (Courchamp et al. 2014; Rhiney 2015). For instance, islands' coasts and surface will change due to sea-level rise, thereby changing ecosystems and decreasing the availability of suitable habitats for some species (Courchamp et al. 2014; Douglass & Cooper 2020).

Geo- and socio-political particularities influencing biodiversity conservation in the insular Caribbean

The insular Caribbean has peculiar cultural, political and environmental features, that deserve to be taken into account in the conservation of its natural heritage. First, the insular Caribbean consist of a complex geopolitical system, including 26 different sovereign countries and 19 overseas territories associated to France, the Netherlands, the United Kingdom or the United States of America. Each island harbours one political system, excepted the island of Hispaniola, which is split between Haiti and the Dominican Republic, and Saint-Martin, which is shared by France (Saint-Martin) and the Netherlands (Sint Maarten). This results in strong differences in terms of economic vulnerability among Caribbean countries, which inevitably influence their investment in the conservation of their own and common biodiversity. Second, all Caribbean countries rely on their natural environment for their economic prosperity, whether for tourism or local people livelihood (Table 1). For instance, in most countries, tourism activities represent more than 50% of the Gross National Product by supporting about 15% of employment in the region compared to 6% worldwide (Dehoorne et al. 2011; Cannonier & Burke 2017, 2019). In parallel, marine ecosystems through fisheries generate approximatively US$ 300 million of revenues per year, while providing food security for millions of peoples (Nurse 2011; de Oliveira Leis et al. 2019). Tourism, economic, and livelihood activities mainly rely on marine and coastal ecosystems, making them particularly exposed to disturbing and destructing activities, that affect the availability, the fragmentation and the health of these ecosystems, as pointed out by numerous studies (Sadovy de Mitcheson et al. 2013; Riegl & Glynn 2020; Giglio et al. 2020). Finally, Caribbean countries share a common societal history through the Amerindians that first colonized the different islands, and, thereafter, through colonisation by Europeans and the slavery trade (Kemp et al. 2020). However, each island also has its own societal peculiarities, which result in different cultural beliefs and tradition regarding Caribbean plants and animal species. For instance, there are traditional religious ceremonials and rituals, inherited from African slaves, which sometimes require the use of local plant and animals, such as in the Haitian voodoo (Apter 2002; Weber 2018) or in the Cuban Santeria (Kerestetzi 2018). Other practices, not necessarily relying on a religion, imply the use of animal and plant species for different purposes. For instance, snake and reptile oils are used to cure inflammatory problems, as observed with the

skin fat of Clouded Boa (*Boa constrictor nebulosus*) in Dominica (Ferreira et al. 2014; CEPF et al. 2019). Many plant species are used for medicinal purposes (Picking et al. 2015; Soelberg et al. 2016). Such traditional practices attract more and more scientific attention in relation to the development of new drugs and medications (e.g., Schmeda-Hirschmann et al. 2014; Ferreira et al. 2014), as it has been the case for plant species several decades ago (e.g., Torres-Avilez et al. 2015; Vardeman & Vandebroek 2021 for Caribbean examples). The exploitation of Caribbean plant species and animals is rooted in cultural traditions of Caribbean people and this societal feature has to be considered for implementing effective conservation plans.

As a consequence of global change, Caribbean governments have to work together to preserve the Caribbean biodiversity, while achieving the highest sustainable economic growth and ensuring the wellbeing of local people. To that end, the Caribbean Community (CARICOM) consists of 15 different member states from the Caribbean basin (including Belize, Guyana, and Suriname outside of the insular Caribbean) and five associated members (UK overseas territories). Although the main objective of the organization is to build the capacity of the region and to improve its economic future, the preservation of the Caribbean biodiversity is also considered as a preeminent part of the quality of life for inhabitants and as a resource that Caribbean countries have to preserve (Hassanali 2018, 2020). Another Caribbean inter-governmental organisation, called The Organisation of Eastern Caribbean States (OECS) and gathering almost all Lesser Antilles islands, aims at “consolidating the single economic space for enhanced economic growth, social inclusion and environmental protection”. As for CARICOM, although main objective of the organization is economic, they also perform actions for preserving biodiversity and ecosystem of the Eastern Caribbean, with the implementation of several projects. Coordinating the efforts of different countries and territories in the perspective of preserving biodiversity at a regional level is a challenging task considering the large number of stakeholders in the Caribbean region and discrepancies in terms of political objectives and economical and societal development.

Table 1 : : Ecosystem services provided by main Caribbean ecosystems and human use. Table obtained from CEPF's Ecosystem profile of the Caribbean islands' biodiversity hotspot (CEPF et al. 2019)

Ecosystem Service	Beneficiaries	Relative Importance within the region
Provisioning Services		
Fresh Water Flows (Artisanal and run off, flows) for drinking, irrigation, industrial use, energy generation	Entire population within the Hotspot	Very important as the area is water stressed
Food Production (Fisheries in freshwater and marine systems)	Local fishers, fish consumers, associated economic activities	Very important for local fisheries communities
Food Production (Crops)	Entire population within the Hotspot	Very important
Food Production (Livestock)	Entire population within the Hotspot	Very important
Non-timber forest products (Honey, handicraft materials, thatch, ornamental and household plants, spices, oils, seeds, tree seedlings, orchids, fruits)	Entire population within the Hotspot	Important, some goods could be obtained from other sources beyond the region
Timber products	Entire population within the Hotspot	Very important
Medicinal plants, pharmaceuticals	Rural communities	Very important
Energy (Solar and wind energy)	Entire population	Important
Supporting Services		
Habitat for species	Global	Very important for global biodiversity
Maintenance of genetic diversity (Source of novel genetic material for crops (e.g. fruits)	Global	Potentially significant
Regulating Services		
Erosion regulation (Reduction of soil erosion and desertification through stabilization of soils)	Local populations, economic activity, especially in mountainous areas	Important, significant in some areas
Air quality regulation (Local climate air quality)	Local populations, economic activity, especially in mountainous areas	Very important, significant in some areas
Water regulation (Reduction of disaster risk (flooding, landslide) through absorption of run-off)	Local populations, economic activity, especially in mountainous areas	Very important, significant in some areas
Water purification and waste management (Absorption of nutrient pollution, other pollutants in wetlands)	Local populations, economic activity	Important, significant in some areas
Natural hazard regulation (Coastal and beach stabilization)	Local fishers, fish consumers, coastal communities, associated economic activities	Very important for local fishers, coastal communities and associated economic activities
Pest regulation (Control of pest species through predation, natural limits on population)	Farmers, livestock herders	Significant in some areas
Pollination	Entire population, farmers, rural communities	Very important for food security
Climate regulation (Carbon sequestration)	Global	Very important
Cultural Services		
Recreation and ecotourism (Recreation)	Local populations, especially urban populations using natural areas	Important mainly in urban coastal areas
Recreation and ecotourism (Tourism using natural spaces (beaches, coastal habitats)	Global tourists, local people engaged in the tourism economy	Very important mainly in coastal areas
Spiritual and religious value (cultural and spiritual experience)	Nature related spiritual, cultural experiences, values	Important

Biodiversity within the Caribbean region

Geographical, climatic and geological peculiarities of the insular Caribbean have resulted in the formation of diverse terrestrial and marine habitats, making the region far from being biologically and ecologically homogeneous (Iturralde-Vinent 2006). This diversity of habitats harbours unique and diverse plant and animal species that, together, shapes important ecosystems, emblematic of the Caribbean region such as coral reefs, mangroves or tropical rain forests. In this respect, the Caribbean region is considered as one of the most important biodiversity hotspots in the world as it harbours exceptional rate of endemism, representing 2.3% and 2.9% of endemic plants and vertebrates among the world biodiversity (Myers et al. 2000), what makes the region and its biodiversity irreplaceable. However, the downside of biodiversity hotspots is that it is highly vulnerable and exposed to high extinction risks due to important threats mostly associated with human activities (Myers et al. 2000; Brooks et al. 2002; Mittermeier et al. 2011, see Figure 8B for locations of biodiversity hotspots). The ecosystems of the Caribbean region, through their high diversity and species richness, provide however many valuable ecosystem services particularly relevant for human well-being (Table 1), such that their sustainable exploitation by humans has become a crucial issue.

Paleogeography and biogeography: an explanation about the current biodiversity within the Caribbean region

The Caribbean region has geographically evolved over time in consequence of tectonic plates movements that resulted in the erection of different islands (Figure 2), *a priori* untouched by any form of terrestrial life when they emerged above the sea level, which is contrasting with the rich biodiversity they currently harbour. In this respect, Caribbean islands form, as well as other oceanic archipelagos, a natural laboratory to understand the evolution of life (Ricklefs & Bermingham 2008; Cox et al. 2016). Indeed, the peculiarities of islands' ecosystems have inspired many biologists, as early as Charles Darwin who famously developed his theory of evolution during the 19th century, partly based on the observation of morphological differences between species of finches in the Galàpagos archipelago (Darwin 1859; Sulloway 1982). Darwin's attention focused on differences in beak shape among island species of finches. Such comparisons and relationships between species' traits and environment particularities are indeed easier to perform in archipelagos than in continental areas (Cox et

al. 2016). That is why, islands provide interesting research opportunities to understand in which way the environment affects and controls evolutionary processes (e.g., Thorpe and Stenson 2002; Thorpe et al. 2008; Carstensen et al. 2013; Stigall 2013; Medina et al. 2016), and, at a more proximate scale, how environmental drivers control population dynamics and the number of species that can live in a given area (e.g., Carstensen et al. 2013; Stigall 2013; Dalsgaard et al. 2014; Shpeley et al. 2017). In addition, islands have allowed scientists to understand how species disperse and colonise a new environment and how biodiversity increases and stabilises (e.g., Dalsgaard et al. 2014; Weaver et al. 2016; Čandek et al. 2019; Woods et al. 2021), leading to the development of Biogeography as a scientific discipline (Wilson & MacArthur 1967).

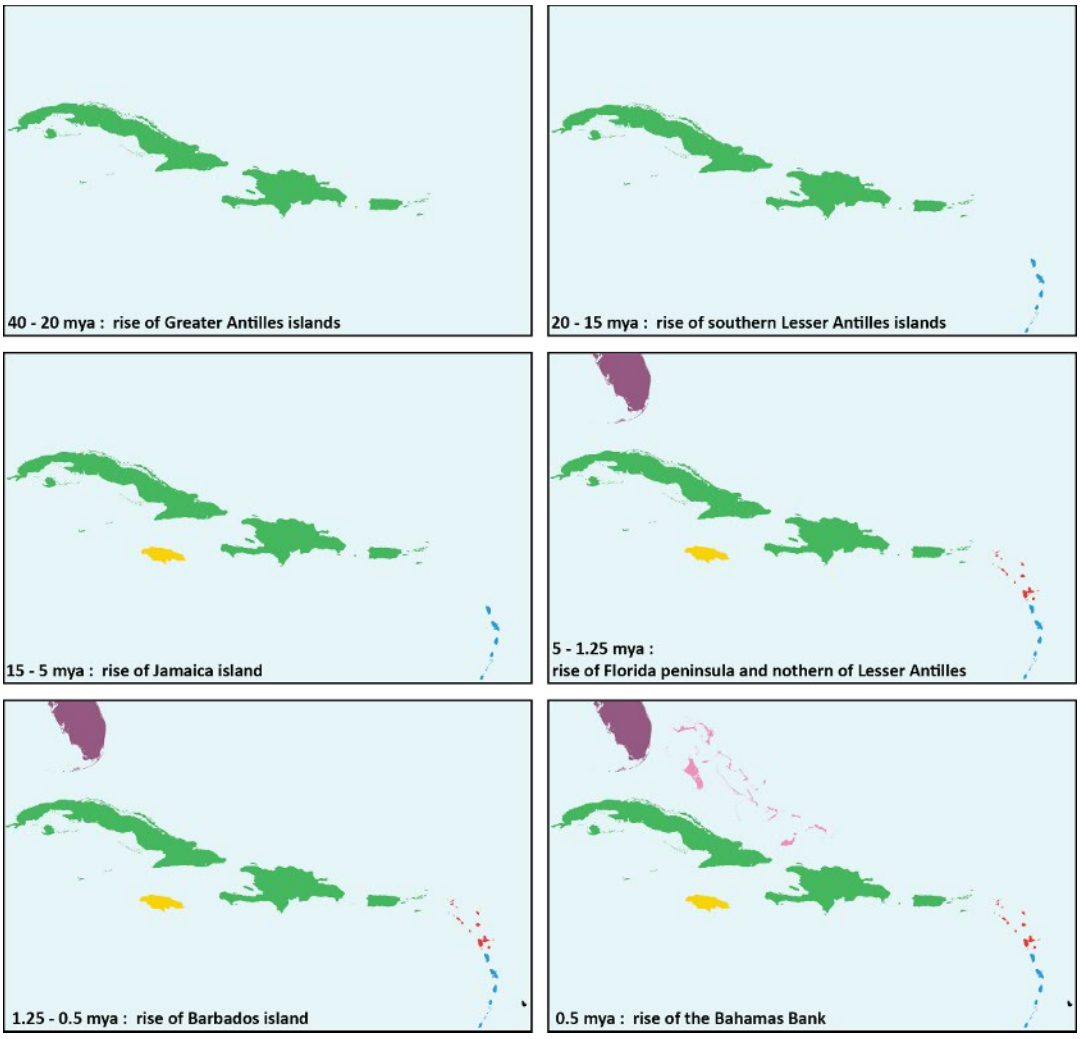


Figure 2 : Chronology of islands rising in the Caribbean region, created from Crews & Esposito (2020)

This research area borrows hypotheses, theories, concepts and tools from other scientific disciplines (e.g., Ecology, Evolutionnary biology, Genetics, Geology, Palaeontology, Geography, Oceanography and Climatology) to understand how physical (e.g., current and past topography or climate) and biological/ecological (e.g., predation, parasitism, disease or competition) features can explain the colonisation history and the current distribution of species at different spatial and time scales (Wilkinson 2002; Ebach et al. 2003; Lieberman 2003; Alfredo Bueno-Hernandez & Llorente-Bousquets 2006; Whittaker & Fernández-Palacios 2007; Ronquist & Sanmartín 2011; Cox et al. 2016). Considering that Caribbean islands are supposed to have emerged above sea level without traces of terrestrial life, and that the region consists of islands varying in terms of age, size range, climate, topography and distance from mainland, Biogeography is a valuable concept to study how Caribbean biodiversity has emerged and evolved. In this respect, retracing the biogeographic history of the Caribbean region is a current stimulating research topic within the scientific community, including both biologists/ecologists and geologists/palaeontologists (Table 2). A substantial body of evidence relying on both palaeontological and genetic data, indicates that the current native Caribbean animal and plant species has diverged from ancestral species originated from North, Central and South America (e.g., Weaver et al. 2016; Nieto-Blázquez et al. 2017; Crews & Esposito 2020; Nieto-Blázquez et al. 2021; Loureiro et al. 2021; Marivaux et al. 2021).

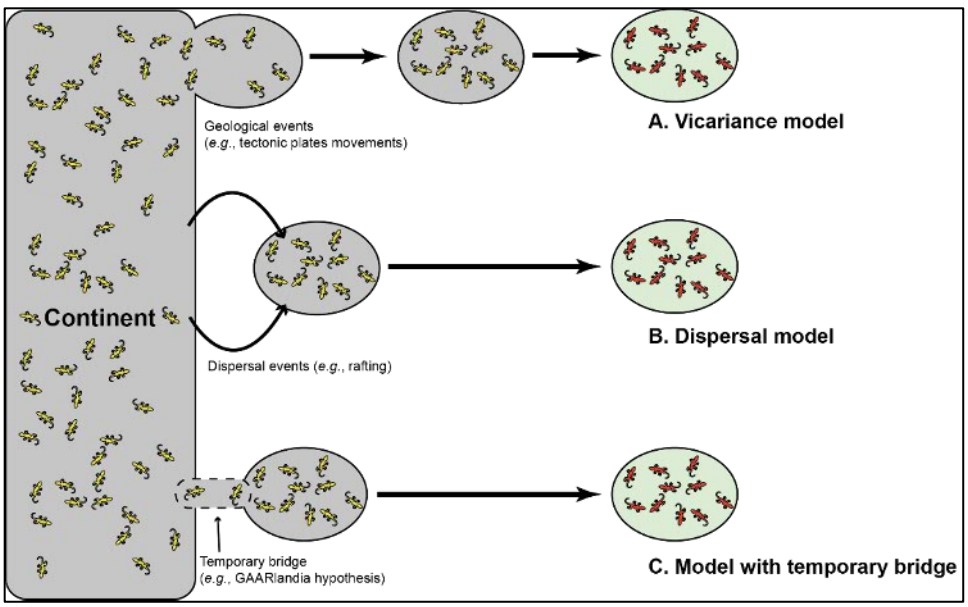


Figure 3 : Dispersion and colonisation scenarios used to explain the colonisation of Caribbean islands

However, many debates still exist about the way these ancestral species reached the Caribbean islands (Table 2). On this precise point, biogeographic and paleogeographic studies suggest four possible scenarios: (i) current geographic barriers (i.e., sea water) that could stop species/population dispersion did not exist in the past and a population of the ancestral species, initially present in a given area, according to their ecological needs, became isolated from other populations due to the rise of the barriers (Vicariance model, Figure 3A), (ii) ancestral species could get through barriers (Dispersal model, Figure 3B and 4), (iii) ancestral species took advantage of a temporary attenuation or removal of geographical barriers to spread (Temporary bridge, Figure 3C) and/or (iv) some islands have been probably colonised according to a "stepping stone" colonisation scheme (Successive dispersal model), with younger islands (e.g., Lesser Antilles) being colonised by species inhabiting the older islands (e.g., Greater Antilles), that themselves were colonized earlier from the continent.

In the core of the scientific debates regarding colonisation pathways of the Caribbean region by terrestrial biodiversity, and in opposition with the dispersal model, there is the rising hypothesis of the past-existence of landmasses that would have connected Caribbean islands with surrounding continents such as the GAARlandia (Greater Antilles Aves Ridge; Iturralde-Vinent and MacPhee 1996; Iturralde-Vinent 2006) and GrANoLA hypothesis (Greater Antilles-Northern Lesser Antilles; Cornée et al. 2020, 2021; Philippon et al. 2020). These landmasses would have helped inter-islands movements and the colonisation of the Greater Antilles by animals and plants originating from south America (but see Garrocq et al. 2021; Ali & Hedges 2021). Regarding the dispersal model of Caribbean islands colonisation, one difficulty lies in

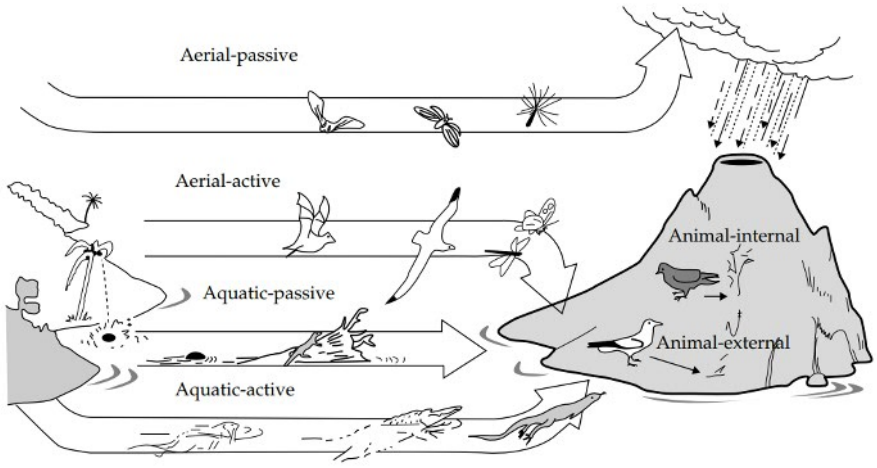


Figure 4 : Different possible modes of dispersal by which species can colonize islands. According to Whittaker & Fernández-Palacios (2007).

the low ability of ancestral colonist species to disperse, contrary to birds for instance, such that they would have reached Caribbean islands using natural rafts (Figure 4, for different models of dispersal). As a by-default explanation, many authors suggest this hypothesis of colonisation for flightless, non-swimming or non-seawater taxa when the estimation of the colonisation period does not overlap the period of the hypothetical existence of GAARlandia land bridge (Hedges 2001; Glor et al. 2005; Weaver et al. 2016; Poe et al. 2017; Čandek et al. 2019; Woods et al. 2021; Marivaux et al. 2021). Main arguments against this colonisation hypothesis of Caribbean islands relied on the biological and physiological abilities of colonist species. Mazza et al. (2019) recently argue against this model, pointing to physiological problems that colonist animals would have been exposed to during such journeys, such as starvation, dehydration and physiological challenges due to fluctuation in temperature and humidity. Although scientific knowledge about the physiology, dynamics and persistence of populations, especially small ones, suggest that overwater dispersion might seem a misleading explanation of colonisation, evidences for long-distance dispersal have been established, especially through phylogenetic analyses (e.g., Chamberland et al. 2018; Shapiro et al. 2022 for a Caribbean species of spider). However, its relative importance across different groups of taxa and compared to other colonisation scenarios still needs further investigations (Cowie & Holland 2006; Whittaker & Fernández-Palacios 2007).

The biogeographic history of the Caribbean species is therefore closely related to its paleogeography. It is easy to consider the Caribbean colonisation through dispersion for species having high abilities to disperse such as birds and most of marine species, bats, insects or even plants, through various efficient seed dispersal strategies. In contrast, for most terrestrial and freshwater species (except for the cases of diadromous species), the colonisation of Caribbean islands through over-sea dispersion is more complicated to consider. Therefore, all biogeographic hypotheses concerning Caribbean colonisation by animals and plants have, in the current state of scientific knowledge, some downsides. This leads us to consider that there is no universal explanation, but that instead all suggested colonisation scenarios probably contribute to explain the complexity of the Caribbean biogeographical pattern (Whittaker & Fernández-Palacios 2007; Hrbek et al. 2007). The confrontation of ideas is however scientifically stimulating and is attesting that science is making progress, what sooner or later may lead to one or several reliable explanation(s)

regarding the biogeographic history of the Caribbean region. Moreover, this research topic highlights the mutual gain that geological and biological sciences have in working together to elucidate this question (Wilkinson 2002; Alfredo Bueno-Hernandez & Llorente-Bousquets 2006).

Insularity, species distribution, richness and endemicity

Likewise colonisation opportunities, the distribution and abundance of a given island species change over time and space as they depend on both its biological and ecological features and requirements (e.g., life history traits, dispersal ability, habitat or resource specificities), its evolutionary capacity (e.g., phenotypic plasticity, adaptation, exaptation), and the ecological changes of its environment over time and space (e.g., paleogeography, changes in weather, of carrying capacity or in biotic interactions). Several hypotheses have been suggested to explain the current biodiversity distribution within islands areas, especially regarding species richness and endemicity. Indeed, species richness in islands may be influenced by both dispersal/colonisation and endemicity.

Several studies showed a positive correlation between Caribbean islands size and elevation, and the richness of both nonendemic and endemic species (e.g., Losos 1996; Losos & Schluter 2000; Ricklefs & Bermingham 2004, 2008; Dalsgaard et al. 2014; Rodriguez-Silva & Schlupp 2021). This pattern is usually interpreted through three theories. The first is simple, as it consists in considering all species as ecologically similar and just stating that the larger an island is, the more species it contains (Whittaker & Fernández-Palacios 2007). The second theory considers differences in species' ecological requirements and differences in environmental heterogeneity and niche partitioning among islands to explain the observed pattern. For instance, it is expected that generalist species or species from lower trophic levels could establish in a larger number of islands than more specialist ones or those from higher trophic levels (Carstensen et al. 2013). The underlying argument is that the larger an island is, the more heterogenous it is in terms of habitats, thus allowing the coexistence of a larger number of species with different ecological requirements (Whittaker & Fernández-Palacios 2007; Hortal et al. 2009). The same is true for elevation, which increases both island area and habitats heterogeneity within islands. The third theory consist in considering that the two

previous ones probably act together to shape observed patterns of diversity across islands (Kadmon & Allouche 2007; Carstensen et al. 2013). The high rate of endemism in larger islands

Table 2 : Selection of seminal articles that contributed to build the current/ongoing hypothesis about the biogeographic history of the Caribbean region. They are chronologically sorted. Geol = Geology, Pal = Palaeontology, BioEcoEvo = Biology, Ecology, Evolution. Asterisks indicate articles that contribute to the debate on how ancestral species reached Caribbean islands.

Research articles	**Main research area of authors**	**Topic**
Malfait & Dinkelman (1972)	Geol	Tectonic study suggesting the existence of temporary emerged lands at the east of the current Lesser Antilles, afterward called Aves Ridge
Woods (1990))	BioEcoEvo	First suggestion that Aves Ridge played the role of corridor between South America and Greater Antilles
Holcombe & Edgar (1990)	Geol	First suggestion that Aves Ridge played the role of corridor between South America and Greater Antilles
Iturralde-Vinent & MacPhee (1996)*	Geol/Pal	First suggestion of the existence of a possible landbridge that connected South America to Caribbean - hypothesis of GAARlandia
Hedges (2001)	BioEcoEvo	A review of the Biogeography of the Caribbean region
Hedges (2006)	BioEcoEvo	A review of possible colonisation pathways of Caribbean teerestrial vertebrates
de Queiroz (2005)*	BioEcoEvo	Argument in defense of over-oceanic dispersal in opposition of the vicariance model
Iturralde-Vinent (2006)*	Geol/Pal	Provide a possible scenario of the Caribbean emergence + hypothesis of GAARlandia
Ricklefs & Bermingham (2008)	BioEcoEvo	A review and perspectives about the biogeograhy study in the Caribbean region
Ali (2012)*	Geol/BioEcoEvo	Argument against the hypothesis of GAARlandia
Carstensen et al. (2013)	BioEcoEvo	Analysis of the influence of bird species traits in shaping species distribution within the Caribbean region
Dalsgaard et al. (2014)	BioEcoEvo	Analysis of environmental drivers that shape Caribbean bird diversity
Lomolino (2016)	BioEcoEvo	Description of biogeographic processes that shape island biodiversity
Nieto- Blázquez et al. (2017)	BioEcoEvo	Analysis of the biogeographic history of Caribbean seed plant
Franklin et al. (2018)	BioEcoEvo	Analysis of the influence of climate and isolation in shaping a Caribbean plant community
Ali and Vences (2019)*	Geol/BioEcoEvo	Response to Mazza et al 's argumentation regarding the reconsideration of natural rafting over ocean and sea as a probable way of oceanic islands colonisation.
Mazza et al. (2019)*	Geol/Pal	Argument against natural rafting over ocean and sea as a probable way of oceanic islands colonisation.
Philippon et al. (2020)*	Geol	Geological evidence of temporary emerged land in the North of Lesser Antilles that could be implied in South-America/Caribbean connection - Hypothesis of GrANoLA
Philippon et al. (2020b)*	Geol	Geological evidence of temporary emerged land in the North of Lesser Antilles that could be implied in South-America/Caribbean connection - Hypothesis of GrANoLA
Cornée et al. (2020)*	Geol	Geological evidence of temporary emerged land in the North of Lesser Antilles that could be implied in South-America/Caribbean connection - Hypothesis of GrANoLA
Crews & Esposito (2020)	BioEcoEvo	Analysis of the biogeographic history of Caribbean arthropod species
Ali & Hedges (2021)*	Geol/BioEcoEvo	Argument against the hypothesis of GAARlandia
Rodriguez-Silva & Schlupp (2021)	BioEcoEvo	Comparison of the biogeographic history between Caribbean terrestrial and aquation fauna.
Cornée et al. (2021)*	Geol	Geological evidence of temporary emerged land in the North of Lesser Antilles that could be implied in South-America/Caribbean connection - Hypothesis of GrANoLA

appears to result from both the effect of evolutionary processes on small colonizing populations (e.g., founder effect, genetic drift) and ecological diversification and radiation. The latter is mainly explained by the presence of unoccupied ecological niches, as widely investigated and evidenced in anoles lizard (e.g., Warheit et al. 1999; Losos et al. 2006; Losos & Schneider 2009; Losos 2011; Mahler et al. 2013), as well as in other taxa (Losos & Ricklefs 2009). Because the probability to find unoccupied ecological niches is higher in larger or mountainous islands than in smaller, flatter ones, ecological diversifications and radiations are more likely to occur in larger islands. In addition to islands size, island biogeographical studies also stated that species richness decrease with isolation of islands either from a mainland or from other islands. The main explanation is based on dispersal ability of species. Indeed, more remote islands are more difficult to reach than closer ones, thus only species having a certain ability to disperse could naturally reach more isolated islands (Sutherland et al. 2000; Jenkins et al. 2007). For instance, body size was found to be positively related with dispersal ability (Jenkins et al. 2007). As a result, richness species, as well as similarity between island communities, decrease with increasing distance from mainland (e.g., in Caribbean bats, Presley & Willig 2008 and in Caribbean birds, Carstensen et al. 2013).

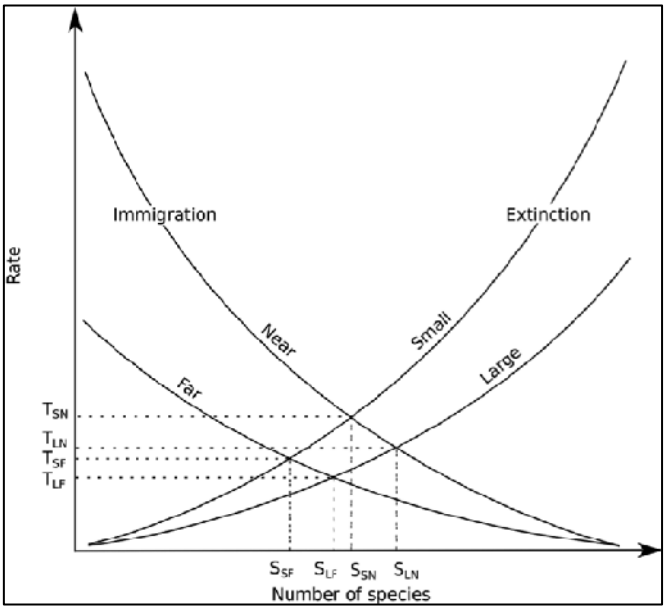


Figure 5 : Equilibrium model of island biogeography (MacArthur & Wilson 1963, 1967). The letter "T" corresponds to species turnover rate. In indices of "T", in y-axis, and "S", in x-axis, "S", "L", "N" and "F" refer to island features and mean small, large, near and far, respectively.

Both island size and isolation have been considered by MacArthur & Wilson (1963, 1967) to elaborate a model, known as the *equilibrium model of island biogeography*, which illustrates community dynamics within islands and how an island's number of species can reach a stable value. According to that model, the species richness on an island over time is a balance between the probability of colonisation (i.e., immigration in Figure 5) and the probability of extinction, which are both related to island size (small or large) and isolation (near or

far; Figure 5). The model shows that the smallest and the most isolated the island is, the lowest is the equilibrium richness species, because the probability of colonisation would be low while the extinction risk of species would be high. The model then suggests that species richness within an island should be relatively stable when the equilibrium is reached, and that there would exist a more or less important turnover (called "T" in Figure 5) regarding species composition within islands, according to their size and isolation (Whittaker & Fernández-Palacios 2007). The main value of this model lies in its simplicity and the fact it considers species richness, and hence biodiversity, within islands as varying over time. However, one weakness of the model is that it does not consider some ecological aspects of species and populations, such as interspecific interactions as well as trophic level and specialist vs. generalist strategy of the species that also influence the probability of colonisation and extinction of a species.

Threats to Caribbean biodiversity

Since the emergence of Caribbean islands, the region has been through some more or less important biodiversity crises, especially due to natural climate change and both direct and indirect effects of human activities (O'Dea et al. 2007; Dávalos & Russell 2012; Cooke et al. 2017; Kemp et al. 2020). Regarding past climate change, the shift from xeric to mesic ecosystems and the increase of sea level that induced loss of island area and flooding of critical habitats according to cycles of glaciation, has driven past extinction of several Caribbean species (Cooke et al. 2017). The Caribbean region has also been impacted by the arrival of humans (Kemp et al. 2020). This began with Amerindians, who are suspected to have induced negative impacts on Caribbean ecosystems due to the translocation of animal and plants (Fitzpatrick & Keegan 2007; Giovas 2016, 2017, 2018; Turvey et al. 2017; LeFebvre et al. 2019; Kemp et al. 2020) as well as hunting and fishing pressures on easy-to-capture species (Steadman et al. 2015; LeFebvre et al. 2019). However, the human fingerprint on natural Caribbean ecosystems have never been so important since the arrival of Christopher Columbus and that of Europeans in the Caribbean region (Fitzpatrick & Keegan 2007; Acevedo-Rodríguez & Strong 2008; Maunder et al. 2011). As a result, all taxa are threatened within the Caribbean region. According to the IUCN Red List, the most threatened ones are

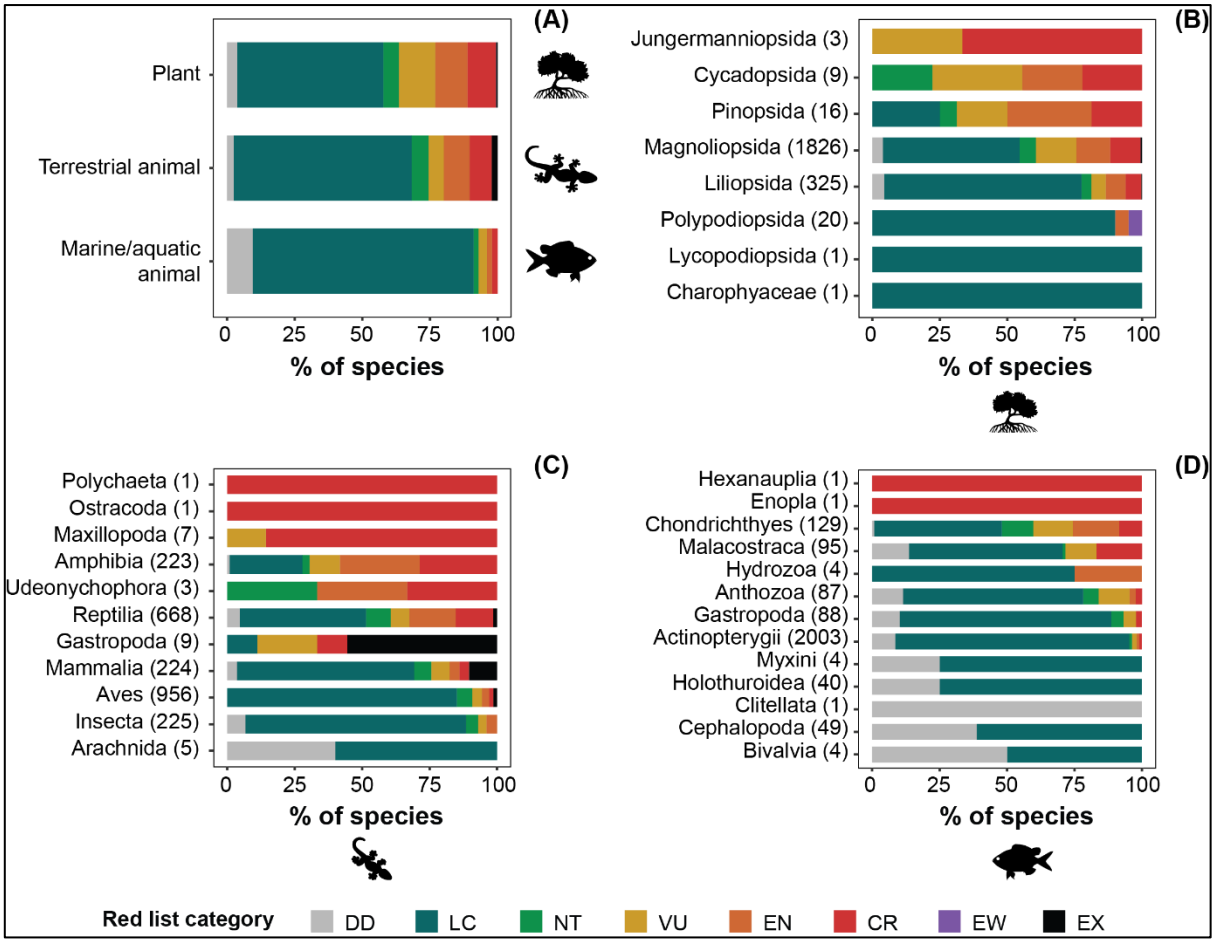


Figure 6 : Percentage of Caribbean species within Classes according to IUCN red list categories. (A) is for each considered group of species, that is Plant, Terrestrial and marine/aquatic animal species. (B), (C) and (D) detailed the percentage of species within species classes according to their IUCN red list categories, respectively. In top-down, species classes are ordered according to the percentage of threatened species (i.e., VU + EN + CR). In parentheses are the number of species assessed by the IUCN and considered here.

terrestrial species, including plants and animals, followed by marine and aquatic animals (Figure 6).

From its biogeographic history, Caribbean animal and plant species are represented by small populations that have evolved in isolation within a restricted area distribution and ecological interactions (Courchamp et al. 2003), making island species much exposed to genetic and demographic stochastic forces (Soulé 1985). In addition, the region is regularly exposed to natural hazards. As a result, Caribbean species are particularly affected by ecological and biological disturbances induced by human expansion and activities (Brooks et al. 2002), but are, in contrast, particularly resilient to disturbances caused by natural hazards such as hurricanes, although the increase in intensity of such events due to the current global change might alter in the future, either positively or negatively, Caribbean ecosystems (e.g., Hass et al. 2012; Precheur et al. 2016; Campos-Cerqueira & Aide 2021). Based on species listed

within the IUCN Red List, Caribbean terrestrial animal species are particularly threatened by agriculture, invasive alien species, urbanisation, deforestation, climate change and over-hunting (Figure 7). In contrast, marine and aquatic species are threatened by over-fishing, pollution, invasive species, urbanisation and climate change (Figure 7). Regarding plant species occurring within the Caribbean region, the main reported threats are agriculture, deforestation, climate change, urbanisation and invasive species (Figure 7).

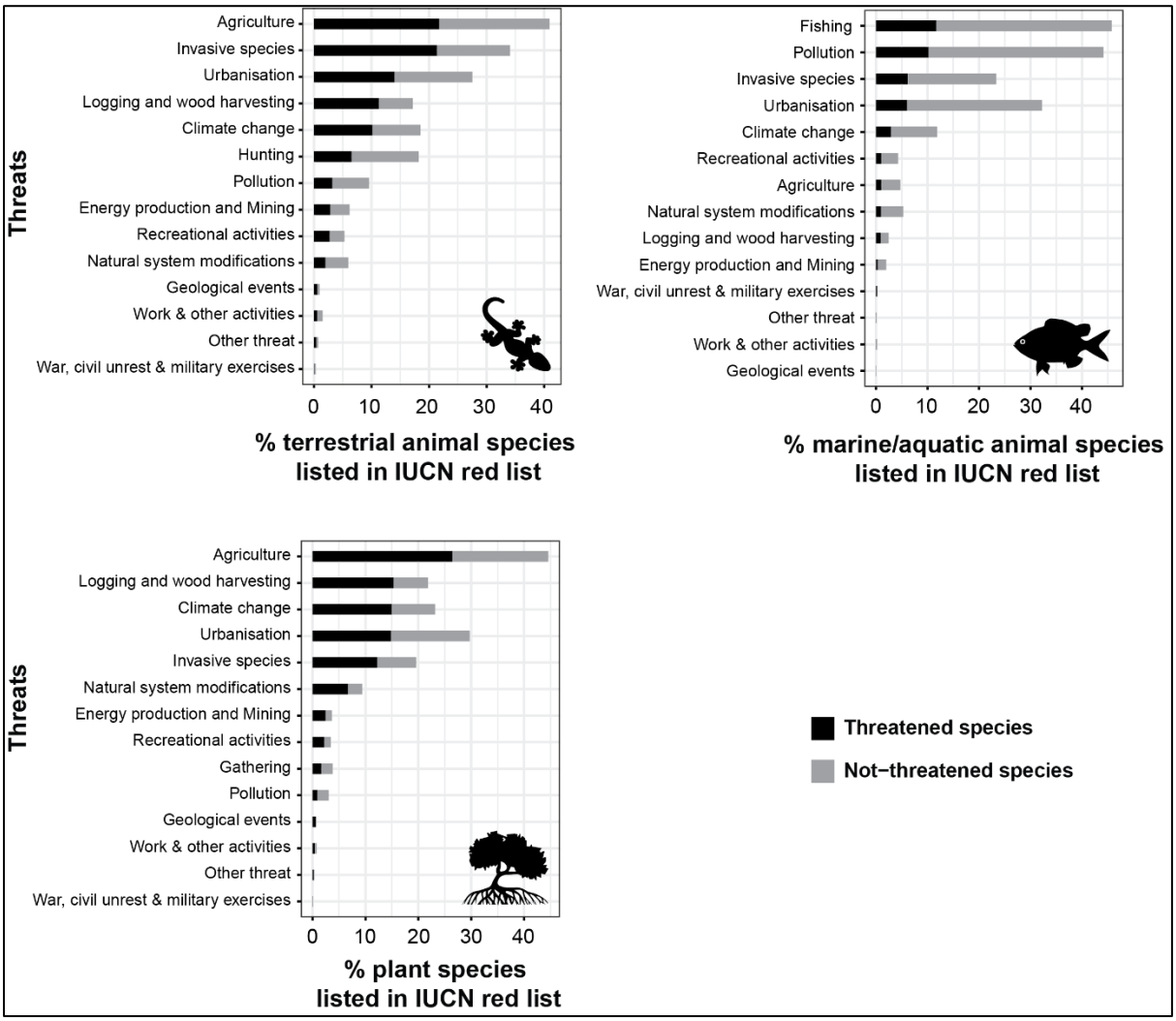


Figure 7 : Percentage of threatened and not-threatened species according to threats within terrestrial animals, marine and aquatic animals, and plant species. Threats are sorted according to the percentage of threatened species. Threats have been extracted from IUCN red list, taking into account the first level of the threat classification scheme, excepted for urbanisation and, hunting, fishing, logging and gathering. Urbanisation referred to both “residential & commercial development” and “transportation & service corridors” in the current threat classification scheme. For the threat level called “Biological resource use”, hunting, fishing, logging and gathering were considered as independent threats. Calculation of percentage were based on the total species listed within the IUCN red list for terrestrial animals, plants and marine/aquatic animals.

Habitat loss and alteration

The loss of natural habitats through agricultural expansion and intensification, deforestation and urbanisation probably constitute the most important threat for plant and animal species. For instance, Caribbean forests are home to a high diversity of trees and flowering plants, including about 12,000 plant species, from which about 7,000 (58%) are endemic (Santiago-Valentin & Olmstead 2004; Francisco-Ortega et al. 2007; Acevedo-Rodríguez & Strong 2008). As a consequence, these forests are also home to numerous animal species, which are for a high proportion endemic to the Caribbean region. According to current scientific knowledge, the percentage of endemism among the 169 Caribbean amphibian species and the 572 Caribbean reptiles is as high as 99% and 93%, respectively (Henderson & Powell 1999). Regarding birds and mammals, it amounts to 26% of 565 species and 49% of 104 species, respectively (CEPF et al. 2019). However, land clearance for agricultural purposes began during the period of Amerindians, who introduced many plant species, such as wild avocado (*Persea americana*), maize (*Zea mays*), cassava (*Manihot esculenta*), sweet potatoes (*Ipomoea batatas*) for livelihood (Cooke et al. 2017). Their impact on natural Caribbean ecosystems is obviously not comparable to the consequences of European colonisation. Large surfaces of natural forests were then cleared for the benefit of sugarcane, cotton, bananas, cocoa, coffee, and tobacco plantations (Acevedo-Rodríguez & Strong 2008; Maunder et al. 2011, see part 3.2.). In addition to this and in response to rapid population growth of human within the region, urbanisation has increased by impinging on and altering natural habitats. As a result, the Caribbean region is particularly impacted by land transformations (Venter et al. 2016), with declining or totally destructed native forests in some islands such as in The Bahamas, Barbados or Antigua (Watts 1990). At the regional scale, only about 3% of the primary forest remains (Hedges et al. 2018; FAO 2021). This is why, the insular Caribbean is currently considered as one of the regions with the highest rate of natural habitat loss (Brooks et al. 2002).

Given the loss of natural forest cover, and past and current rate of urbanisation in the Caribbean region (Barragán & de Andrés 2016; Duque et al. 2019), urban areas deserve particular attention to understand their negative impact on biodiversity (though natural habitat loss and facilitating the spread of invasive species, Jesse et al. 2018), as well as their potential importance in the maintenance of local biodiversity (Enedino et al. 2018; Exantus et

al. 2021). In addition, human made infrastructures can directly be home to species that have ecologically and behaviourally evolved to live in urban areas (Johnson & Munshi-South 2017). This is why, although global urbanisation has contributed to the decline of biodiversity across the world (McKinney 2002; McIntyre 2014; Silva-Junior et al. 2018), increasing attention has been given by ecologists to urban environments during the last decades in order to identify how urban biodiversity is structured and how species can exploit the urban habitat. For instance, a recent study conducted in Port-au-Prince, Haiti, being the most densely populated country in the Caribbean region, highlighted the relevance of urban agroforestry areas and remnant forests for the maintenance of Haitian avian density (Exantus et al. 2021).

Overexploitation

Overexploitation is a major threat for Caribbean plant and animal biodiversity, as the same time it is closely associated to human livelihoods and food security, such as for fishery resources (McConney et al. 2015; Sherman et al. 2018). For plants, overexploitation includes all kind of logging and plant gathering for food, medicinal or horticulture, while for animals this includes legal and non-legal hunting/fishing of adults, juveniles or eggs and capture for pet trade. Due to significative illegal exploitation, the impact of such behaviours is often under-estimated. Collecting plant or animals induce direct mortality within populations and decrease growth rate of the population, especially when many individuals are harvested in a short period of time, thus affecting their persistence. Within the Caribbean region, many animals and plants are harvested for food, sport or medicinal and cultural purposes. Often, these species are traditionally hunted, consumed or used by local peoples for long time ago. For instance, bone remains of Caribbean native species are found in archaeological excavations dating back to slavery (Wallman 2018, 2020) or Amerindians periods (Krigbaum et al. 2013; Giovas 2016; Bochaton et al. 2016), allowing anthropologists to identify diets of local human populations at the time. In particular, bones of Caribbean Giant frogs (*Leptodactylus spp.*), Lesser Antilles iguanas (*Iguana delicatissama*) and several bird species (Bochaton et al. 2016, Wallman 2018, 2020) have been found, suggesting that these animals were historically consumed. Therefore, the impact of humans on Caribbean native species, although minor, had already begun at the period of Amerindians (Fitzpatrick & Keegan 2007, Cooke et al. 2017). Regarding Caribbean native plant, their consumption is more for medicinal

purposes than for subsistence, and this is why Amerindians had early developed maize and tuberous root crops agriculture (Lane et al. 2008; Mickleburgh & Pagán-Jiménez 2012). Such historical evidence indicates that the use of native Caribbean plant and animal species is deeply rooted in traditions of Caribbean people of African and Amerindian descent.

Invasive species

Another significant cause of extinction among Caribbean species is invasive alien species. Since the arrival of Amerindians, exotic species have been accidentally or intentionally introduced to the Caribbean islands (Giovas 2017, 2018, Kemp et al. 2020), inducing new pressures of predation, parasitism, and competition on native plant and animal species (Mack et al. 2000; Courchamp et al. 2003; Sax & Gaines 2008). In addition, hybridization may occur between close native and exotic species, thus affecting genetic integrity of the native species (e.g., *Iguana delicatissima* and *I. iguana*, Vuillaume et al. 2015; van den Burg et al. 2018; Pounder et al. 2020). At a larger scale, invasive species may disrupt ecosystem processes and functions (Mack et al. 2000). Once again, the contribution of Amerindians in introducing exotic species within the Caribbean region is much lower than that of contemporary humans (Kemp et al. 2020). The arrival of Europeans was in fact associated with the introduction of a wide range of exotic plants and animals for both commercial and non-commercial purposes (Cooke et al.2017, Kemp et al. 2020). Moreover, the arrival of European ships is synonymous with the introduction of the most invasive species known nowadays, that is rats (*Rattus spp.*) and cats (*Felis catus*; Kemp et al. 2020*),* which are both responsible for more than 30% of all known contemporary extinction across the word (Doherty et al. 2016). In oceanic islands, the impact of such invasive species is particularly significant, more than in continents (Sax and Gaines 2008), because island species, because of their biogeographical history, have evolved in isolation, under particular selection pressures, such as relaxed predation and parasitism (Courchamp et al. 2003, Ricklefs & Bermingham 2008). In addition, they usually have smaller geographic range, which decreases the availability of suitable habitats and of refugia in case of perturbations as induced by invasive predators, competitors or pathogens. This is why the Caribbean region is particularly impacted by invasive alien species (Walsh et al. 2012; Doherty et al. 2016).

Pollution

Pollution is an important threat for Caribbean species. It most often results from human practices such as urbanisation, tourism or agriculture. As a result, there are many types and sources of pollution that affect all life compartments, that is air, freshwater, seas and oceans, or soil, and even animal senses such as visual, earing or spatial orientation. Contrary to other threats, except for global change (see next paragraph), pollution may have more direct incidences on human health, and from this perspective, has attracted more attention from politicians and the civil society. Indeed, contaminant agents often enter and spread in food webs (e.g., Goss et al. 2018), ultimately reaching apex predators such as humans (Roopnarine 2014). For instance, contamination of soils by chlordecone (a carcinogenic chemical molecule intensively used to control an insect species devastating banana plantations) is known to directly contaminate animals and plants living in environments that were directly exposed to this molecule (Coat et al. 2011; Dromard et al. 2016). The molecule is transmitted to humans through water consumption or through the food web (Dereumeaux et al. 2020). In addition to chemical pollutions, other kinds of pollution occur in the Caribbean. Plastic and microplastic pollution mainly affect the marine biota and food webs (Zarfl et al. 2011; Monteiro et al. 2018). Air pollution due to urbanisation expansion and volcanic eruptions (Jury 2017) affects local weather and contributes to global change. Light pollution is known to affect various animal and plant species (Longcore & Rich 2004), while noise pollution affects ecological inter and intra-specific interactions (Ferrier-Pagès et al. 2021; Bertucci et al. 2021). Pollution also comes from domestic waste products, including pharmaceuticals, artificial sweeteners, household products, steroid hormones, and current-use pesticides (Edwards et al. 2018). Caribbean animal and plant species are all exposed to at least one of these pollution sources. This may directly affect individuals by modifying their metabolism or physiology, inducing diseases and/or death, or indirectly through decreasing the quality of their environment and, the availability and quality of resources.

Global change

The last but not least threat on Caribbean biodiversity is global change. This threat directly concerns biodiversity as well as human wellbeing (Rhiney 2015; Stennett-Brown et al. 2019). Since the emergence of Caribbean islands, several climate change events related to glaciation

cycle have shaped the Caribbean region and its biodiversity, inducing extinction and cycles of colonisation/extirpation (Higuera-Gundy et al. 1999; Getty et al. 2001; Dávalos & Russell 2012). In addition, regional climate also changed as a consequence of the Panamanian isthmus emergence (Molnar 2008). In contrast to past "natural" climate changes, the current global change is induced, or at least promoted, by human activities (IPCC 2014). Expected critical environmental changes include increasing temperature of air and oceans, as well as more frequent extreme weather events and sea level rise (IPCC 2014). These environmental changes are not homogenous across the globe and the Caribbean region has been identified as particularly vulnerable, with direct consequences for both biodiversity and human populations (Rhiney 2015; Stennett-Brown et al. 2019). Climate change affects both species and ecosystems through geophysical modifications (e.g., air or sea temperature, pluviometry). Most vulnerable species are those directly depending on such geophysical features such as plants, corals or planktonic species (Higuera-Gundy et al. 1999, Getty et al. 2001, Molnar 2008, Precht et al. 2020). These environmental changes may disrupt all kind of interactions among species and modify their survival rate in climate-altered habitats (Visser & Gienapp 2019; Schippers et al. 2021). As response to climate change, species may shift their spatial distribution in order to reach more suitable environment (Tomiolo & Ward 2018, for a plant example), or maintain populations in a changing environment depending on their ability to adapt (i.e., first through phenotypic plasticity and then through natural selection; (Visser et al. 2009; Visser & Gienapp 2019). When the species does not have possibility to escape or fails to adapt, it is doomed to become extinct or extirped (Keith et al. 2008; Schippers et al. 2021). This is the risk for most Caribbean plants and animal species because oceanic islands are by definition small land areas enclosed by sea that, accordingly, do not usually offer wide and diversified habitats. In addition, island species often have a low potential of biological adaptation due to the colonisation process and geographical isolation that characterise their biological evolution history and the biogeographic history of the region. Therefore, Caribbean plant and animal species, as well as in other insular region, are particularly vulnerable to climatic change, especially those having low ability to disperse (Courchamp et al. 2014).

Conservation in the Caribbean region: between academic research and citizen initiative

Shortly following the discovery of the Caribbean region by Europeans (1492) and before the iconic scientific expeditions conducted by Charles Darwin (1839, 1859) and Alfred Russel Wallace (1910) during the 19th century, European naturalists explored the world, taking advantage of commercial vessels implied in the triangular trade. During which time they made various scientific observations benefiting astronomy, geography and natural history, including regular visits to Caribbean colonies (Dew 2010). From the point of view of academic research in Ecology and Conservation Biology, research leadership has not changed much within the region, as most of contemporary studies are conducted by scientists coming from European and North American research institutions (Wilson et al. 2016b; Reboredo Segovia et al. 2020; Vallès et al. 2021). This is in accordance with the observed inverse relationship among countries between research effort and levels of biodiversity (Figure 8, Wilson et al. 2016). As a result, and despite the involvement or European and North American researchers within the region, there is still a lack of knowledge regarding many Caribbean plant and, marine and terrestrial animal species, as highlighted by several studies (e.g., Donovan et al. 2002; Wilson et al. 2006; Maunder et al. 2008; Latta 2012; Kobara et al. 2013; Nelson et al. 2018; Devenish-Nelson et al. 2019; Barona et al. 2020). Besides, there is a lack of Caribbean specialists in conservation sciences and the few local expertise available is heterogeneously distributed across Caribbean islands, with current overseas territories, such

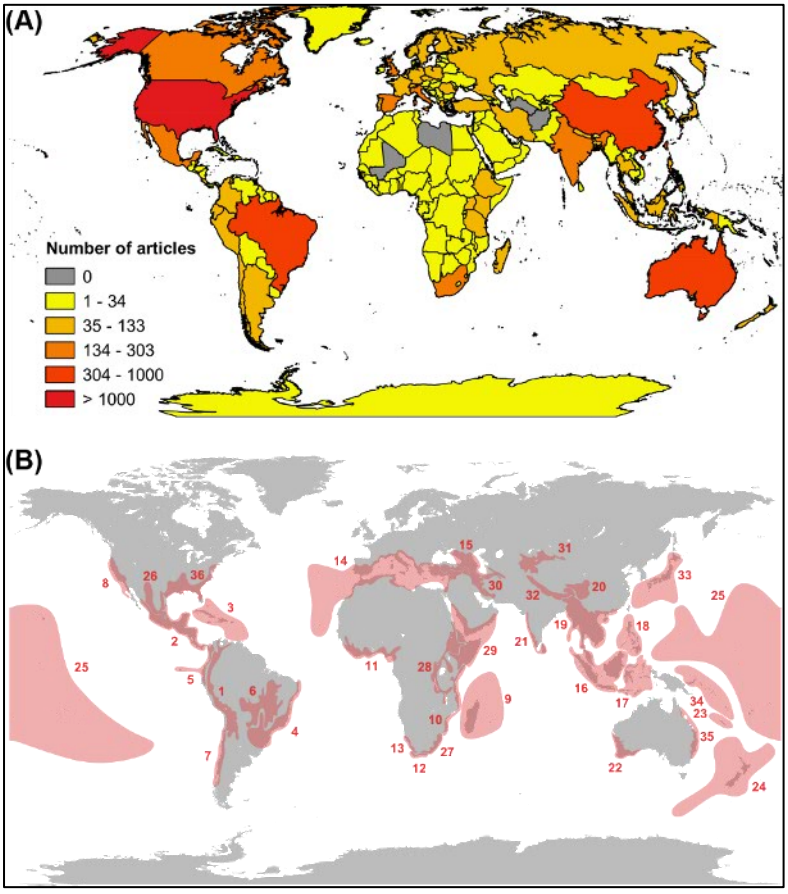


Figure 8 : (A) Global distribution of biodiversity conservation studies, published in international peer-reviewed journals, according to Wilson et al. (2016). (B) Distribution of biodiversity hotspots, according to Myers et al. (2000), Brooks et al. (2002) and CEPF website. Map was downloaded and modified from Wikipedia.org. See Appendix 1 for legend of map (B), that is names of biodiversity hotspots

as Puerto-Rico and the French West-indies, producing more peer-reviewed papers than sovereign islands (Vallès et al. 2021).

In contrast, local initiatives have been undertaken throughout the Caribbean region, led by local wildlife officers or local organizations, who work to raise public awareness about local biodiversity and ways to preserve it (e.g., Shapiro et al. 2016). For instance, an impressive conservation education program has been implemented in Saint-Lucia to assist with the conservation of the Saint-Lucia Amazon (*Amazona versicolor*). It consisted in educating a large audience of Saint-Lucians, across all ages and social status, to increase the popularity of the Saint-Lucia parrot. Such conservation approaches may result in encouraging results as the population of parrot has increased due to a decrease in anthropogenic pressures (Christian et al. 1996; Jenks et al. 2010). Throughout the Caribbean region, other conservation educational campaigns promoting one or several endemic species have also been implemented such as in Saint-Vincent and Dominica for their own vulnerable and critically endangered parrot species (*A. guildingii* and *A. imperialis*, respectively; Colmore et al. 1996) or in Grenada for the critically-endangered Grenada Dove (*Zenaida wellsi*) or sea turtles (Rosenberg & Korsmo 2001; Frame et al. 2021). Another way to involve the Caribbean civil society in conservation program consists in relying on the economic sector that, directly or indirectly, depends on their surrounding biodiversity to prosper, such as tourism and agriculture. The aim is to conciliate such economical activities, necessary for the economic health of Caribbean countries and for Caribbean people livelihood, and the preservation of local biodiversity through adjustments in various practices. For instance, a study showed that an educative message explaining to snorkellers how to properly behave during their trips to avoid to damage corals is an easy way to reduce damages caused by this activity in Puerto-Rico (Webler & Jakubowski 2016).

The success of conservation program often relies on a synergic effect of several conservation actions (Soulé 1985). Educational programs dedicated to civil society can for instance be combined with one or several direct actions targeting specific species or their associated ecosystems, such as the monitoring of ecosystem health and species populations, habitat/ecosystem protection or restoration, managing threats, resource supplementation, reinforcement of populations through translocation or captive breeding. The objectives of such methods consist in assessing population viability and improving one or several

demographic parameters, such as survival probability, fecundity, emigration/immigration, and/or genetic diversity. However, prior to undertaking such conservation actions, relevant knowledge about the ecology of the species or ecosystems and associated threats has to be collected to guarantee the efficiency of the resulting program (Soulé 1985). For instance, a conservation program has been implemented for a Jamaican-endemic reptile, the Jamaican Iguana (*Cyclura collei*). The methodology consisted in monitoring populations using abundance indexes and mark-recapture methods, monitoring nest sites to estimate the number reproductive females within the population and reproductive success, constructing artificial nesting sites, implementing a captive breeding program and reintroducing individuals within the natural population, and controlling exotic predators such as rats (*Rattus sp.*) and mongooses (*Herpestes sp.*). To that end, ecological research had to be performed to support conservation initiatives, through preliminary assessing the vulnerability of the species (Woodley 1980), monitoring its demographic evolution (e.g., survival, dispersal, home range; Lewis et al. 2008) and evaluating threats affecting its persistence in its natural habitat (e.g., hunting, invasive species and habitat loss; (Woodley 1980; Tole 2002; Lewis et al. 2011; Veen & Wilson 2017). This research effort allowed conservationists to evaluate the potential effectiveness of different actions, such as captive breeding and reintroduction (Lewis et al. 2008; Searcy et al. 2009). As a result of such conservation-oriented research programs, the number of reproductive females and the number of hatchlings has been multiplicated by five between 1991 and 2013 (Wilson et al. 2016a). However, such integrative conservation programs remain limited in the insular Caribbean and local biodiversity continues to decline (Hoffmann et al. 2010).

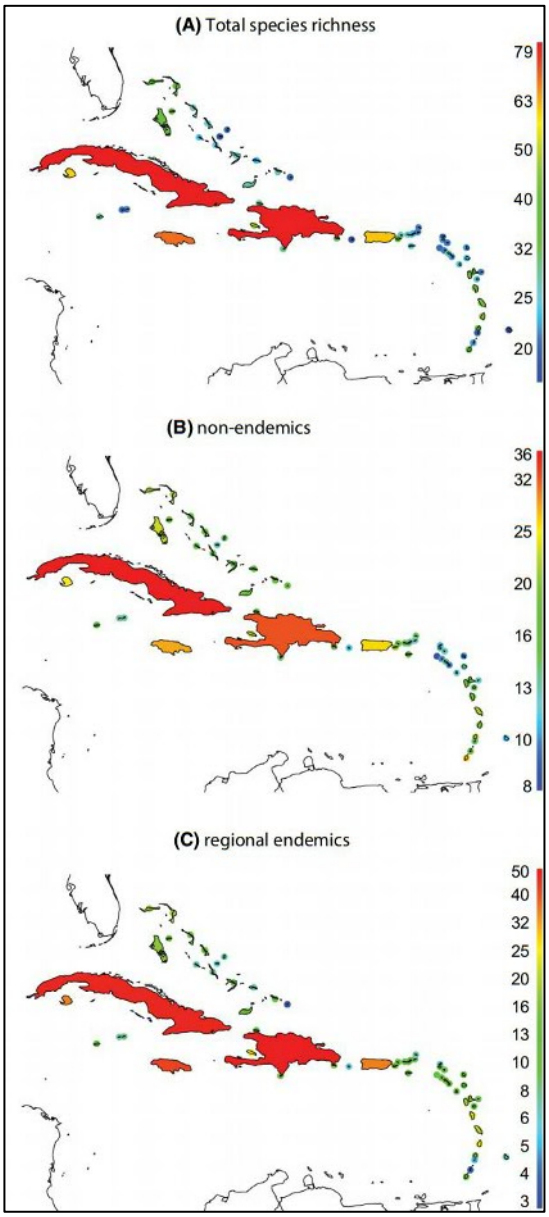


Figure 9 : Map representing the geographical richness distribution of (A) total species, (B) nonendemic and (C) regional endemic. Richness legends are in logarithmic scale. Figure extracted from Dalsgaard et al. (2014, Ecology and Evolution, 4:4019-31)

Avian conservation in the insular Caribbean

Nowadays, the Caribbean region is home to about 738 extant bird species according to the complete checklist of the Birds of the West Indies (Gerbracht & Levesque 2019), including at least 13 extinct species and six extirped. Among extant species, nearly half are residents, i.e., year-round species, including half of which are endemics. About the same percentage of extant species is non-resident (Table 3). Bird species are not homogeneously distributed across the region, with Greater Antilles islands having higher species richness, whether considering regional endemics or not (Dalsgaard et al. 2014, Table 3, Figure 9). In this respect, Dalsgaard et al. (2014) showed that the richness of Caribbean bird species and level of endemicity are positively related to the surface and elevation of islands, and the amount of rainfall. In contrast, they found a negative relation between avian species richness and island isolation and ambient temperature.

Table 3 : Number of bird species within the Caribbean region according to their status. Table was extracted from Gerbracht & Levesque (2019). See this report for complete table.

Category of species	**Living**	**Extinct**	**Total**
Residents	347	10	357
Regional endemics	*171*	*9*	*180*
Bahamas bank endemics	*6*	*1*	*7*
Greater Antilles endemics	*109*	*4*	*113*
Lesser Antilles	*23*	*4*	*27*
Non residents	334	3	337
Vagrants	*145*	*1*	*146*
Introduced species	100	-	100
Breeding species	362	10	372
Extirped species	6	-	6
Total in Caribbean region	**738**	**13**	**749**

The 738 extant species are mainly represented by Passeriformes (~40%), Charadriiformes (15%), Anseriformes and Caprimulgiformes (6% each). However, higher proportions of threatened species occur within less represented order such as Psittaciformes (43%), Procellariiformes (31%) and Columbiformes (21%; Figure 10). In addition, among the 13 extinct species, nine are endemic to the Caribbean region (Table 3) and mainly belonging to Psittaciformes (four species). Caribbean bird species are mainly exposed to habitat loss and alteration (including agriculture, urbanisation, logging and wood harvesting, natural system modifications and energy production and mining), invasive species, climate change and hunting (Figure 10A). These threats are more likely to increase extinction risk of year-round

species, including regional endemic species, as showed by statistical analyses performed using data extracted from IUCN Red List (Figure 11; Gerbracht & Levesque 2019).

As about 26% of Caribbean birds depend on forests to live (CEPF et al. 2016), it is not surprising that habitat loss and alteration, which mainly affect forests in Caribbean terrestrial ecosystems, is the main threat to which Caribbean bird species are exposed. In addition, similar to hunting and invasive species, habitat loss started as early as humans arrived on Caribbean islands and was intensified when Europeans colonised the archipelago. In response of habitat loss and degradation, initiatives have been taken in an attempt to regulate deforestation. Numerous national parks have been created in different Caribbean islands and Important Bird & Biodiversity Areas (IBAs) have been identified, a type of Key Biodiversity Areas dedicated to birds (Donald et al. 2019; for Caribbean examples see Cañizares & Reed 2020; Voskamp et al. 2021). IBAs are areas inhabited by vulnerable bird species and for which regular surveys should be carried out to detect threats and/or assess conservation actions at the level of areas and bird populations. For instance, the Bahama's Abaco National Park that was created to protect the habitat of the island-endemic Bahama Parrot, *Amazona leucocephala bahamensis* (Stahala 2008). In the Greater Antilles, the Sierra del Cristal National Park, in Cuba, harbours numerous species, including about 51 bird species out of which 10

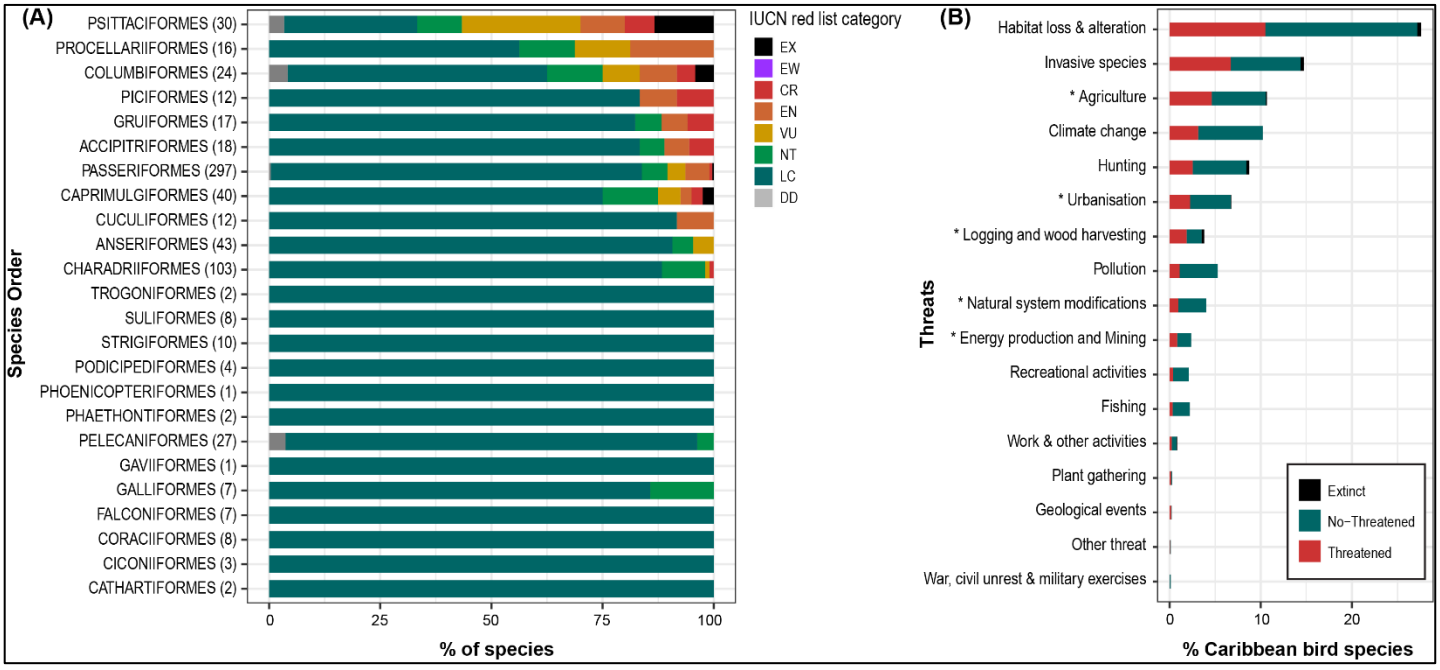


Figure 10 : (A) Percentage of species within bird Orders occurring in the Caribbean region according to their IUCN red list categories. Bird Orders are sorted from the Order having the higher proportion of threatened species (CR+EN+VU) to lowest ones. (B) Occurrence percentage of threats within Caribbean bird species. Threats with "*" are included in the threat named "Habitat loss and alteration"." Figures were performed using data of IUCN and Gerbracht & Levesque (2019)

are endemics (Fa et al. 2002). Hunting is often prohibited in such areas, thus offering potential refuges for bird species.

Regarding hunting, this activity is widespread in Caribbean islands and is a part of the activities that can be qualified as cultural and traditional. It includes both legal and illegal hunting, and recreational, sport and subsistence hunting. As a result, species having certain features are preys for hunters depending on the type of hunting practiced. For instance, hunters practicing recreational or sport hunting tend to target species that offer some shooting challenge, or that are appreciated for their meat, such as most of columbid species or waterbirds species, which are mainly hunted in the Caribbean region. In contrast, subsistence hunting is more opportunistic, or selects species that are easily killed or provide large quantity of proteins. In response to population declines, initiatives have been taken in order to attempt to regulate hunting pression, such as legislations regulating hunting, which limit the number of collected birds and/or limits spatially or temporally the hunting activity. For instance, in 2021, Puerto Rican hunters could shoot up to five scaly-naped pigeons and 30 Zenaida doves per hunting day, limited to Fridays, Saturdays, Sundays, Mondays and public

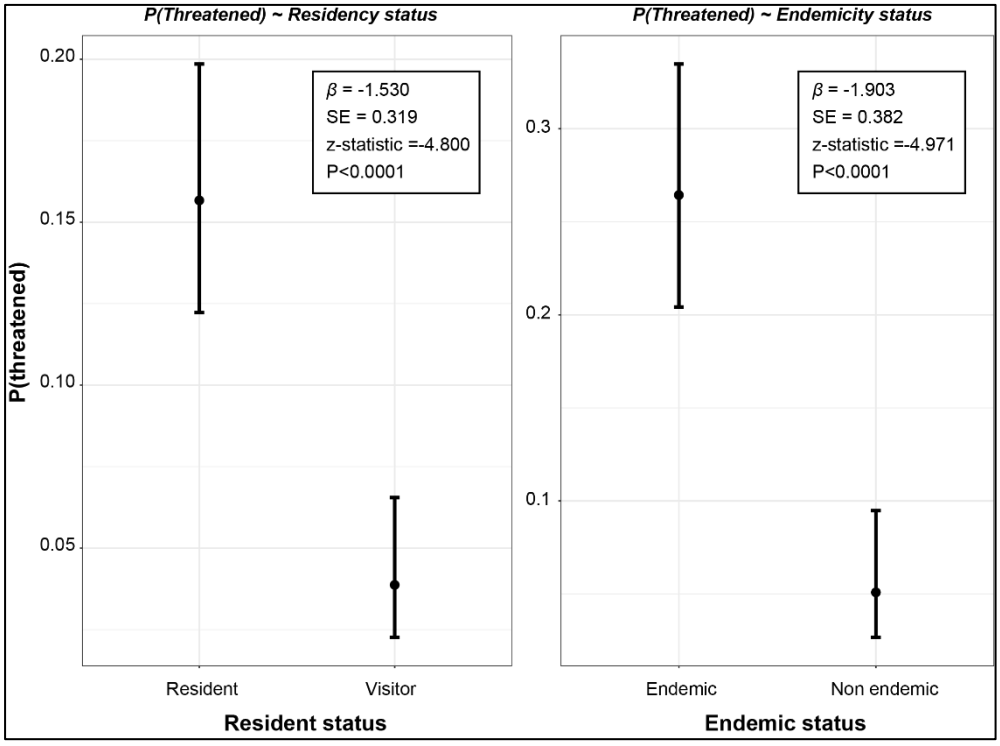


Figure 11 : Probability to be threatened of Caribbean birds according to residency and endemicity status. Error bars represent 95% confidence intervals. These results were obtained by performing a logistic regression relying on data extracted from IUCN red list and Gerbracht & Levesque (2019). For a complete explanation of the method having led to these results; see Appendix 2.

holidays in September and October. Such documents, regulating hunting activity, can be easily found on the internet from agencies in charge of implementing regulations.

Exotic species are another major threat to biodiversity, as a result of predation, parasitism, competition, hybridization with native species and the disturbance of native ecosystems (Mack et al. 2000; Sax et al. 2002; Sax & Gaines 2008; Walsh et al. 2012). Although rarely quantitively estimated, Caribbean birds are particularly exposed to predation by invasive species (see I.3.3). Invasive alien species are responsible of more than 30% of all known contemporary extinctions, especially in islands (Doherty et al. 2016). They can predate native animals or their offspring (Tidemann et al. 1994; James & Clout 1996; Fraser & Whitehead 2005) and they may also compete them for resources (Duron et al. 2017; Bond et al. 2019).

PhD thesis objectives

Although considerable research has been carried out on Caribbean birds, there still exists some disparities across bird taxa, geographical areas and research topics. In this respect, overall, the biology and ecology of most Caribbean bird species are often under-documented (Latta 2012). As a result, conservation management plans of vulnerable Caribbean birds are often implemented without having a good appreciation of the species ecology and biology in relation to threats and extinction risks. This is especially the case of most of Caribbean columbid species. In this context, this PhD work aimed at providing additional information about two regional endemic pigeons, the White-crowned Pigeon (WCPi - *Patagioenas leucocephala*) and the Scaly-naped Pigeon (SNPi - *P. squamosa*). The two species are exposed to several threats such as habitat loss, hunting, invasive species and global change. However, ecological and demographic data concerning these two species remain fragmentary and sparse. In addition, the genetic diversity of these two species and connectivity among populations at the scale of the Caribbean region are not documented. Literature search on these two species and on *Columbidae* in general, revealed a lack of information regarding actual threats weighing on pigeons and doves over the world. Up to now, only one article (Walker 2007) considered the geographic distribution of columbid species across the world according to their extinction risk and associated threats, as listed in the IUCN Red List. After a presentation of the *Columbidae* family, the first chapter of this

thesis provides an update on Walker (2007). We particularly went further by quantitatively assessing to what extent various biological, ecological, environmental and socio-political drivers influence extinction risk and population trends of columbid species. In addition, interspecific variation in levels of scientific attention was also investigated. The aim of this review was to provide some guidelines that would help to prioritize research on columbid species according to their extinction risk and in relation to their ecology, biology and environment. In contrast, the next chapters are dedicated to the White-crowned Pigeon and the Scaly-naped Pigeon. The second chapter presents two methodological studies. The first article proposes an efficient survey method for monitoring population trends of both the Scaly-naped Pigeon and the White-crowned Pigeon. The second is a comparison of two types of DNA samples, i.e., buccal cells and blood, for genetic study purposes. The third chapter documents a GPS tracking attempt to study white-crowned pigeons' movement. Collected data were however insufficient to assess movement patterns of the species as GPS devices stopped working, most probably due to technical and environmental issues. In the fourth chapter/article, the prevalence and diversity of haemosporidians, i.e., avian malaria, found in several white-crowned pigeon populations is presented. The fifth chapter consists of two articles addressing the genetic diversity and exiting connectivity among Caribbean populations of the White-crowned and the Scaly-naped Pigeon. Finally, the sixth chapter consists in a discussion of the results obtained during this PhD and perspectives for future conservation-oriented research on the two species.

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Appendices

Appendix 1 – Name of biodiversity hotspots, corresponding to legends of the Figure 8B

Legend on map	Name of biodiversity hotspots
1	The Tropical Andes
2	Mesoamerica
3	The Caribbean Islands
4	The Atlantic Forest
5	Tumbes-Chocó-Magdalena
6	The Cerrado
7	Chilean Winter Rainfall-Valdivian Forests
8	The California Floristic Province
9	Madagascar and the Indian Ocean Islands
10	The Coastal Forests of Eastern Africa
11	The Guinean Forests of West Africa
12	The Cape Floristic Region
13	The Succulent Karoo
14	The Mediterranean Basin
15	The Caucasus
16	Sundaland
17	Wallacea
18	The Philippines
19	Indo-Burma
20	The Mountains of Southwest China
21	Western Ghats and Sri Lanka
22	Southwest Australia
23	New Caledonia
24	New Zealand
25	Polynesia and Micronesia
26	The Madrean Pine-Oak Woodlands
27	Maputaland-Pondoland-Albany
28	The Eastern Afromontane
29	The Horn of Africa
30	The Irano-Anatolian
31	The Mountains of Central Asia
32	Eastern Himalaya
33	Japan
34	East Melanesian Islands
35	The Forests of East Australia
36	North American Coastal Plain

Appendix 2 – Method for the logistic regression performed for making the Figure 11.

We evaluated the extinction risk of Caribbean bird species according their residency and endemicity status. To that end, we relied on a logistic regression, considering as explained variable whether species are considered Threatened (Vulnerable to Critically Endangered) or Not-Threatened (Least Concern and Near threatened), and as explanatory variables their residency or their endemicity status. We used the R software to run the logistic model and the command *glm()*.

CHAPTER 1

Pigeons and doves (*Columbidae*)

The Columbidae family

Columbidae is the only extant family of Columbiformes and consists of 369 extant and extinct species of pigeons and doves that are distributed on all continents, except for the high Arctic and Antarctica (del Hoyo et al. 1997; BirdLife International 2021; Figure 12). This large family is easily recognisable with a general body shape consistent across species. However, they present a high variation in color patterns, from highly colored species, such as the Many-colored Fruit-Dove (*Ptilinopus perousii*), which has white, orange, purple, pink, yellow and green feathers, to duller, brownish species like most of *Macropygia sp.* In addition, they present a diversity of plumages, with some species being particularly extravagant such as *Goura spp.*, which are topped with a crown of fine and elegant feathers. There is also a large variation in size and weight among columbid species. The smallest described species is the Common Ground Dove (*Columbina passerina*) weighing between 22 and 40 g, while the largest and heaviest species is the extinct Dodo (*Raphus cucllatus*), that could weigh more than 10 kg. The largest extant species is the Western Crowned Pigeon (*Goura cristata*) weighing more than 2 kg (Gibbs et al. 2001).

Columbid species inhabit diverse ecosystems, from primeval ecosystems, such as lush virgin rainforests or arid deserts, to man-made ecosystems such as urban areas and agricultural lands (Gibbs et al. 2001; Winkler et al. 2020). However, there is a large disparity among species in the extent of their area of distribution. Some species are widely distributed, being found in all continents like the Feral Pigeon (*Columba livia domesticus*), whereas others have a very restricted spatial distribution, such as the Negros Fruit Dove (*Ptilinopus arcanus*),

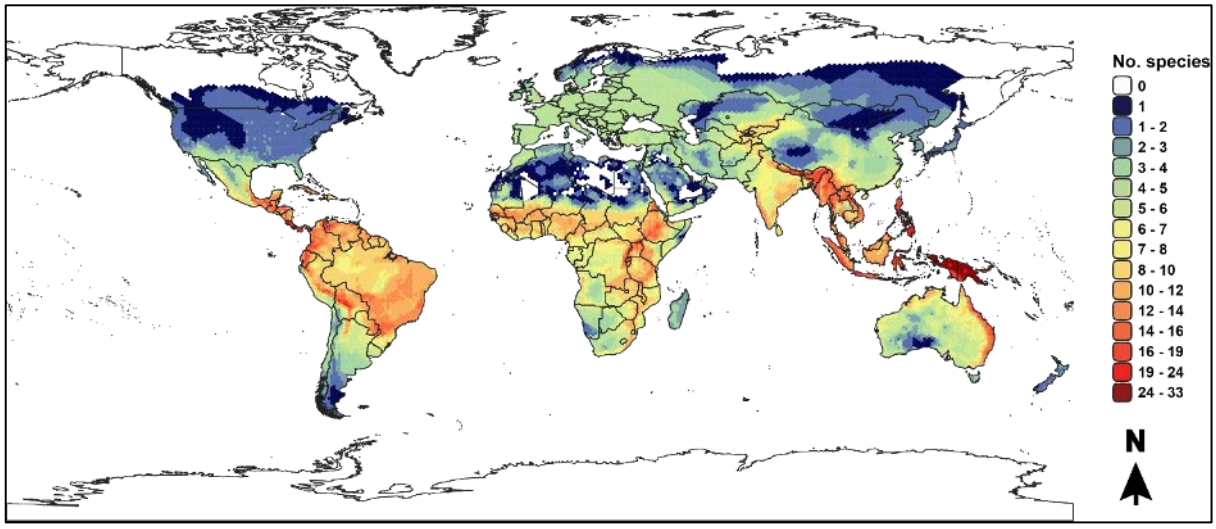


Figure 12 : Geographical distribution of species richness among the Columbidae family. Species richness were calculated into a grid cell of 1°x1°. Data used were extracted from BirdLife International (2021).

which is only found in a locality of a Filipino island (Baptista et al. 2020b). Columbid species are often categorized as granivorous and frugivorous, although some species can include invertebrates such as insects and snails in their diet (del Hoyo et al. 1997; Winkler et al. 2020). In addition, most of columbid species have conserved a high-flying ability (Winkler et al. 2020). Consequently, several species of pigeons and doves play an important role in the maintenance of plant diversity through seed dispersal, especially in tropical ecosystems (Meehan et al. 2005; Wotton & Kelly 2012; Costan & Sarasola 2017; Ando et al. 2021).

Overall, columbid species are threatened by habitat loss, hunting and invasive predators (Walker 2007). Among the extant species, 233 are listed as least concern in the IUCN Red List, 47 as near-threatened, 39 as vulnerable, 20 as endangered, 12 as critically endangered and one is data deficient. Besides, 17 species are currently extinct, including one that is extinct in the wild (BirdLife International 2021), the Socorro Dove (*Zenaida graysoni*). Among these extinct columbids, all of them were insular species, except for the Passenger Pigeon (*Ectopistes migratorius*). In addition, the two largest columbid species ever known, the extinct iconic Dodo and the extinct Rodrigues Solitaire (*Pezophaps solitaria*) were both endemic to Mauritius islands and flightless. Their extinction was probably due to a combination of factors, including large body size, island endemism, low population growth, small population size and small geographic range size, making them particularly vulnerable to habitat destruction, hunting and invasive species (Duncan et al. 2002; Fisher & Owens 2004; Hume 2006; Hilbers et al. 2016; see Article I).

ARTICLE 1

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Identifying global research and conservation priorities for *Columbidae*: a quantitative approach using random forest models.

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Draft manuscript

Introduction

Understanding global patterns of biodiversity is of crucial importance for assessing conservation research, priorities, and policies (Pimm *et al.* 2014; Costello & Chaudhary, 2017). The rate of biodiversity loss is currently increasing at an unprecedented rate, as a direct consequence of habitat loss, fragmentation and degradation, biological invasions, and climate change (Balmford, Green, & Jenkins, 2003; Barnosky *et al.* 2011). The overall decline in biodiversity results in the concomitant loss or reduction of several important ecosystem services that negatively affects human livelihoods (Worm *et al.* 2006; Tilman, Isbell, & Cowles, 2014; Bogoni, Peres, & Ferraz, 2020). However, the extent and rate of biodiversity loss are not uniform around the globe (Howard, Flather, & Stephens, 2020), nor across taxa (Pimm *et al.* 2014). As available funding for conservation research and actions is limited (Evans *et al.* 2012; Waldron *et al.* 2013), there is an obvious need to define priorities for optimizing resource allocation (Brooks *et al.* 2006).

Ideally, prioritization mechanisms should be based on sound and reliable scientific evidence. However, although research activity in conservation biology has markedly increased over the last three decades (Williams, Balmford, & Wilcove, 2020), important gaps in knowledge still exist. This is particularly true of many biodiversity-rich countries, where ongoing conflicts and political instability severely hamper the development of field research and monitoring programs (Hanson *et al.* 2009; Conteh, Gavin, & McCarter, 2017). More broadly, in several hotspots of biodiversity, local researchers and conservationists have only weak access to facilities and face the problem of irregular or insufficient funding (Ahrends *et al.* 2011; Barber *et al.* 2014; dos Santos *et al.* 2020; Vallès *et al.* 2021). In addition to spatial bias, the conservation literature tends to give a disproportionate attention to emblematic, charismatic, and flagship species (Andelman & Fagan, 2000; Brambilla, Gustin, & Celada, 2013; Mammides, 2019; Habel, Gossner, & Schmitt, 2021). Consequently, there is a certain mismatch between research efforts and conservation priorities (Christie *et al.* 2021). For instance, research on coral reefs tends is positively related to *per capita* gross domestic product and negatively to coral species richness, such that it is, overall, largely unrelated to conservation needs (Fisher *et al.* 2011). Similarly, research on felids is highly biased towards large, charismatic species, rather than being allocated in relation to actual conservation status (Brodie, 2009).

Despite the large attention given to bird species by professional and amateur ornithologists, the avian conservation biology research is no exception to the rule, and various taxonomic and geographic biases and knowledge gaps have been pointed out in recent years (de Lima, Bird, & Barlow, 2011; Ducatez & Lefebvre, 2014; Murray *et al.* 2015; Roberts *et al.* 2016; Buechley *et al.* 2019). Overall, the research effort in avian biology is significantly influenced by phylogeny, geographic range and ecological life history traits, with non-threatened species being studied twice as much as threatened ones (Ducatez & Lefebvre, 2014). This is also true within groups of charismatic bird species. For instance, although the Andean Flamingo, *Phoenicoparrus andinus*, is the least abundant flamingo in the world and is listed as Vulnerable by the IUCN Species Survival Commission (Derlindati *et al.* 2014), it has received far less attention than the Least Concern Greater Flamingo, *Phoenicopterus roseus* (Cézilly *et al.* 1997; Johnson & Cézilly, 2007; Geraci *et al.* 2012). From a geographic point of view, the majority of avian conservation research is conducted in developed countries. In addition, not surprisingly, avian conservation research is achieved mainly by North American and western European researchers, confirming the mismatch between priorities and capacity for research and conservation (Brito & Oprea, 2009; see also Vallès *et al.* 2021).

Columbidae, the only extant family of columbiformes, in particular, appear to be particularly understudied, despite their patrimonial value and their high ecological and conservation relevance (Walker, 2007; McKenzie & Robertson, 2015; Yarwood, Weston, & Symonds, 2019; Devenish-Nelson *et al.* 2019; Wood & Cerbini, 2021). Pigeons and doves are one of the oldest and most diverse extant lineages of birds. However, their evolutionary radiation remains poorly resolved (Soares *et al.* 2016). Their phylogeny is subject to frequent modifications, with the creation of new genus or species and the slimming of other genus based on molecular or paleontological evidence (*e.g.* Johnson *et al.* 2001; Banks *et al.* 2013; Cibois *et al.* 2014, 2017; Dourado *et al.* 2014; Sangster, Sweet, & Johnson, 2018; Steadman & Takano, 2020; Liu *et al.* 2020). As a result, the number of species belonging to this family is regularly updated. Currently, it includes about 369 extant species of pigeons and doves that are distributed on all continents, except for the high Arctic and Antarctica (del Hoyo *et al.* 1997; BirdLife International, 2021). They are found in various habitats, mainly in tropical rain forests, but also in dry forests, mangroves, deserts, mountains, as well as in human-altered habitats, including the urban environment (del Hoyo *et al.* 1997; Gibbs, Barnes, & Cox, 2001;

Carvalho *et al.* 2015; Fey *et al.* 2015; Monterrubio-Rico *et al.* 2016). Columbid species are often divided into granivorous and frugivorous species, although some species can include invertebrates such as insects and snails in their diet (del Hoyo *et al.* 1997). Consequently, several species of pigeons and doves play an important role in the maintenance of tropical or semi-arid forest diversity through seed dispersal (Meehan, Mcconkey, & Drake, 2005; Wotton & Kelly, 2012; Costan & Sarasola, 2017; Ando *et al.* 2021). Columbids are also an important source of proteins for human populations in different parts of the world (del Hoyo *et al.* 1997), and are often much appreciated by local hunters (da Silva *et al.* 2021).

However, a significant proportion of columbid species are endangered or threatened with extinction (Bennett & Owens, 1997; Walker, 2007; Rivera-Milán *et al.* 2015; Lees *et al.* 2021; Wood & Cerbini, 2021). The decline of columbid species and populations usually results from habitat fragmentation and degradation, introduced predators, competition with invasive columbid species, excessive hunting pressure and ineffective management, and trade (Owens & Bennett, 2000; Walker, 2007; Carvalho *et al.* 2015; Rivera-Milán, Boomer, & Martínez, 2016; Camacho-Cervantes & Schondube, 2018; Lamelas-López *et al.* 2020; da Silva *et al.* 2021; Moreno-Zarate, Arroyo, & Peach, 2021). The conservation status of several species remains however uncertain, mainly due to lack of data (del Hoyo *et al.* 1997; Walker, 2007; Wood & Cerbini, 2021). This is particularly true of secretive species or species with very restricted geographical range for which traditional study methods might not be appropriate (Cambrone, Bezault, & Cézilly, 2021). More to the point, little effort has been made so far to synthetise the available information on the factors influencing the conservation status of columbid species and research effort. To the best of our knowledge, only Walker (2007) provided a short review of the main drivers of extinction risk for columbid species based on data from BirdLife International, emphasizing the vulnerability of insular species.

Here we assess to what extent environmental, life history and socio-political factors may drive the extinction risk of pigeons and doves and explain differences in scientific attention among species. To that end, we relied on random forests (RFs) using a database especially compiled for this study. This machine-learning technique has been increasingly used in ecological and macro-ecology studies (Cutler *et al.* 2007; Kampichler *et al.* 2010) that aimed at assessing the impact of various biological or ecological drivers on the extinction risks of a given group of species (Davidson *et al.* 2012; Di Marco *et al.* 2014, 2018; Buechley *et al.*

2019). In particular, this modelling method is not affected by phylogenetic inertia, can include both continuous and categorical variables, and its predictions are not much affected by outliers (Murray *et al.* 2011; Davidson *et al.* 2012; Buechley *et al.* 2019). This technique is therefore particularly suitable for columbids given that their phylogenetic relationships are not fully resolved due to scarce sampling of taxa and limited availability of sequence data (Khan & Arif, 2013; Bruxaux *et al.* 2018).

Materials and Methods

Database creation

Information about Columbid species

We compiled available data for the 369 species based on three main sources: Birdlife international (BirdLife International, 2021), the website *Birds of the World* (https://birdsoftheworld.org) and *Pigeons and Doves: A Guide to the Pigeons and Doves of the World* (Gibbs et al. 2001). We extracted information on biological, ecological and environmental traits that are likely to influence extinction risk (see Peñaranda & Simonetti, 2015; Buechley *et al.* 2019). We classified those traits into four categories, namely "environment", "behaviour and life history", "anthropogenic pressure" and "socio-political indicators" (see Table 1). In order to estimate extinction risk of columbid species, we relied on IUCN Red List categories and global population trends of each species (BirdLife International, 2021). We classified species as threatened (critically endangered *CR*, endangered *EN* and vulnerable *VU*), non-threatened (near-threatened *NT* and least-concern *LC*), extinct (extinct *EX* and extinct in the wild *EW*), or data deficient. Because non-threatened species do not necessarily show stable or increasing population trends (Fig. 1), we used global population trends of species (a demographic trait that is included in the IUCN status assessment of species, Mace & Lande, 1991; Rodrigues *et al.* 2006) as a second proxy of extinction risk. Species were thus classified as increasing, stable or decreasing according to (BirdLife International, 2021).

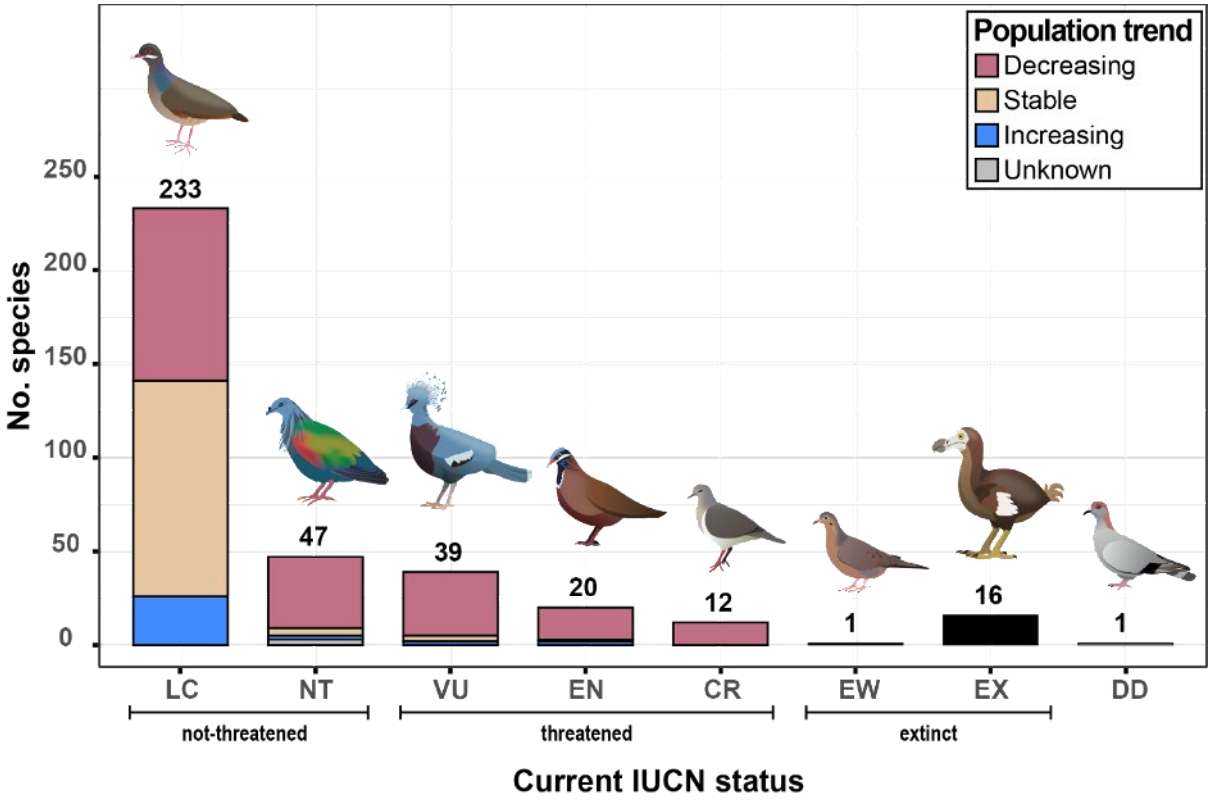


Fig.1 : Distribution of columbid species according to current IUCN status and current population trends. From left to right, columbid species illustrations correspond to: Bridled Quail-Dove (*Geotrygon mystacea*), Nicobar Pigeon (*Caloenas nicobarica*), Victoria Crowned-Pigeon (*Goura victoria*), Blue-headed Quail-Dove (*Starnoenas cyanocephala*), Grenada Dove (*Leptotila wellsi*), Socorro Dove (*Zenaida graysoni*), Dodo (*Raphus cucullatus*) and Somali Pigeon (*Columba oliviae*).

Scientific attention

In order to quantify the scientific attention given to each columbid species, we relied on the number of peer-reviewed articles. To that end, we performed an extensive bibliographic search on all databases of the Web of Science (on the mid-2021). We performed searches using current and past binomial scientific names, vernacular names in English, French and Spanish and all recognised synonyms. To compile all possible names of species, we used the websites of Avibase (https://avibase.bsc-eoc.org) and Birds of the World. We considered various "research areas", as defined in the Web of Science, as all studies bringing ecological knowledge about a species may be helpful for its conservation (Courchamp *et al.* 2015). Each search was conducted using the following procedure:

"TS="
(in order to search following terms in title, abstract and keywords of articles)
+
Full binomial scientific names, vernacular and all recognised synonyms in independent quotes, separated by "OR"
+

“AND SU=("Ecology" OR "Biodiversity and Conservation" OR "Ornithology" OR "Evolutionary Biology" OR "Zoology")”
(to limit searches to studies related to following science disciplines: Ecology, Conservation Biology, Ornithology, Evolutionary Biology/Ecology and Zoology)
+
“NOT SU=("Veterinary Sciences" OR "Neurosciences & Neurology" OR "Anthropology")”
(to exclude any study related to veterinary sciences, neurosciences and neurology, and anthropology).

We also performed a global search in order to estimate national contributions to research on *Columbidae,* assessed from the affiliations of authors of peer-reviewed articles. To that end, we used the same search nomenclature, except that we replaced names of species and their synonyms by “*Columbidae*”, “Columbiformes”, “Pigeon” and “Dove”, separated by the Booleans operator “OR”. In order to exclude all articles on Pigeon pea (*Cajanus cajan*), we added: NOT TS=(“Pigeon*pea*” OR “*Cajanus cajan*”).

Spatial analyses (GIS)

We used QGIS (3.10.11) to calculate spatial statistics for explanatory variables related to extinction risk and scientific attention (Table 1). We obtained various raster and polygon files from several sources (Table 1). Polygons of historical species’ range were obtained from Birdlife International (BirdLife International and Handbook of the Birds of the World, 2018). We calculated Governance Stability Index (GSI) and Human Development Index (HDI) per country, and imported their values in a polygon file representing countries. After mapping country polygons onto the historical ranges of each columbid species, we calculated the geometric mean of HDI and GSI for each species. In order to determine whether species range only consists of islands, we relied on polygons representing continents rather than islands because island boundaries and their locations were not accurate and not exhaustive. Thus, all species for which the historical range did not overlap continents were considered as strict island species. For these species, we thereafter estimated the distance to the closest continent using the v.distance GRASS (7.8.4) function implemented in QGIS. For all species, we calculated the proportion of their historical range on islands. In the same way, we calculated the proportion of the species range overlapping biodiversity hotspots and tropical areas, using, respectively, a shapefile from Hoffman *et al.* (2016) and a polygon manually created that overlayed the intertropical area. From raster files for Human Footprint (HFP) and Human density values, we calculated the mean of pixel values within the historical range of

each species. Based on estimates of hurricane frequency, we calculated the frequency of occurrence within each species range of hurricanes ranked at least 3 on the Saffir-Simpson scale. We considered hurricanes occurring since 1980 in order to have an accurate event record (Knapp *et al.* 2010). We obtained hurricane trajectories from the International Best Track Archive for Climate Stewardship (IBTrACS v4, Knapp *et al.* 2010). To estimate the frequency of events (number of events per year) per species, we considered a range of 200km around each hurricane trajectory (thus including the eye and outer cores), based on studies of the boundary layer wind structure of tropical hurricanes (Schwendike & Kepert, 2008; Kalourazi *et al.* 2020). In the case a given hurricane trajectory overlapped more than once with the distribution range of a species, this was counted as a single event for the considered species. We then calculated the mean hurricane frequency per species from 1980 to 2020. We also obtained geographical maps outputs using QGIS. To that end, we rasterized polygons representing area distribution of species using scientific attention (log[number of articles + 1]) as value for the raster unit (square of 400km²). Since, polygons of several species may overlap, we beforehand calculated the mean of scientific attention per raster unit.

Random forest models

For our analyses relying on random forest models (hereafter RFs), we excluded extinct species and data deficient species (Fig. 1). We thus performed three different random forest models (hereafter RFs) to identify the factors influencing the extinction risk of columbid species, population trends of columbid species and scientific attention given to columbid species. Several studies on birds, mammals or amphibians have relied on this modelling approach for the same purpose (e.g., Davidson *et al.* 2009, 2012; Murray *et al.* 2011; Di Marco *et al.* 2014; Buechley & Şekercioğlu, 2016; Buechley *et al.* 2019). RFs are built into two steps, which are repeated a given number times. The first step consists in bootstrapping the original dataset and the second consists in building a classification tree (CT) from this bootstrapped dataset. A CT consisted of successive dichotomous nodes containing a high proportion of statistical individuals belonging to the same class (i.e., in our case, level of extinction risk). At each node, a random subset of explanatory variables or column is selected as candidate for the node formation. The number of randomly selected variables is determined by the experimenter or automatically by the program (see below for our models). Among these variables, the one

able to split statistical individuals into two groups as homogeneous as possible is used to create the node. The others are then randomly selected subsequently for the formation of the following nodes. In the case of quantitative explanatory variables, the program splits statistical individuals by finding a threshold value allowing their partitioning in two homogenous groups. The formation of nodes goes on until the program cannot split individuals homogenously enough into two groups. The end of each branch corresponds to a class of the explained variables, and hence the prediction of the CT (e.g., in our case, threatened or no-threatened). The program repeats the two steps a given number of times, thus creating hundreds or thousands of CTs from which the forest develops. The final result of a RF is the average of predictions estimated by all CTs (Genuer and Poggi 2019). The accuracy of a RF is measured based on the statistical individuals that have not been selected by the bootstrap process during the first step. Once CT is created at the end of the second step, the program predicts the class of these unselected statistical individual (*e.g.,* in our case, threatened or no-threatened) based on the CT just created, and compare them with their actual class. The percentage of error in predicting the class of these statistical individuals across all CTs of the RF provides a estimate of RF accuracy, or Out Of Bag errors (OOB). This process therefore allows predicting outcomes based on the associations existing among explanatory variables (Davidson *et al.* 2009). Examples of CTs (Hothorn, Hornik, & Zeileis, 2006) based on our original data and aiming at classifying species according their extinction risk, population trends and scientific attention are presented in Appendix S1 (Fig. S1-S3) in order to illustrate the building process of a random forest.

We ran the three random forest models by including all traits listed and detailed in Table 1 as explanatory variables. We thus considered a wider range of threats than the one considered by IUCN, thus reducing the risk of circularity when using IUCN status as a proxy of extinction risk of columbid species. All models were performed using the R software (v4.1.1, R Core Team, 2021), using RandomForest package (Liaw & Wiener, 2002). Forests consisted of 10,000, 10,000 and 1,000 CTs for models implying respectively extinction risk, population trends and scientific attention, in order to reach the lowest value of OOB, which was also stable throughout the formation of the forest. To evaluate the accuracy of our classification models, we also relied on the percentage of species correctly classified, Cohen's kappa statistic that tests the consistency between the actual and predicted classifications, and the

sensitivity and specificity of models (Prasad, Iverson, & Liaw, 2006; Allouche, Tsoar, & Kadmon, 2006; Gamer *et al.* 2019). In the case RF models assign statistical individuals to one of two categories, such as non-threatened or threatened in our study, sensitivity is the percentage of actually non-threatened species correctly identified by the model, while specificity corresponds to the percentage of actually threatened species correctly classified by the model. In contrast, when RF models imply more than two levels of classification in the explained variable, as for population trends in the present study (i.e., decreasing vs. stable vs. increasing), sensitivity is the percentage of species having a given population trend correctly identified by RF model (percentage of true positive), while specificity is the percentage of species correctly classified by the RF model as not belonging to the considered population trend (percentage of true negative). For the RF implying scientific attention (quantitative variable), we included the current IUCN category, status (threatened/non-threatened), and population trends as explanatory variables and we relied on the R-squared as accuracy metric of our model, which estimates the percentage of variance explained by the model. The number of variables tested at each split was determined by the program (*mtry* ; root-squared of the total number of explanatory variables). We estimated the relative importance of each explanatory variables implied in RF models based on the mean decrease Gini (when the dependent variable is qualitative or classes) or increase in node purity (when the dependent variable is quantitative), which measures how much the model accuracy decreases when we removed the considered explanatory variable. The calculation is based on the Gini/node impurity value estimated from CTs composing the forest. We graphically visualised relationships between explanatory variables and dependent variables relying on partial dependence plots. The area was restricted to the eight first most important variables, as the other variables were much less meaningful and did not present enough variation (see Appendix S1, Fig. S4-S7). In order to compare and statistically assess the relative importance of explanatory variables and partial plots, we calculated 95% confident interval (95%CI) by performing 1,000 independent bootstraps from the original dataset (Cumming & Finch, 2005). Results of inferential statistical tests (i.e., tests of Wilcoxon, Kruskal-Wallis, Kendall's correlation or Fisher) are presented in Appendix S1 (Table S1-S3), for all explanatory variables

Table 1 : Traits used as explanatory variables in random forest models

Class	Variables	Description	Source
Environment	Altitude	Averaged altitude at which species occurs (x10² m).	BirdLife International, 2021
Environment	Dist. to continent ‡	Distance to the closest continent (km). Species being even partially on continent are set at 0. This variable was normalized using log (x+1) transformation in order to improve graph quality.	BirdLife International, 2021 ; Sayre *et al.* 2019 ; data.gouv.fr
Environment	Endemicity ‡	Species occurring in one country (Yes / No).	BirdLife International, 2021
Environment	Hurricane frequency ‡	Number of hurricanes ranked at least 3 in the Saffir-Simpson scale per year, since 1980. Hurricanes are known to affect avifauna (Wunderle 1995).	Knapp *et al.* 2010
Environment	IBA	Overlap between historical species range and Important Bird and Biodiversity Areas (Yes/No).	BirdLife International, 2021
Environment	Prop. in islands ‡	Proportion of historical species range on islands. Islands species are usually more prone to extinction (Wood et al. 2017)	Sayre *et al.* 2019 ; data.gouv.fr
Environment	Prop. in biodiv. Hotspot ‡	Proportion of historical species range overlapping with biodiversity hotspots.	Hoffman *et al.* 2016
Environment	Range size ‡	Historical geographic range size of species (km²). This variable was normalized by log transformation in order improve on graph quality. This variable is a strong predictor of species vulnerability (Staude et al. 2020, Newsome et al. 2020)	BirdLife International, 2021
Environment	Island endemic ‡	Restriction of historical species range to a single country consisting of one or several islands. (Yes/No). Island- endemic species are usually more prone to extinction (Wood et al. 2017)	BirdLife International, 2021; Sayre *et al.* 2019; data.gouv.fr
Environment	Prop. in tropical ‡	Proportion of historical species range between the northern and the southern tropics.	Manually created
Behaviour and Life history	Fecundity	Average clutch size.	Birds or the world; Gibbs *et al.* 2001
Behaviour and Life history	Diet	Seven diet categories: granivorous, frugivorous, herbivorous (parts of plants other than seeds or fruits. e.g., buds), invertebrates eaters, meat, fungi and/or reptiles. The last three diet are very rare.	Birds or the world; Gibbs *et al.* 2001
Behaviour and Life history	Diet breadth	Number of diet categories per species	Birds or the world; Gibbs *et al.* 2001
Behaviour and Life history	Forest dependency	Forest dependency of species. (No forest sp. (No) < Low (Lo) < Medium (Mid) < High (Hi))	BirdLife International, 2021
Behaviour and Life history	Generation length	Generation length of species from Birdlife International. The variable is known to impact extinction risk in birds (Bird et al. 2020).	BirdLife International, 2021
Behaviour and Life history	Sociality	Sociality of species. (Gregarious (Greg.) / Occasionally gregarious (Occ.) / Solitary (Sol.))	Birds or the world; Gibbs *et al.* 2001
Behaviour and Life history	Life stratum	Stratum in which the species live (Arboreal (Arb.)/ Ground (Gr.) / Both).	Birds or the world; Gibbs *et al.* 2001
Behaviour and Life history	Mass	Averaged body mass of species (g). This variable was normalized using log transformation to improve graph quality.	Birds or the world; Gibbs *et al.* 2001
Behaviour and Life history	Migration	Migration status: Full migrant (Full.) / Vagrant (Vag.)/ Sedentary (Sed.)	BirdLife International, 2021; Birds of the world; Gibbs *et al.* 2001
Behaviour and Life history	Habitat breadth	Number of different habitats used, based on Habitat level 2 from Birdlife International.	BirdLife International, 2021

Table 1 (next): Traits used as explanatory variables in random forest models

Class	Variables	Description	Source
Pressure	Subsistence use	Species hunted for subsistence purpose, as defined by IUCN Red List (Yes / No).	BirdLife International, 2021
Pressure	Handicraft use	Whole or parts of the species used for crafting jewellery, ornament, or any items (Yes / No).	BirdLife International, 2021
Pressure	HFP ‡	Geometric mean of human footprint values estimated in 2009. (see Di Marco (2018) and Venter et al. (2016) for detailed explanation)	Venter *et al.* 2016, 2018
Pressure	IAS birds	Species impacted by invasive alien bird species (Reported/Not Reported).	BirdLife International, 2021
Pressure	IAS mammals	Species impacted by invasive alien mammals species (Reported/Not Reported).	BirdLife International, 2021
Pressure	IAS reptiles	Species impacted by invasive alien reptiles species (Reported/Not Reported).	BirdLife International, 2021
Pressure	Pets use	Use of species used as pet or display (Yes/No).	BirdLife International, 2021
Pressure	Sport hunting use	Species exposed to sport hunting (Yes/No).	BirdLife International, 2021
Socio-political indicator	GSI ‡	Governance stability index. This index is the average of six indices that measures governance (voice and accountability, political stability and absence of violence, government effectiveness, regulatory quality, rule of law, and control of corruption (Baynham-Herd et al. 2018).	World Bank database
Socio-political Indicator	HDI ‡	Human Development Index. This index is based on life expectancy, years of schooling and gross national income per capita.	World Bank database
Socio-political political indicator	Human density ‡	Number of humans per km². Averaged over estimates for 2000, 2005, 2010 and 2020.	CIESIN, 2018

Results

Among the 369 described columbid species, 76% are considered by IUCN as non-threatened and 19% as threatened, and 5% as extinct or extinct in the wild (Fig. 1). In terms of population trends, 52% of species are declining, while 33% are stable and 9% are increasing. As expected, the relative proportions of declining, stable, and increasing species differed between threatened and non-threatened ones (threatened species: 89%, 6%, and 5%, respectively; non-threatened species: 46%, 43%, and 11%, respectively; Fisher's exact test, $P < 0.0001$).

Extinction risk

Threatened vs. non-threatened species

The RF model classifying species either as non-threatened or threatened correctly classified 87% of the species based on the 38 explanatory variables used (Table 1). Sensitivity and specificity of model respectively indicated that 87% of actual non-threatened species and 82%

of actual threatened species were correctly identified by the RF model. Cohen's kappa statistics further indicated that the classification of species significantly differed from a random classification (Cohen's kappa = 0.51, P < 0.0001), while the OOB error was 13%. The eight most important variables influencing extinction risk were in decreasing order: species historical range size, impact of invasive alien mammals, distance to the closest continent, altitude distribution of the species, human footprint on ecosystems, human density, proportion of species historical range in current biodiversity hotspots, and species body mass. Based on the 95% CI comparison, only historical geographic range size had a relative importance significantly higher than the other variables (Fig. 2A, see Appendix S1, Fig. S4 for all variables importance values). Based on partial dependence plots, species with the smallest historical range size, exposed to invasive alien mammals, living the farthest to a continent and/or at the lowest altitudes were associated to a greater risk of extinction (Fig. 3A). Although other variables were relatively important in the prediction of species' risk extinction based on the RF model, their variation did not seem to be directly associated to extinction risk (Fig. 3A; see Appendix S1, Fig. S5 for all variables).

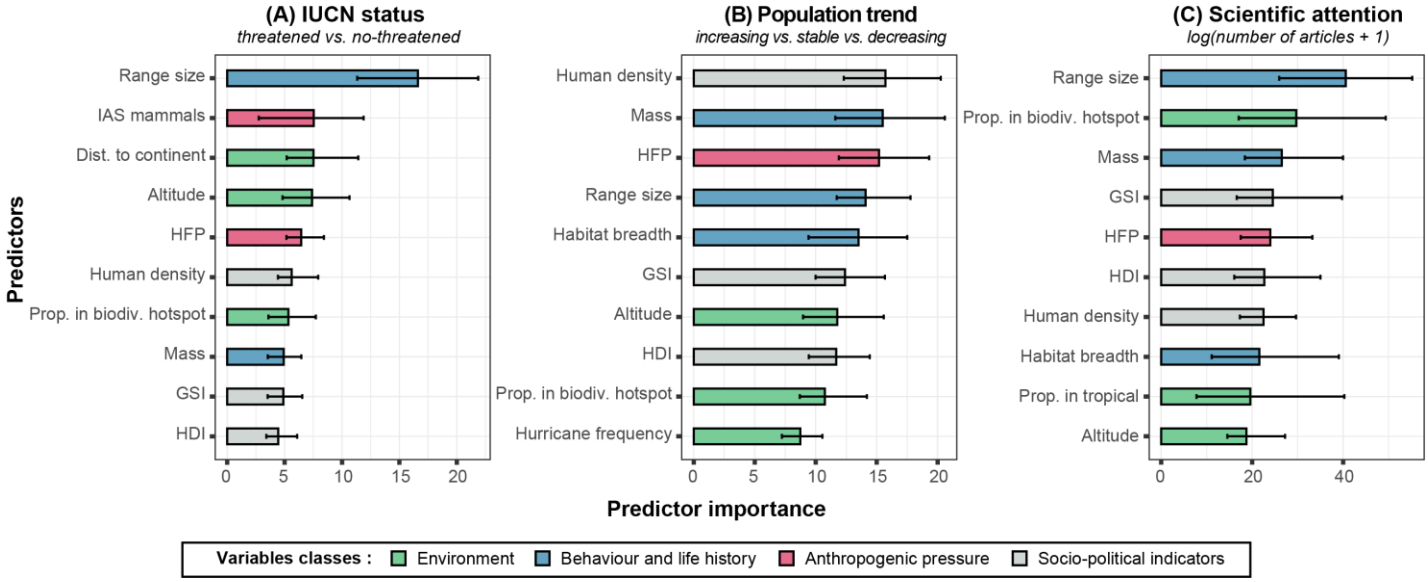


Fig.2 : The relative importance of each explanatory variables included in RF models with (A) IUCN status, (B) population trend and (C) scientific attention as explained variables. Only the ten most important variables are presented here. See Appendix S1, Fig. S4 for all explanatory variables.

Population trends

The RF model correctly classified 73% of species according to population trends, with a OOB error of 27%. In addition, the percentage of true positive was of 74%, 69% and 64% for decreasing, stable and increasing species, respectively. On the other hand, the percentage of true negative was of 80%, 89% and 94% for decreasing, stable and increasing species' population trends, respectively. Although goodness-of-fit was lower than for the previous model, Cohen's kappa still indicated that the classification of species differed from a random classification (Cohen's kappa = 0.48, $P < 0.0001$). The eight most important variables to predict population trends were in decreasing order: human density within the species historical geographic range distribution, species body mass, human footprint on ecosystems, species historical geographic range size, species habitat breadth, governance stability index, species altitude distribution, and human development index (Fig. 2B). Based on the comparison of 95%CI, these eight variables did not seem to be significantly different in terms of importance (Fig. 2B, see Appendix S1, Fig. S4 for all variables importance values). Based on partial dependence plots, the risk of population decline was significantly lower for species with a low body weight living in areas characterized by high human density (Fig. 3B). In addition, a higher risk of decline was associated with species with narrow habitat breadth and living at low altitudes (Fig 3B). Although the other variables were important in predicting species' risk of decline based on RF model, partial dependent plots suggest that they are not directly associated to species risk of decline (Fig. 3B; see Appendix S1, Fig. S6 for all variables).

Scientific attention

Our literature survey resulted in a total of 3,537 research articles across the 369 Columbidae species, in scientific disciplines related to environment, ecology and evolution. From these articles, 405 concerned multiple species. Scientific attention (assessed from the number of published articles) differed markedly between columbid species, ranging from 0 to 1363 (median = 1, interquartile = 3). Overall, 10 species (about 3% all species) accounted for 77% of all research articles published since 1966. The more commonly studied species were the Common Wood Pigeon (*Columba palumbus*, 1363 articles), the Rock Pigeon (*Columba livia*, 1079), the Mourning Dove (*Zenaida macroura*, 309), the European Turtle-Dove (*Streptopelia turtur*, 121), the Eurasian Collared-Dove (*Streptopelia decaocto*, 117), the Common Ground

Dove (*Columbina passerina*, 115), the Malagasy Turtle Dove (*Nesoenas picturatus*, 93), the Zenaida Dove (*Zenaida aurita*, 79), the White-winged Dove (*Zenaida asiatica*, 65) and the Plain-breasted Ground Dove (*Columbina minuta*, 46). Among them, only the Eurasian Turtle Dove is listed as vulnerable with a high risk of extinction, whereas the nine other species are considered of least-concern. In contrast, we did not find any article for 151 (41%) species.

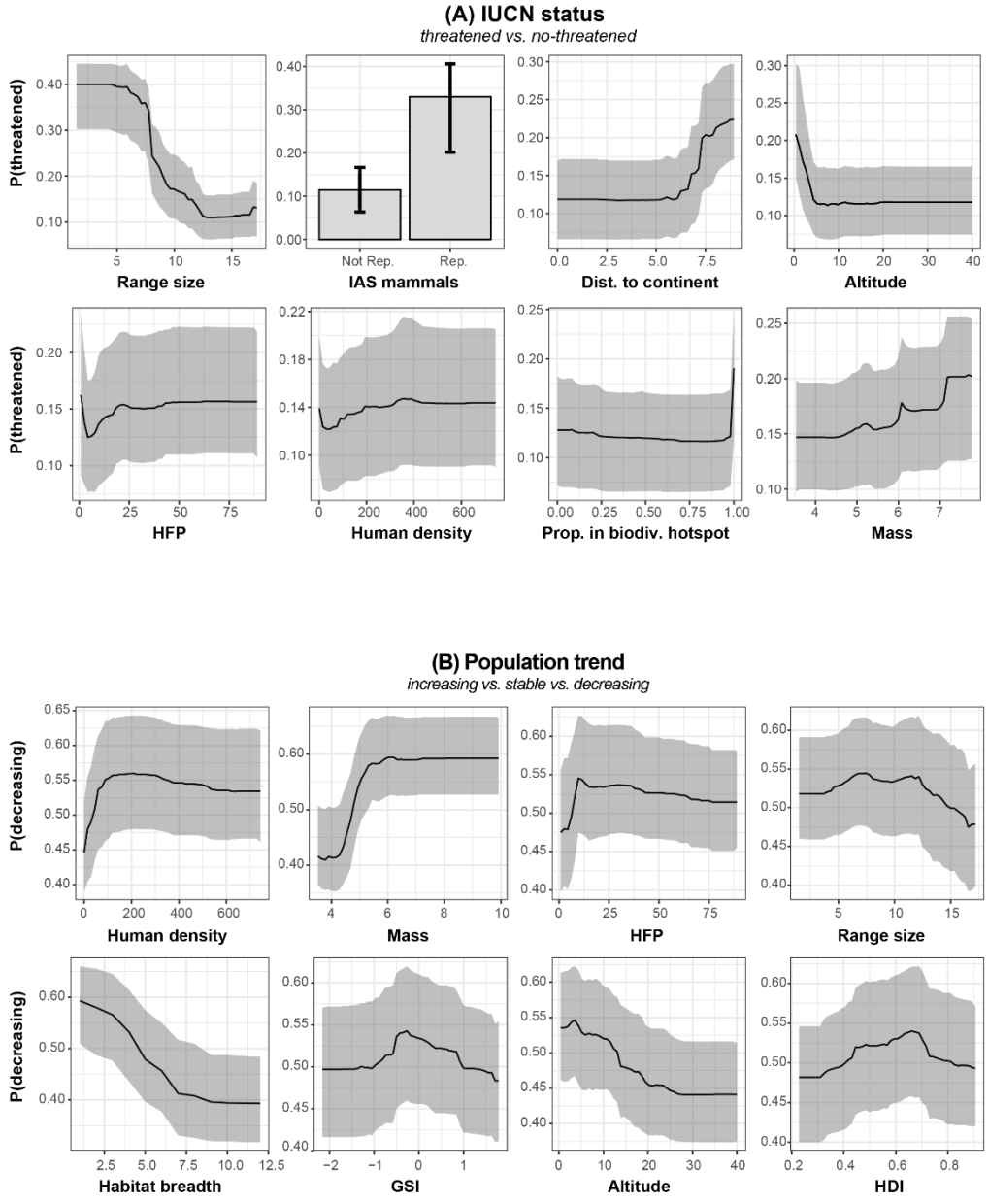


Fig.3: Partial dependence plots illustrating (A) the probability for a species to be threatened and (B) the probability of decreasing population trend in relation to the top eight predictors according to their relative importance (Fig. 2). See Appendix S1, Fig. S5-S6 for partial dependence plots of all explanatory variables.

Overall, scientific attention was negatively associated with IUCN status (Jonckheere-Terpstra test, JT= 13864, P = 0.006).

Research labs in North America and Europe contributed the most to research on columbid species, amounting to, respectively, about 43% and 32% of articles published in peer-reviewed journals (Fig. 4A). Scientific attention by country was positively correlated with GSI and HDI, but negatively with HFP (Fig. 4). Based on geographic distribution of species, species inhabiting close to "hotspots" of research on columbid species were the most studied (Fig. 4). The geographic distribution of scientific attention was also positively correlated with GSI and HDI, and, contrary to scientific attention per se, to HFP (Fig. 4). Accordingly, the vast majority of unstudied species were located in the southern hemisphere, while the top 10 most studied species mostly were all located in the northern hemisphere (Fig. 4 and Appendix S1, Fig. S8).

Based on RF models, explanatory variables accounted for 45% of the observed variation in scientific attention among columbid species. The most important variables were species historical geographic range size, proportion of species range in current biodiversity hotspots, species body mass, governance stability index, human footprint on ecosystems, human development index, human density within species range, and species habitat breadth (Fig. 2C). In terms of relative importance, the historical range size of species did not differ from the proportion of species range in current biodiversity hotspots, species body mass of species, GSI, and species habitat breadth, as evidenced by their 95%CI (Fig. 2C). The importance of the other variables was lower (Appendix S1, Fig. S4). Partial dependence plots show that species with the largest range size, largest species, species occurring in countries with highest values of governance stability index, and species located in ecosystems highly impacted by human activities (high HFP value) have been the most studied (Fig. 5). Although no clear association was observed with the proportion of species range within hotspot biodiversity, there was a trend for species exclusively occurring in biodiversity hotspots to be less studied than others. In addition, we observed a positive trend relating scientific attention to species habitat breadth (Fig. 5). Although the others variables were more or less important to explain scientific attention variation based on RF model, their variations were not directly associated to scientific attention (Fig. 5; see Appendix S1, Fig. S7 for all variables).

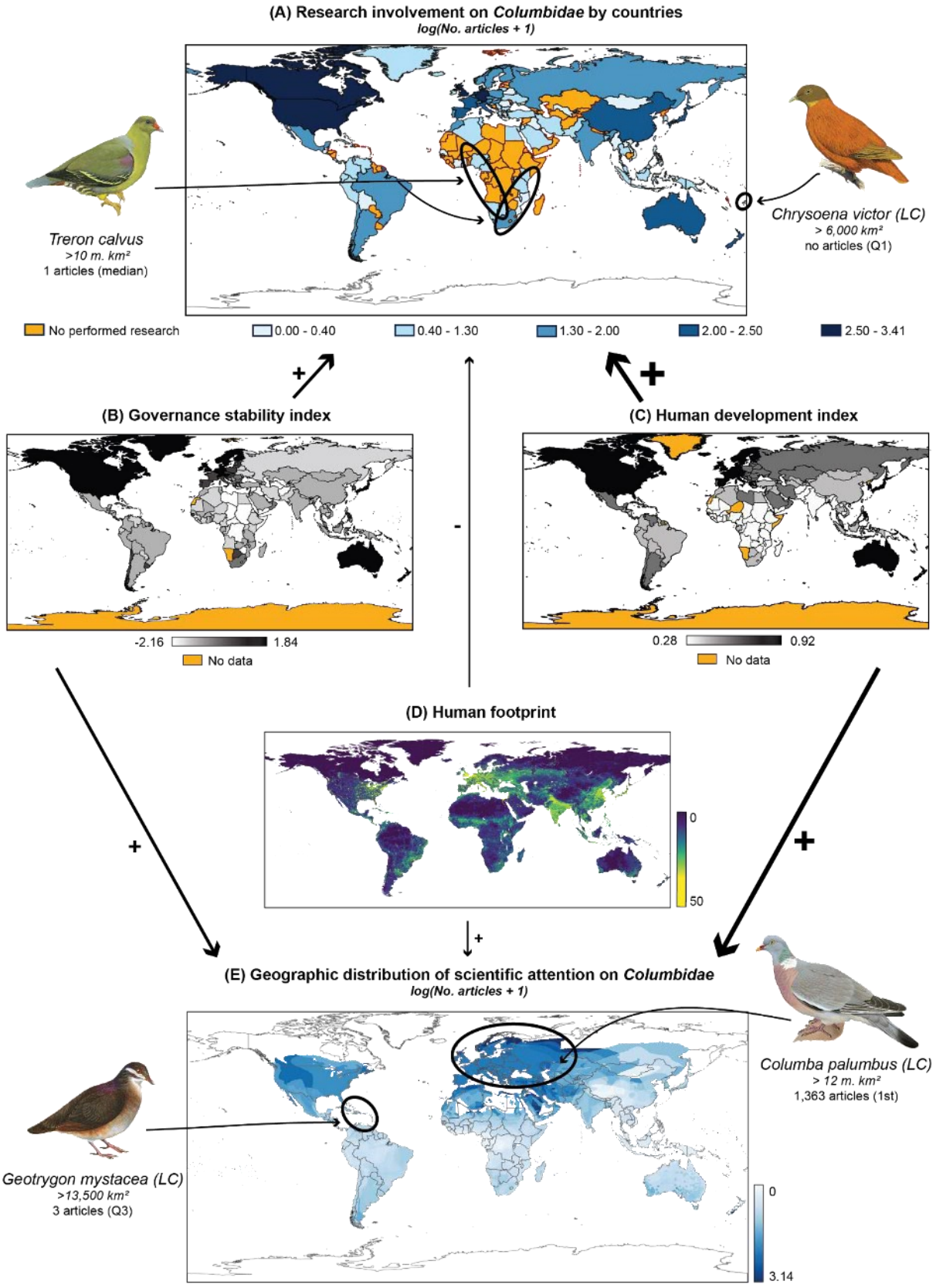


Fig.4: Maps illustrating (A) the research on columbid species by country and (E) the geographical distribution of scientific attention on columbid species based on spatial distribution of columbid species. These maps are linked by arrows with other maps representing geographical distribution of three important socio-economic predictors (Fig. 2C): (B) Governance stability index, (C) Human development index and (D) Human footprint. Arrows illustrate Spearman's correlation test results, as detailed in Appendix S1, Table S4. Thickness of arrows is proportional to the absolute value of the correlation's effect size rho and, positive and negative signs correspond to its sign.

Discussion

Since Walker (2007), the number of identified species of *Columbidae* has been extended from 317 to 369 species. Meanwhile, the proportions of extinct (4.30% in the present study and 3.70% in Walker 2007), threatened (19.24% and 18.61%, respectively) or non-threatened species (75.88% and 77.29%, respectively) have however remained stable. Walker (2007) described the geographic distribution of columbid species across the world according to their extinction risk and associated threats, as listed in the IUCN Red List. We went further by quantitatively assessing to what extent various biological, ecological, environmental and socio-political drivers influence extinction risk and population trends of columbid species as well as interspecific variation in levels of scientific attention. In addition, the present study confirms the growing importance of RF models in avian conservation science (Buechley *et al.* 2019; Wang *et al.* 2021; Hernández-Yáñez, Kim, & Che-Castaldo, 2022; Jungandreas *et al.* 2022). The present study was intended to provide some guidelines that would help to prioritize research on columbid species according to extinction risk, ecology, biology and habitat.

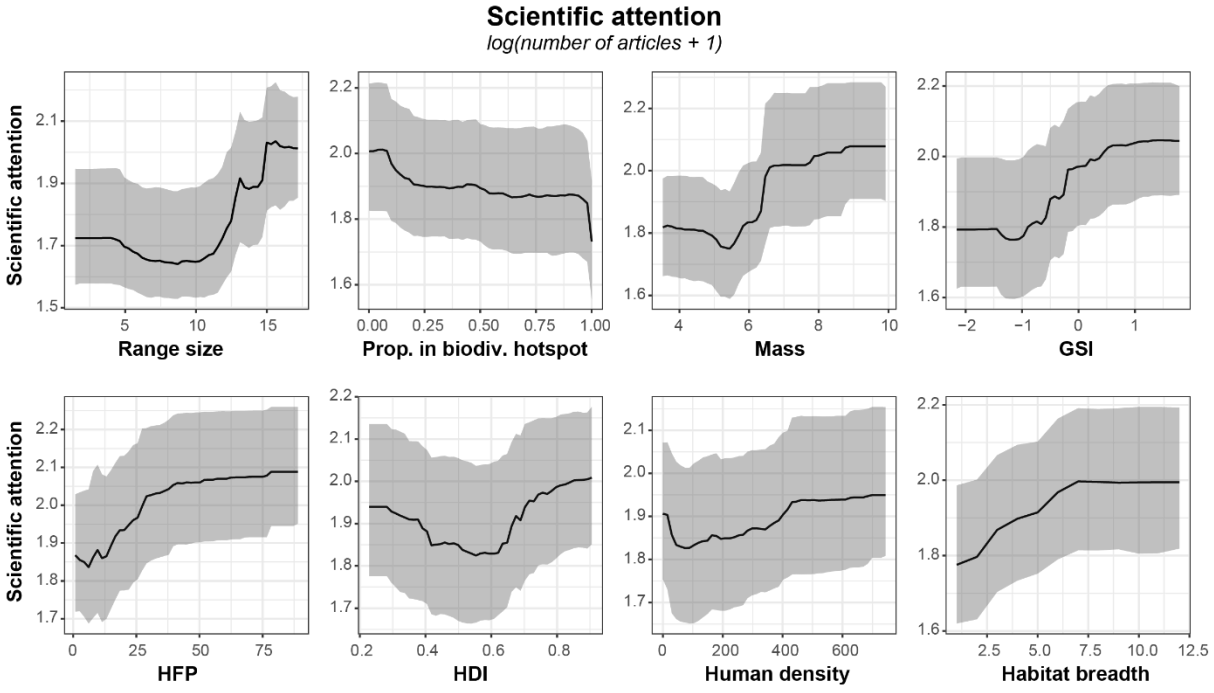


Fig. 5: Partial dependence plots the relationship between scientific attention and the top eight predictors according to relative importance (Fig. 2).

Extinction risk

Ecological and biological drivers

Overall, our analysis confirms previous findings (Walker, 2007) about the main drivers of extinction risk. However, we further show that the use of both IUCN status and the population trends are complementary in the identification of these drivers. Using both of them allowed us to identify several biological and ecological traits that make columbid species more prone to extinction. First, range-restricted species appeared more prone to extinction than more geographically widespread ones. Such relationship might seem obvious when using IUCN status as a proxy of extinction risk because geographic range size is an important factor taken into account in its assessment (Fisher & Owens, 2004). However, geographic range size of columbid species was also positively associated with risks of population decline, thus confirming that geographic range size is an important predictor of extinction risk. Although the impact of geographic range size on extinction risk are complex, small range size is often associated with restricted ecological niche and small population sizes. Indeed, the number of habitats or resources required for survival and reproduction of individuals and, hence, population growth, often covary with geographic range size (Gaston, 2003; Peterson *et al.* 2011; Saupe *et al.* 2015). Moreover, large-range species are less likely to be affected by human disturbance or destruction of their natural environment, or by stochastic events, in their entire geographic range at once (Gaston, 2003, Fisher & Owens, 2004). The negative relationship between extinction risk and geographic range size has been evidenced in a large range of species, including squamate reptiles (Böhm *et al.* 2016), raptors (Buechley *et al.* 2019), terrestrial mammals (Di Marco *et al.* 2014), or marine mammals (Davidson *et al.* 2012), and at different geological time scales (*e.g.,* Payne & Finnegan, 2007; Kiessling & Aberhan, 2007; Harris & Pimm, 2008; Harnik, Simpson, & Payne, 2012).

Second, island columbid species appeared to be more prone to extinction than continental ones, and even more so the further the distance of islands to continents. Indeed, in addition to usually having smaller geographic range size than continental species, island-species have evolved in isolation, under particular selection pressures (e.g., relaxed predation and parasitism), making them particularly vulnerable to disturbances such as overexploitation and any kind of negative interactions with invasive species (Purvis *et al.* 2000a; Purvis, Jones, & Mace, 2000b; Boyer, 2010; Loehle & Eschenbach, 2012). In mammals and birds, 95% of

known extinctions concerned island-species (Loehle & Eschenbach, 2012). However, smaller geographic range size of most insular species might be the most important variable in explaining species extinction risk (Manne, Brooks, & Pimm, 1999). Among columbid species, all known extinct species totally inhabited islands, excepted for the Passenger Pigeon (*Ectopistes migratorius*), suggesting that the features of being an island-species is at least as much important as the geographic range size, especially when added other sources of extinction such as overexploitation, invasive species or climate change (Courchamp, Chapuis, & Pascal, 2003; Courchamp *et al.* 2014; Wood *et al.* 2017). For instance, the two largest known and now extinct columbid species, the iconic Dodo, *Raphus cucullatus*, and the Rodrigues Solitaire, *Pezophaps solitaria*, were both endemic to Mauritius islands and flightless. Their extinction was probably due to a combination of factors, including large body size, island endemism, low population growth, small population size and small geographic range size, making them particularly vulnerable to habitat destruction, hunting and invasive species (Duncan, Blackburn, & Worthy, 2002; Fisher & Owens, 2004; Hume, 2006; Hilbers *et al.* 2016). Our results indeed suggest that the extinction risk of columbid species increases with their body mass, as also observed in diverse taxa (e.g., Gaston & Blackburn, 1995; Owens & Bennett, 2000; Cardillo, 2005; Fritz, Bininda-Emonds, & Purvis, 2009; Davidson *et al.* 2012; White & Bennett, 2015; Hilbers *et al.* 2016; Böhm *et al.* 2016). The influence of body size could actually be multifactorial, and be exacerbated by other external factors like overexploitation. Indeed, larger species are often preferred as game species (Duncan *et al.* 2002; Whytock *et al.* 2016; Ripple *et al.* 2017). Moreover, about 77% of threatened columbid species are exposed to overexploitation (Walker, 2007). On the other hand, we did not find any influence of clutch size on extinction risk among columbid species, possibly due to low interspecific variability, with 1.44 ± 0.50 (mean±SD; min = 1, max = 2) eggs per species.

Habitat breadth also appeared to influence the extinction risk of columbid species, confirming a general trend (Norris & Harper, 2004; Devictor, Julliard, & Jiguet, 2008; Clavel, Julliard, & Devictor, 2011; Callaghan *et al.* 2019). Although ecological specialisation does not always limit the capacity of species to persist (Benito, Gil-Romera, & Birks, 2020), several ecological studies support the idea that ecological specialisation tends to be evolutionary disadvantageous in environments exposed to changes and perturbations (Davies, Margules, & Lawrence, 2004; Colles, Liow, & Prinzing, 2009; Clavel *et al.* 2011; Ducatez, Tingley, & Shine, 2014). More recently, Morelli, Benedetti, & Callaghan (2020) highlighted that the degree of

ecological specialisation is a proxy of the adaptability of species exposed to environmental perturbations. Indeed, specialist species often tend to be associated with small population size, restricted geographic range size and limited use of resources and habitats, what may make them particularly vulnerable when exposed to environmental changes and disturbances (McKinney, 1997; Davies *et al.* 2004; Colles *et al.* 2009).

Anthropogenic drivers

Not surprisingly, our analysis points to alien invasive species as an important factor affecting the risk of extinction of columbid species (see (Rodríguez-Estrella *et al.* 1991; Walker, 2007). Exotic species are indeed amongst major threat to biodiversity, as a result of predation, parasitism, competition, hybridization with native species and the disturbance of native ecosystems (Mack *et al.* 2000; Sax & Gaines, 2008; Walsh *et al.* 2012). Invasive mammals are the most damaging group of alien species across all ecosystems and on all animal groups, especially rodents (e.g., rats *Rattus sp.*) and cats (*Felis catus*). Both are responsible of more than 30% of all known contemporary extinctions, especially on islands (Doherty *et al.* 2016). They can predate native animals or their offspring (Tidemann, Yorkston, & Russack, 1994; James & Clout, 1996; Fraser & Whitehead, 2005; Duron *et al.* 2017), and may also compete with them for resources (Matsui, Hisaka, & Takagi, 2010; Shiels *et al.* 2014; Duron *et al.* 2017; Bond *et al.* 2019), or may carry pathogens (Stimmelmayr *et al.* (2012) for an example in columbid species). Based on IUCN data, 9% of all columbid species have been reported to be exposed to invasive rats, 8% to cats, and 12% of any mammals (IUCN, 2021). Among non-threatened species, 3% of species have been reported to be exposed to invasive mammals, compared to 35% for threatened species and 77% for extinct species (*e.g.,* Tidemann *et al.* 1994; James & Clout, 1996; Bond *et al.* 2019; Fea, Linklater, & Hartley, 2021). However, the precise impact of invasive mammal species on the demography of columbid species of patrimonial interest in the wild remains to be quantitatively assessed and compared to that of other threats such as, for instance, hunting pressure.

Landscape heterogeneity tends to decrease with increased elevation, and species found at elevation tend to have a higher degree of ecological specialization than those found at lower elevation (Reif, Marhoul, & Koptík, 2013; Rivas-Salvador, Hořák, & Reif, 2019). One would then have expected to observe a positive relationship between extinction risk and elevation in columbids. However, our analysis concluded to the opposite. Our results thus

contradict to some extent predictions of climate change scenario stipulating that global warming may increase extinction risk among species living at high altitude (*e.g.*, Williams, Bolitho, & Fox, 2003; Shoo, Williams, & Hero, 2005; Sekercioglu *et al.* 2008; Bässler *et al.* 2010; La Sorte & Jetz, 2010). One possibility is that lower elevations are also associated with higher anthropogenic transformation, associated with all induced negative effects on local biodiversity (Ellis *et al.* 2010; Reif *et al.* 2013; White & Bennett, 2015; Smith *et al.* 2019). On the other hand, the consequences of global warming on extinction risk at higher elevation might not be felt yet (Bellard *et al.* 2012) in columbid species or might have been hidden in our analysis by the consequences of human activities at lower elevations. Further attention should then be given to the influence of elevation on demographic trends of columbid species in relation to climate change and habitat modification.

Several other drivers of anthropogenic origin appeared to contribute, directly or indirectly, to increase the extinction risk of columbid species, such as low levels of governmental stability and poor socioeconomics, the presence of invasive mammals and/or the loss and disturbance of natural habitats through the development of HFP. HFP provides an index of human conversion of natural terrestrial ecosystems based on the measure of eight different anthropogenic pressures related to urbanization, agriculture and human density (Venter *et al.* 2016), and can therefore be regarded as a proxy of habitat degradation or loss. Urbanization (Wackernagel *et al.* 2006; Rees & Wackernagel, 2008), habitat fragmentation (Carrete *et al.* 2009; Hagen *et al.* 2012; Reino *et al.* 2013) and farming development and practices (Donald *et al.* 2001, 2002; Vickery *et al.* 2004; Sanderson *et al.* 2013) have been previously identified as drivers of population declines. In terrestrial mammals, Di Marco *et al.* (2018) showed that HFP and its variation over years were strong predictors of extinction risk. Although some columbid species have adapted to live in landscapes modified by humans, sometimes living in close association with them such as the widespread Feral Pigeon, *Columba livia domestica* (Carlen, Li, & Winchell, 2021), the Zenaida Dove, *Zenaida aurita* (Wolff *et al.* 2018), or the invasive Eurasian Collared-Dove, *Streptopelia decaocto* (Luna *et al.* 2018), most of them rely on their natural habitats to persist. Following Walker (2007), the present study confirms that the loss of natural habitat is the greatest cause of extinction and populations decline in columbid species.

Governmental and socioeconomic factor of human communities were also found to predict extinction risk of columbid species, with columbid species inhabiting in developing

countries being more exposed to extinction risk. Public policies can have negative or positive influences on biodiversity, through supporting or not reliable environmental and conservation projects and by protecting or not most vulnerable species and/or sensitive ecosystems (Chapron, 2014; Ripple *et al.* 2016). For instance, the Pink Pigeon, *Nesoenas mayeri*, have been downlisted twice, in the IUCN Red List, from critically-endangered to vulnerable thanks to conservation projects lead by conservationists, private foundations and the Mauritian government (Tatayah, 2019). Globally, the socioeconomic level of countries tends to positively influence the persistence of species (Forester & Machlist, 1996; Dietz & Adger, 2003). For instance, Bösch (2021) showed that the level and speed of countries' economic-institutional development tend to be negatively associated with illegal logging, and hence, with habitat loss and decline of biodiversity. In line with our results, they found that countries with high economic growth rates (i.e., developing countries) were more exposed to illegal logging, and hence, habitat loss. The same is true of wildlife trafficking and hunting pressure, in developing countries where wildlife can be the sole source of proteins for isolated and/or low-income communities (e.g., (Robinson & Bennett, 2002; Fa, Currie, & Meeuwig, 2003; Fa *et al.* 2009; Borgerson *et al.* 2016; Lescuyer & Nasi, 2016). The exploitation of natural resources can be complex and multifactorial, as shown by legal and illegal hunting that can take place for the purpose of finding food, social stature, tradition, sport or traditional medicine (Phelps, Biggs, & Webb, 2016; Stirnemann *et al.* 2018). Indeed, wildlife meat consumption is not always associated with poverty and can also be a sign of richness (Fa *et al.* 2009). This is for instance the case in the Pacific islands where poor people hunt both the Pacific Pigeon, *Ducula pacifica*, and the Tooth-billed Pigeon, *Didunculus strigiristris*, to sell their meat to high-income populations (Phelps *et al.* 2016; Stirnemann *et al.* 2018).

Scientific attention

Geographical bias

Scientific attention on columbid species appeared to be geographically biased and independent of species extinction risk. Research labs within North-America and Europe regions are the most invested in research on columbid species, accounting respectively for about 43% and 32% of all articles published in peer-reviewed journals. These same regions harbour the most studied species. The same geographical bias has been found in several studies focusing on diverse taxa (e.g., Ducatez & Lefebvre, 2014; Tydecks *et al.* 2018; Melles

et al. 2019). It might be explained by logistic constraints met is some habitats and countries, local poverty, political instability, geographic isolation and lack of local expertise (Clark, 1985; de Lima *et al.* 2011; Amano & Sutherland, 2013; Wilson *et al.* 2016; Reboredo Segovia, Romano, & Armsworth, 2020), which may all limit research productivity on columbid species. The influence of HDI and GSI on scientific attention evidenced in the present study argues in that direction (Livingston *et al.* 2016; Tydecks *et al.* 2018; Melles *et al.* 2019). This was reflected in the negative association between scientific attention and proportion of species in biodiversity hotspots. Indeed, the most important hotspots of biodiversity (Brooks *et al.* 2002) are areas often isolated from Europe and North-America with a lack of local expertise and research productivity (Livingston *et al.* 2016; Tydecks *et al.* 2018; Melles *et al.* 2019; Vallès *et al.* 2021). In addition, we found that island-endemic columbid species were on average less studied than continental species, and even more so when the further they were from continents. In accordance with our findings, Brooks *et al.* (2008) found that passerines and "near passerines" island-species are less studied than continental ones. For instance, the most important hotspot of threatened columbid species and diversity, as described by Walker (2007), is in the Pacific Islands, between Fiji and Indonesia. This area is also one of the most important biodiversity hotpots (Brooks *et al.* 2002). However, columbid species living in this area are among the least studied ones, probably due to local poverty, political instability, geographic isolation and/or lack of local expertise.

Ecological and biological bias

In addition, we found that widespread columbid species, in terms of geographic distribution or in terms of number of different habitats used, and large-bodied species were more studied than others, as highlighted by Ducatez & Lefebvre (2014), who focused their research on bird Orders. More widespread species are presumably more accessible and have thus more chance to be studied than less widespread ones (Morales & Traba, 2016; Yarwood *et al.* 2019), while large species are usually easier to locate and observe than more discrete and smaller species (Ripple *et al.* 2017; Yarwood *et al.* 2019). Furthermore, we found that scientific attention on columbid species was positively associated with human density and human footprint, suggesting that columbid species inhabiting areas with high human density and highly urbanized were preferentially studied. As explained by Ibáñez-Álamo, Rubio, & Bitrus Zira (2017), close-human species may be more studied by scientist for several reasons. First,

this kind of species may have particular traits being of interest for scientific purposes. Second, they may be interesting for economic and public health issues, such as feral pigeons (*e.g.,* Perez-Sancho *et al.* 2020; Lindahl & Magnusson, 2020). Finally, such species are more studied because of logistical and/or monetary constraints. They are in fact more accessible and they often require less logistic to be studied. Moreover, most scientists, research agencies and universities are located in urban areas.

Contrary to Ducatez & Lefebvre (2014), we did not find any association between scientific attention and clutch size or generation time, certainly due to the lack of variability since our study focused on one single avian family. In addition, migratory behaviour was not of prime importance in RF model for explaining variation in scientific attention, although a difference was found using inferential statistics, with full migrant species being more studied than vagrant ones, themselves more studied than sedentary species, in line with Ducatez & Lefebvre (2014). In strong contrast with the ubiquity of the family *Columbidae* throughout the world, the order Columbiforms is among the least studied orders in the Aves class (8^{th}/25, Ducatez & Lefebvre, 2014). The same trend has been observed, at a lower geographical scale, in the Caribbean region (Devenish-Nelson *et al.* 2019). One reason for the low attention given to columbids could be that this family does include any charismatic species (Seddon, Soorae, & Launay, 2005; Devenish-Nelson *et al.* 2019), excepted for the iconic Dodo and the iconic Passenger Pigeon that became textbook cases due to their extinction history (Kyne & Adams, 2017).

Conclusions

- There is an important disparity in scientific attention towards columbid species, with the most threatened ones being the least studied. This unequal acquisition of knowledge, both quantitatively and qualitatively, creates gaps that deserve to be filled in order to have a good appreciation of species extinction risk and associated threats. In that respect, a more balanced scientific coverage of columbid species, across countries and species, should be encouraged, for instance through capacity building

in conservation biology and ornithology outside of Europe and North America (Reboredo Segovia *et al.* 2020; Vallès *et al.* 2021).

- Particular attention should be taken to specialist species, restricted-range species, particularly insular ones, and/or large species in relation to habitat disturbance and loss, in order to better estimate direct and specific extinction risk.
- Investigations on the particular features of urban columbid species may allow determining the ability of species to adapt to urbanization and global change. This may help to refine the estimation of species extinction risk.
- Estimating the relative importance of sources of mortality for pigeons and doves in the wild, particularly through comparing the impact of legal and illegal hunting to that of predation by exotic invasive predators is of prime importance. Columbids are indeed particularly appreciated by hunters but are also exposed to various introduced potential predators in various regions. Although the impact of invasive mammal predators is widely acknowledged as an important threat to native wildlife, precise quantitative assessment is often lacking (Doherty *et al.* 2016), particularly for columbid species. To that end, cooperation among hunters, scientists, wildlife officers and agencies should be encouraged, particularly where hunting traditions are an important part of local culture. Although difficult to implement, the regulation of invasive predators or their total eradication may have immediate positive impacts on native populations and ecosystems (*e.g.,* Le Corre *et al.* 2015; Miller-ter Kuile *et al.* 2021).
- The evidence for socioeconomic factors affecting variation in both extinction risk and scientific attention among columbid species points to the necessity of developing coordinated efforts to better inform governments, public agencies and the general

public of the key ecological role played by columbids and of the importance of conserving populations and associated habitats. In that respect, both extinct and extant columbid species could be used more widely in education programs about the erosion of biodiversity.

- Finally, transnational collaborations should be encouraged, especially between small island developing states and developed countries, in order to develop academic training and fair cooperation in conservation-oriented research on columbids.

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Supporting information

All supplementary materials can be downloaded here:
https://docs.google.com/spreadsheets/d/1Qc1WP8UXh5wgohgBhl87mDWa_fHoINkv/edit?usp=sharing&ouid=106548614141078802157&rtpof=true&sd=true

Fig. S1. Example of a classification tree based on our original data based on extinction risk of columbid species (threatened vs. non-threatened)

Fig. S2. Example of a classification tree based on our original data based on population trends of columbid species (decreasing vs. stable vs increasing)

Fig. S3. Example of a regression tree based on our original data based on scientific attention on columbid species

Fig. S4. Relative importance of all predictors used in random forests

Fig. S5. Partial dependance plots of all predictors used in the random forest model aiming at determining drivers influencing the probability to be threatened among columbid species

Fig. S6. Partial dependance plots of all predictors used in the random forest model aiming at determining drivers influencing the probability to habe a declingin population size among columbid species

Fig. S7. Partial dependance plots of all predictors used in the random forest model aiming at determining drivers influencing the scientific attention on columbid species

Fig. S8. Map showing the geographical repartition of the top 10 most studied columbid species and those being not studied

Table S1. Inferential statistics between predictors used in random forest models and the extinction risks of species (threatened vs. no-threatened)

Table S2. Inferential statistics between predictors used in RFs and the population trend of species (decreasing vs. stable vs. increasing)

Table S3. Inferential statistics between predictors used in random forest models and the scientific attention of species

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END OF ARTICLE 1

The White-crowned Pigeon (WCPi) and the Scaly-naped Pigeon (SNPi)

Taxonomy and distribution area

The WCPi (*Patagioenas leucocephala*, Photo 1) and the SNPi (*Patagioenas squamosa*, Photo 2) are closely-related new world pigeons belonging to the *Patagioenas* genus. This genus is a sister taxon of the Passenger Pigeon (*Ectopistes migratorius*; Fulton et al. 2012; Figure 14). The SNPi is endemic to the Caribbean islands, while the WCPi is endemic to the Caribbean basin (Figure 13). The first one is a year-round resident from Cuba to Grenada, except in Jamaica (BirdLife International 2016). The Barbados population has a special status as it was recently formed by captive individuals escaped from a rooftop aviary in Bridgetown (Buckley et al. 2009). In contrast, the WCPi has a larger geographical range extending from the Keys (Floride, U.S.A.) and The Bahamas to Saint-Lucia, including the coasts of Central America and Cayman Islands (BirdLife International 2020). Occasionally, the species is observed beyond Saint Lucia in the south, particularly after climatic hazards, such as hurricanes. Contrary to the SNPi, the WCPi is a year-round resident only in the Greater Antilles, and occurs seasonally in the Lesser Antilles.



Photo 2 : The White-crowned Pigeon (*Patagioenas leucocephala*). © Christopher Cambrone



Photo 2 : The Scaly-naped Pigeon (*Patagioenas squamosa*). © Frank Cézilly

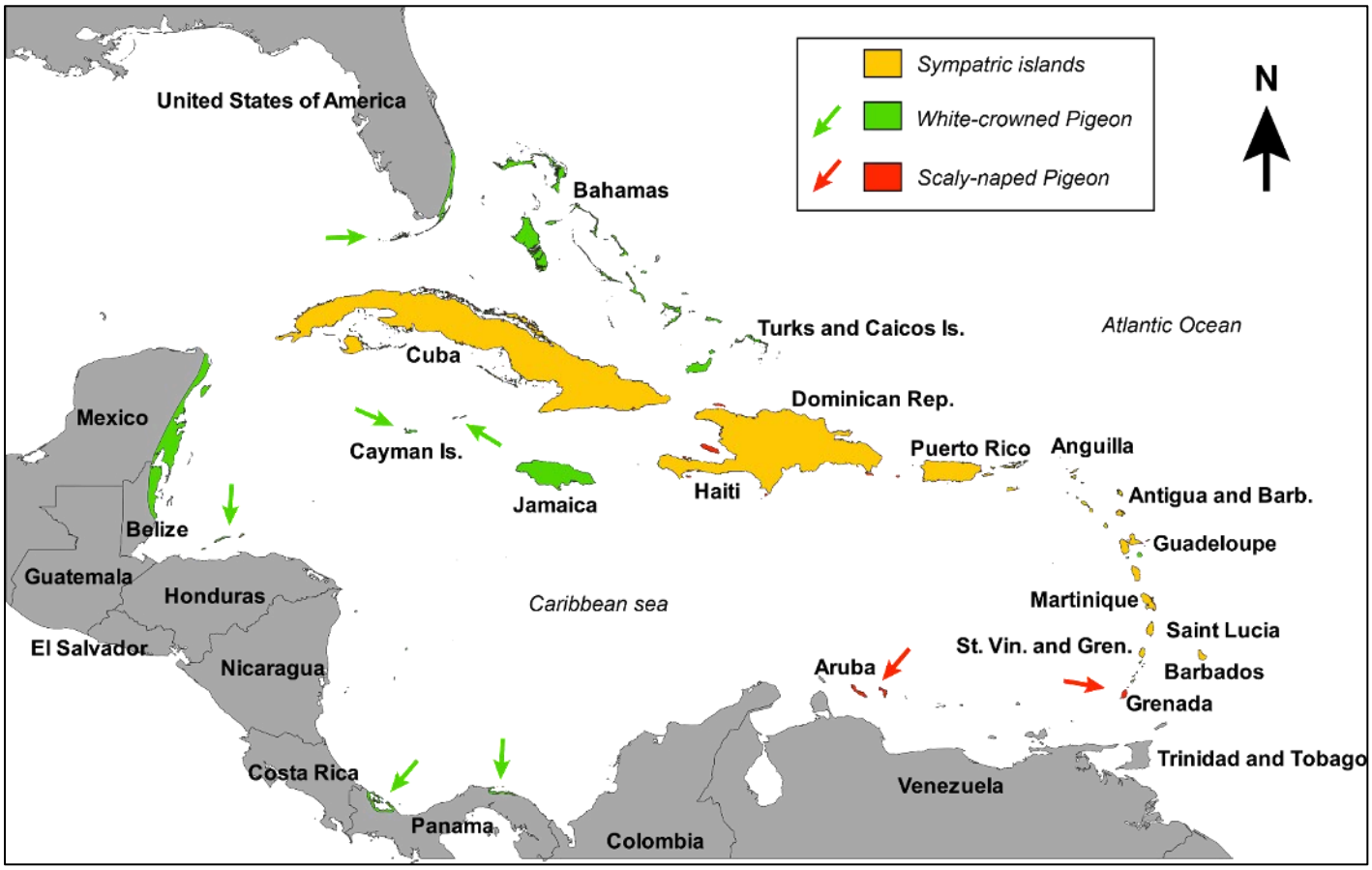


Figure 13 : Geographical distribution of the White-crowned Pigeon and the Scaly-naped Pigeon. Map made using BirdLife International (2021).

Life history traits and ecology of species

In addition to sharing the same areas, the two species are ecologically close and have very similar life history traits. The WCPi, weighing between 230 and 280 g, is smaller than the SNPi, that weighs between 250 and 360g. The two species are arboreal and frugivorous, and, thus, depend to a large extent on forested habitats. However, the SNPi preferentially occupies upland and inland moist forests, while the WCPi is more commonly found in coastal forests, using mangroves and swamp forests to nest and dry evergreen forests to feed. However, both species show some ability to live in urban areas. The SNPi lives year-round in residential areas in Barbados, behaving almost like feral pigeons (Buckley et al. 2009; Baptista et al. 2020a). Similarly, WCPis can be observed next to beaches and hotels on some islands (Bancroft et al. 2020). Both species mostly consume fruits and berries, although they can also feed insects and terrestrial molluscs. SNPis can also exploit human food waste in urban areas (F. Cézilly, pers. com.). In addition, although movement patterns of these two species are not clearly understood, they are considered as vagrant, especially in response of fruit availability. In both species, movements between islands have been observed (Bancroft et al. 2020; Baptista et al. 2020a). In this respect, the two species are suspected to play an important role as agents of seed dispersal and in forest regeneration (Pérez-Rivera 1978; Strong et al. 1994; Strong & Bancroft 1994; Ware 1997; Strong & Johnson 2001; Bancroft et al. 2020; Baptista et al. 2020a). Their seasons of reproduction coincide with the period of maximum availability of fruits (Bancroft & Bowman 1994; Bancroft et al. 2000, 2020; Baptista et al. 2020a). The SNPi breeds between March and June, while the WCPi breeds in colonies from May to September. At least for the WCPi, there can be up to two clutches per season, particularly if the first one is laid early (Bancroft et al. 2020). In both species, nests are mostly built in trees, sometimes in shrubs (Wiley & Wiley 1979; Gibbs et al. 2001; Bancroft et al. 2020; Baptista et al. 2020a). In areas exempt of predators like rats, mongooses or cats, the WCPi can breed in low shrubs or even on the ground (pers. obs., Wiley & Wiley 1979, Bancroft et al. 2020). For the WCPi, main reproductive populations are located in the Greater Antilles (Gibbs et al. 2001, Bancroft et al. 2020).

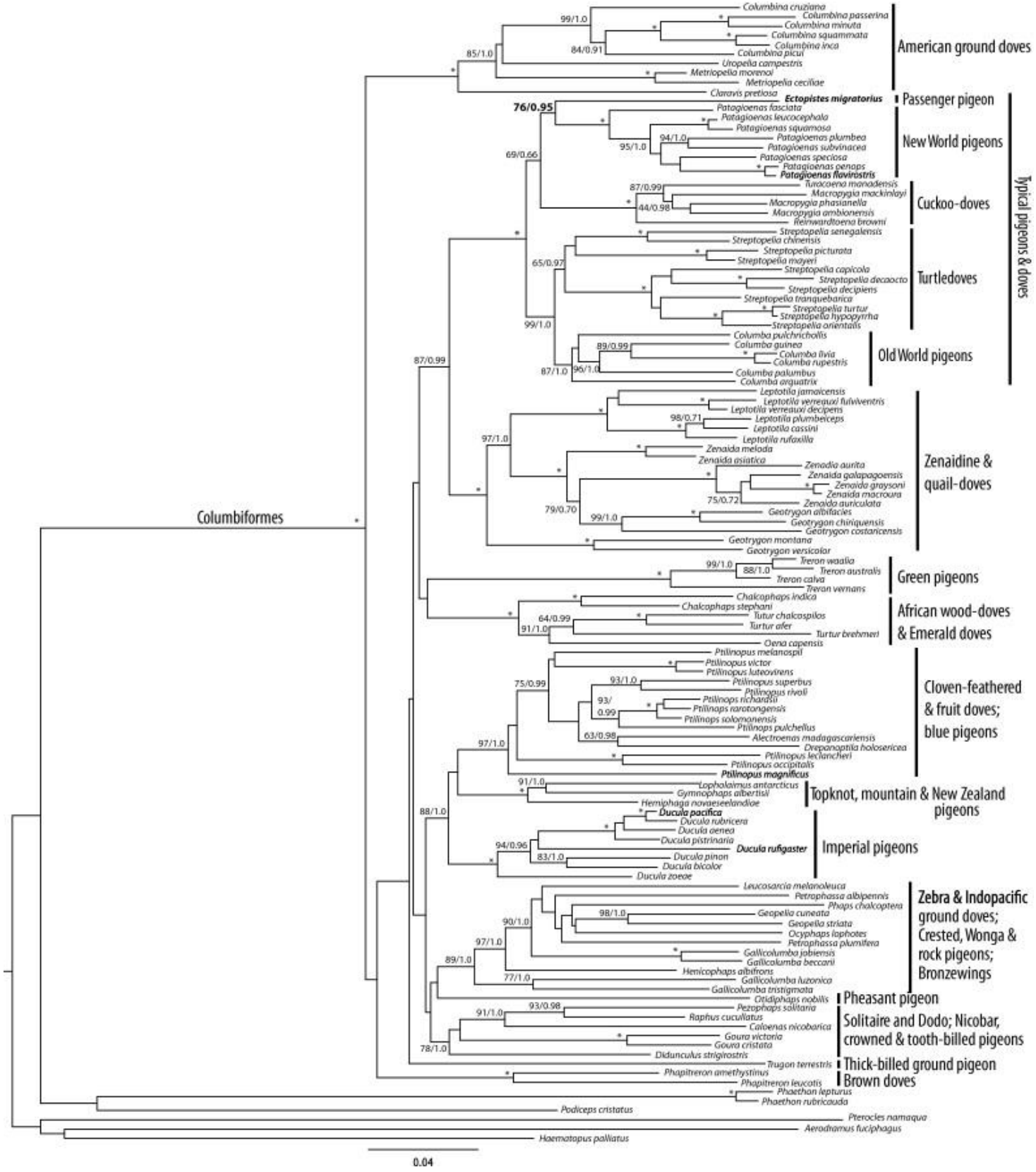


Figure 14 : Molecular phylogeny of Columbidae based on maximum likelihood analysis of nuclear and mitochondrial genes (12S rRNA, cytochrome b, ATP8 and FGB intron). This phylogeny was extracted from Fulton et al. (2012, Annals of Anatomy 194:52-57).

Threats and conservation status

Like other columbid species and most Caribbean birds, the WCPi and SNPi are exposed to habitat loss, unregulated or poorly regulated hunting and invasive predators (Bancroft et al. 2020, Baptista et al. 2020, BirdLife International 2016, 2020). The two species are indeed much appreciated by local hunters. However, squabs can also be harvested, especially in the case of the WCPi, where humans can have access to colonies. Although this practice has gradually decreased, a large number of squabs have been collected in nests for both being eaten or used for livestock food (Bancroft et al. 2020). I personally met people in rural areas of the Dominican Republic who confirmed to me that this practice was still prevalent. As a result, global population sizes of WCPi and SNPi are declining (Pinchon 1976; Feldmann 1998; BirdLife International 2016, 2020), with a number of pigeons estimated at 550,000 for the WCPi. The global population of the SNPi is unknown, but studies in Puerto-Rico estimated the island population size at more than 260,000 pigeons (Rivera-Milán et al. 2014). Therefore, the WCPi and the SNPi are classified as near-threatened and least concern at the global scale, respectively. However, there is a lack of information about their reproductive phenology in the French West-indies and the extent of connectivity between islands populations at the region scale is still unclear for both species. Consequently, their conservation status has been recently and locally reconsidered, thus reclassifying the WCPi as endangered in Guadeloupe and Martinique, and the SNPi as near threatened in Martinique (UICN et al. 2012, 2020).

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CHAPTER 2

Methodological developments

ARTICLE 2

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Efficiency of the call-broadcast method for detecting two Caribbean-endemic columbid game species

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ORIGINAL ARTICLE

Efficiency of the call-broadcast method for detecting two Caribbean-endemic columbid game species

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Abstract

The white-crowned Pigeon (WCPi), *Patagioenas leucocephala*, and the scaly-naped Pigeon (SNPi), *P squamosa*, are two Caribbean endemic species of patrimonial and cynegetic interest. Although both species are under the threat of habitat destruction and hunting pressure, population trends remain undocumented in a large part of their geographical range. Here, we used both the "auditory and visual" and "call-broadcast" census methods to assess the occurrence and relative abundance of both species in Guadeloupe (French West Indies). The call-broadcast method was found to be more efficient as it reduced the problem of "false absence" while increasing the probability of detection. Results from our surveys showed that both WCPis and SNPis were at low density and showed complete habitat segregation. SNPis were only encountered in rainforest, whereas WCPis could be observed at count stations located in dry and swamp forests, mangroves, agricultural lands and wet meadows. We recommend the use of the call-broadcast method for monitoring the two species on islands where they occur at low density, under which conditions distance sampling may be poorly reliable. The general relevance of the call-broadcast method to other species of pigeons and doves deserves further attention, especially to document population trends in elusive game species of conservation interest.

Keywords Call-broadcast · Monitoring · *Patagioenas leucocephala* · *Patagioenas squamosa* · Playback · Point counts

Introduction

The genus *Patagioenas* includes 19 different species of New World pigeons (Johnson et al. 2010; del Hoyo et al. 2014; BirdLife International 2020), of which five are of conservation concern (Walker 2007; BirdLife International 2020). In the Caribbean region, the white-crowned Pigeon (WCPi), *P. leucocephala*, and the scaly-naped Pigeon (SNPi), *P. squamosa*, are considered, respectively, as near-threatened and least-concern in the IUCN Red List (BirdLife International 2016a, b), being both threatened by habitat destruction, climate change, intensive (legal and illegal) hunting pressure as well as harvesting of squabs (Hay 2008; Latta et al. 2010; Latta 2012; Rivera-Milán et al. 2014, 2016; BirdLife International 2016a, b). Although quantitative data are lacking, an overall decline in population size for both species is suspected (Raffaele et al. 1998; Hay 2008). The WCPi's natural range extends from the Florida Keys (USA) and Bahamas to a part of the Lesser Antilles (up to Saint-Lucia), also including the coasts of Central America and the Cayman Islands (Gibbs et al. 2001; Bancroft et al. 2020). In contrast, the SNPi's natural range includes all the Antilles islands, plus the islands off the coast of Venezuela in the south (Gibbs et al. 2001; Baptista et al. 2020). At the regional scale, both species are considered as vagrant (Bancroft et al. 2020; Baptista et al. 2020; Cambrone et al. 2021). These closely related arboreal species are mainly frugivorous and are supposed to play an important role in forest regeneration (Pérez-Rivera 1978; Strong and Bancroft 1994; Ware 1997; Strong and Johnson 2001). According to Gibbs et al.

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(2001), WCPis tend to occupy coastal, lower-lying forests such as swamp forests, mangroves or dry evergreen forests, whereas SNPis are mostly found in upland and inland moist forests. However, so far, no quantitative data exist on habitat partitioning by the two species in sympatry. In addition, for both species, data on demographic trends and local abundance are scarce and limited to a few islands (Rivera-Milán 1996; Brooks et al. 2002; Acevedo and Restrepo 2008; Hay 2008; Florida Fish and Wildlife Conservation Commission 2013; Rivera-Milán et al. 2014, 2016).

In the French West Indies, data about the spatial distribution and ecology of these two popular game species is presently limited to observations made by wildlife officers from the *Office Français de la Biodiversité* (formerly *Office National de la Chasse et de la Faune Sauvage*) and local ornithologist associations. The SNPi is observed year-round in the French Antilles. The species is moderately abundant in Guadeloupe, following a marked decline during the twentieth century associated with intensive hunting pressure (especially on La Désirade, small Eastern island of the Guadeloupe archipelago; Pinchon 1976; Feldmann 1998). In contrast, the abundance of the WCPi in Guadeloupe varies seasonally, with a peak from April to December and a recent increase in the number of reported observations (Delcroix et al. 2016). Based on this information, the IUCN local status of two species has been recently reconsidered, with the WCPi being reclassified as endangered in Guadeloupe and vulnerable in Martinique, while the SNPi remained of least concern in Guadeloupe but was reclassified as near-threatened in Martinique (UICN France et al. 2012, 2020). There is little information on the breeding status of the two species in Guadeloupe and Martinique, apart from anecdotal observations of pairs, nest construction behaviour, reproductive attempts and juveniles (Delcroix et al. 2016 for the WCPi). Further evidence suggestive of local reproduction comes from the regular presence of juveniles in the hunting bags of local hunters (a reliable source of information for demographic monitoring; Christensen and Fox 2014). The closest important breeding areas (> 70 km) to Guadeloupe and Martinique is Antigua for the WCPi and Montserrat and Dominica for the SNPi.

In the absence of quantitative data on the demography of both species in Guadeloupe and Martinique, and considering a recent population genetic study suggesting the existence of one single panmictic population of SNPi at the regional scale (Cambrone et al. 2021), reliable estimates of population abundance are fundamental to identify population fluctuations and changes. Refined estimates of population density, such as those obtained through mark–recapture methods, are available but can be time-consuming and expensive to implement (Thompson 2013; Turlure et al. 2018). In addition, capture of wild birds can be a source of stress and/or trauma, thus limiting its use when dealing with sensitive and/or threatened species (Powell and Proulx 2003; Pauli et al. 2010). Consequently, game bird populations are often monitored using relative abundance indices, obtained from index counts or hunting statistics (e.g., Cattadori et al. 2003; Evans et al. 2007; Lande et al. 2010; Tillmann et al. 2012; Small et al. 2012; Guillemain et al. 2016; Cretois et al. 2020). Although both indices suffer from some limitations (Rosenstock et al. 2002; Christensen 2005), their combined use can provide improved information on population status (Soininen et al. 2016).

In Guadeloupe, data on hunting bags have been regularly collected by the *Office Français de la Biodiversité* for several years, whereas surveys of WCPi or SNPi populations have been seldom conducted. Indeed, the secretive behaviour of both species and their tendency to perch on trees high in the canopy makes the estimation of their local abundance difficult. It is therefore of particular importance to preliminary assess the performance of census methods in order to obtain, in the future, reliable estimates of the local abundance of the two species in Guadeloupe, and, possibly, in other Caribbean islands. Two main methods are currently used in surveys of forest bird species. The first and more conventional method simply consists in detecting birds by sight and/or by hear along a point or line transect (i.e., auditory/visual method). The second method adds the broadcasting of calls of the species of interest to induce behavioural responses, such as vocalisations or movements, from nearby individuals, such that they become easier to detect (i.e., call-broadcast method). The use of call-broadcast has proved to be an efficient method to census various bird species, through increasing the number of detections per unit of sampling effort, reducing the problem of false absence and decreasing temporal variation in detection probability (Sutherland et al. 2004; Sutherland 2006).

The call-broadcast method could be particularly suitable to survey population of *Patagioenas* species, as Kirkpatrick et al. (2007) found that the probability of detecting a cooing band-tailed pigeon, *P. fasciata*, increased by 27% compared to auditory surveys. However, this method comes with some limitations. First, the type of habitat or the weather conditions may alter sound propagation (from the loudspeaker to birds), such that the efficiency of the method may vary according to local conditions (Schieck 1997; Buckland et al. 2005; Simons et al. 2007; Pacifici et al. 2008; Yip et al. 2017). More importantly, the method may lead to overestimate population size if birds are attracted to the calls and move around the observer, thus increasing the risk of double counting (Buckland et al. 2008; Fuller et al. 2012). However, Fuller et al. (2012) showed that this potential bias can be accounted for in statistical analyses and concluded that the method appears especially useful for the survey of elusive forest birds, at least to better ascertain their occurrence in various habitats.

In this context, the main aim of this study was to compare the efficiency of the "auditory and visual" (AV) and

"call-broadcast" (CB) census methods for estimating the presence and the relative abundance of both *P. leucocephala* and *P. squamosa* in Guadeloupe. In addition, we analysed the dominant vegetation type associated with the presence and relative abundance of each species, and assessed to what extent the two species occur in similar habitats. Based on results previously obtained with the congeneric *P. fasciata fasciata* (Kirkpatrick et al. 2007), we expected that the CB method would enhance our ability to detect and improve the estimation of relative abundance of both *P. squamosa* and *P. leucocephala*. Furthermore, we expected that each species would be detected in transects dominated by different vegetation formations, based on previous observations in other Caribbean islands (Bancroft et al. 2020; Baptista et al. 2020).

Methods

Study sites

We compared the efficiency of the AV and CB survey for assessing the presence and abundance of both *P. leucocephala* and *P. squamosa* in Guadeloupe (Fig. 1). This archipelago, located in the Lesser Antilles, consists of two main islands climatically, geologically and ecologically different (Rousteau 1996). The eastern part, Grande-Terre island, is the oldest and flatest, with a calcareous soil occupied by fragmented evergreen and semi-deciduous forests. The Basse-Terre island, to the west, is montainous and largely covered by tropical rainforest. The two islands are separated by a sea inlet called Rivière Salée, where mangroves and swamp forests are concentrated (Fig. 1, Rousteau 1996), making Guadeloupe suitable for the two *Patagioenas* species (Gibbs et al. 2001; Bancroft et al. 2020; Baptista et al. 2020).

Survey design

The study was conducted over two consecutive years, 2016 and 2017. We defined location of transects using aerial photographs and topographic maps, complemented by a mapping of the vegetation of Guadeloupe from *Géoportail* and *Google Earth*. As we aimed at comparing two detection methods, we optimized our sampling effort by positioning the transects in places where each species is known to occur. To that end, we assessed the likely presence/absence of the two pigeon species from observations made by local hunters (a reliable source of information for demographic monitoring; e.g., Lande et al. 2010; Tillmann et al. 2012; Christensen and Fox 2014; Cretois et al. 2020), wildlife officers and data available from ebird (https://ebird.org; Callaghan and Gawlik 2015; Callaghan et al. 2017; Walker and Taylor 2017; Fletcher et al. 2019). Twelve different line transects were considered across Guadeloupe to study both species between April and May 2016 (Fig. 1), each one including 10 count stations, regularly separated by 300. Such a distance reduces the risk of spatial autocorrelation between sampling points (Thomas et al. 2010). Transects' observations were replicated twice (R1: 11 to 27 April 2016 and R2: 28 April to 18 May 2016) in the morning, starting at sunrise, and twice again in the evening, finishing just at sunset, the two periods corresponding to the time at which columbid species are usually the most active (Robbins 1981a; Rivera-Milán et al. 2015). For each replication, the order of transect visits was independently randomized for the morning surveys and for the afternoon surveys. Therefore, a given transect was not systematically visited in the morning and the afternoon on the same day. At each count station, the observer always started with the AV method for a 5-min period, followed by a period during which the calls of the two species were broadcasted one after the other, for a 5-min duration each, and during which cues of the presence of individuals were recorded. During the CB method, the order of presentation of the two species call was systematically reversed between each count station and replication. At each count station, and for each detection method, all individuals seen and heard were recorded, corresponding here to our estimate of relative abundance. For each species, the same 38-s recording was diffused twice for each session of CB method, at the beginning and once again after 150 s. Calls were obtained from "Oiseaux des Antilles" (CEBA/Fremaux et Associés), modified and cleaned (i.e., removing background noise) using the free software Audacity. Calls were broadcasted in four major directions (N, E, S, W) through a Bluetooth-connected nomadic speaker (4 W, Philips BT2600) and a smartphone at the maximum volume without distorsion noises. We did not perform surveys during strong rainy weather in order to reduce biases due to altered propagation of calls (from loudspeaker to birds and from birds to the observer) or reduced activity of individuals (Robbins 1981b; Buckland et al. 2005; Overton et al. 2005). We also recorded the global meteorological condition prevailing during each transect survey, according to four categories, from best to worst: "sunny", "cloudy", "overcast" or "scattered rains".

Based on the results obtained in 2016 (see below) and in order to increase detection rate and, hence, statistical power, a decision was made to conduct a more intensive survey in 2017, focusing exclusively on transects where WCPis were likely to be detected. To that end, we added to the survey design five new transects and decided to remove two transects surveyed in 2016, where no pigeon had been detected (transects 14 and 16, as represented in Fig. 1 and Supplementary materials Table S2). The survey protocol was conducted in the same way as the year before, except that only the call of the WCPi was broadcasted. In addition, three replications were performed later in the season,

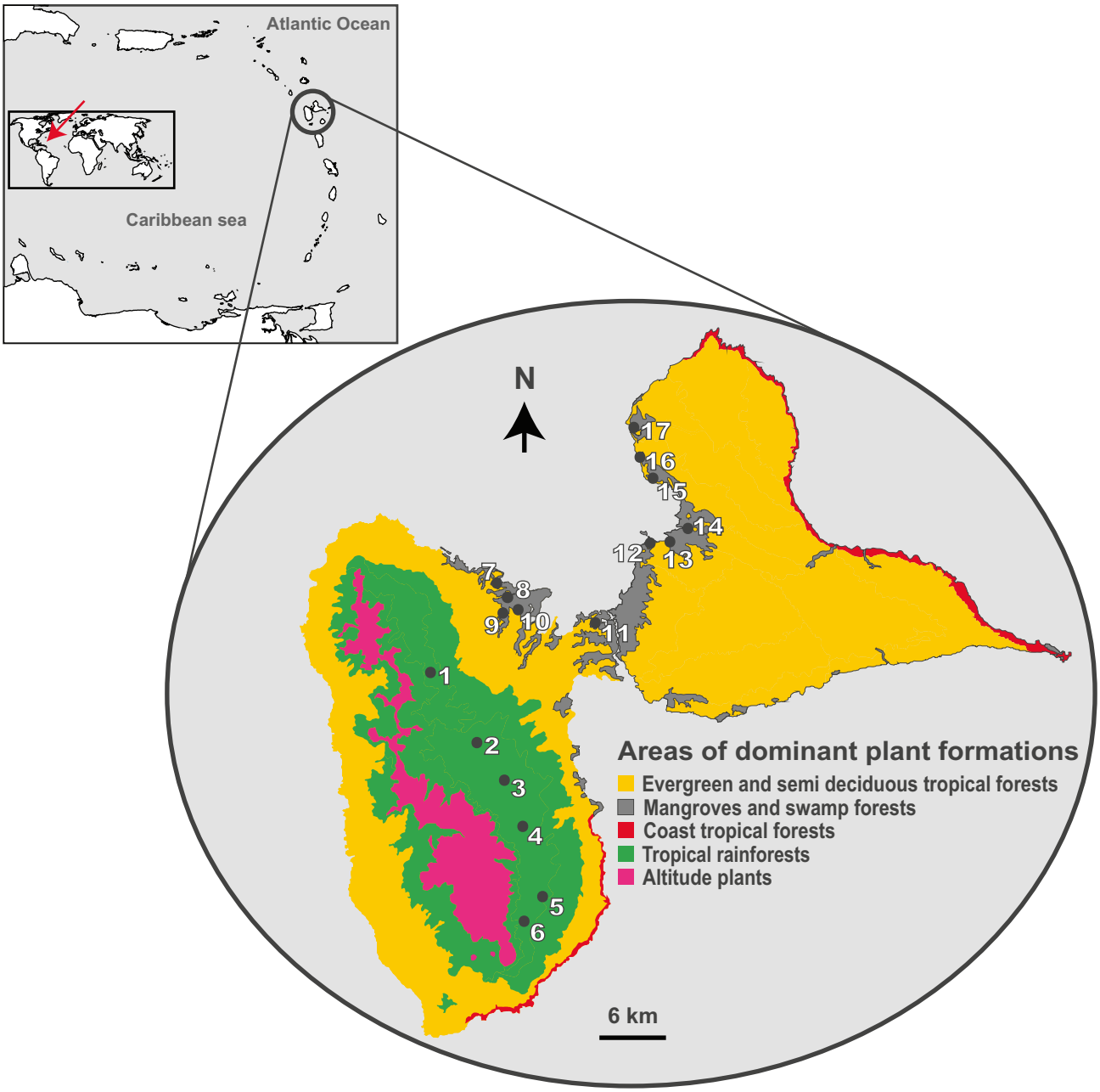


Fig. 1 Map of Guadeloupe, simplified from Rousteau (1996), indicating main vegetation types and the location of transects represented by dots. GPS locations are indicated in Table S2. The red arrow represents the location of the Caribbean region into a world map

in the morning and in the late afternoon, instead of two in 2016 (R1: 1 to 17 May, R2: 22 May to 24 June and R3: 27 June to 11 July). Similar to 2016, the circumtances of detection (Table 1) were recorded for all detected individuals, excepted for the "scanning flight" which was only recorded for the WCPi during the second and third replicates in 2017. Concerning SNPis, scanning flight and "perching" were not recorded because it was difficult to detect such behaviours in response of the broadcasted call, as all individuals were detected in closed and dense tropical rain forest, contrary to WCPis. Circumtances of detection were determined from the first cue allowing the observer to detect the bird.

Habitat characterisation

After having mapped all count stations using Google Earth, we drew a circle with a radius of about 125 m (accuracy of measurements (mean ± SD) 125.45 m ± 0.28) from the centre of each count station. We chose this distance according to our survey methodology (see above) assuming that

Table 1 Details and explanations of circumstances of detection recorded during surveys

Circumstances of detection level 1	Circumstances of detection level 2	Explanations	Recorded for	
			SNPi (2016)	WCPi (2017)
Seen	Seen	The bird was already perched near to the count station	No*	Yes
	Flight	The bird was seen flying next to or distant to the count station	Yes	Yes
	Perching	The bird came to perch near to the count station	No*	Yes
Heard	Heard	The bird was detected through its callings	Yes	Yes
Scanning flight		• The bird deviated from their straight flight line to come near the count station to perch or not • Or, it took off its perch during the counting to fly over the count station to perch not far away • Or, it took off its perch performing a flight over the count station before going away from the observer.	No *	Yes

*The circumtance of detection was recorded during the survey, but no pigeon was detected in these way

a 300-m distance between count stations significantly reduces the risk of spatial autocorrelation and that of double counting between successive count stations by the observer (Thomas et al. 2010). In addition, we estimated the sound propagation of call-broadcast using both a sound meter (measure of sound intensity à 25 m) and observer hearing capacity (maximum distance broadcast detection). The intensity of bird's song broadcasts played by the speaker was standardized at a level/volume about 60 dB as measured at 25 m distance of the source. This sound intensity allowed us to distinguish the bird calls from background noise at a distance between 100 and 150 m, depending on the vegetation type surrounding the loudspeaker and level of background noise in the environment. Previous studies based on domestic pigeons' hearing capacity (e.g., Schwartzkopff 1955; Kreithen and Quine 1979; Heffner et al. 2013) suggest that columbids have a shorter hearing distance than humans. Circles of vegetation determination were imported in *Géoportail* (French web platform for national geographic information; Institut National de l'Information Géographique et Forestière—IGN, 2006), in order to characterize vegetation from aerial photographs (2013). We mapped the vegetation surrounding each count station according to the major vegetation types occurring in Guadeloupe (Fig. 1): tropical rainforest, mangrove, swamp forest, dry forest (i.e., evergreen and semi-deciduous tropical forest) and others (i.e., all non-forest structures such as agricultural lands, wet meadows, sea or habitations; Supplementary materials Table S1 and S2). The identification of vegetation types was achieved from the different texture and green colours in aerial photographs (see an example in Supplementary materials Fig. S5). Vegetation types were subsequently confirmed by direct observations in the field. We then estimated for each circle around each count station the absolute and relative surface covered by each vegetation type using the surface area tool in the *Géoportail* platform.

Data analysis

All calculations, models and statistical tests were performed using the R software 3.6.2 (R Core Team 2019). For inference statistics, significance level was set at 0.05. In 2016, our statistical power was too low for the WCPi (see Supplementary materials Table S4). Therefore, analyses on SNPis were based on data collected in 2016, whereas analyses on WCPis relied on data collected in 2017. We assessed the reproducibility of our data from pairwise Spearman's correlation tests for each species and each detection method. We adjusted *p* values for repetitive tests by using Benjamini–Yuketiely's correction (Benjamini and Yekutieli 2001). Confidence intervals (CIs) for effect sizes were determined through 10,000 bootstraps.

White-crowned pigeon We calculated the diversity of habitat (Hd) using the Shannon index for each count station:

$$Hd = -\sum p_h \times \log_2(p_h)$$

where p_h is the proportion of habitats. We relied on a principal component regression in order to manage colinearity between variables describing habitats (proportions of each habitat type at count station and *Hd*; Supplementary materials Table S1), using the R package *FactoMineR* 2.2 (Husson et al. 2020) to reduce variation in habitats to two principal components (PC1 and PC2).

We used generalized linear mixed models (GLMMs; Bolker et al. 2009; Stroup 2012) in order to compare the detection methods using both relative abundance and presence/absence data. We considered PC1 and PC2, meteorological conditions (including sunny/cloudy vs. overcast/scattered rains) and time of day (coded as morning vs. afternoon) as explanatory variables interacting with detection methods in order to assess their influence on the effectiveness of the CB method. We thereafter relied on two other GLMMs using data obtained through the CB method in order to assess to what extent PC1, PC2, meteorological conditions and time of day

could explain changes in the presence/absence or abundance of WCPis. We also considered PC1 and PC2 interacting with other variables. We relied on random effect models, such as GLMMs, to avoid pseudoreplication (Harrison et al. 2018) as our sampling scheme consisted of three temporal replications. For all models, we then considered replica nested within station ID as random effect parameters. GLMMs were fitted with Poisson distribution (log link) for the estimated relative abundance data and with binomial error distribution (logit link) for presence/absence data. We ran models using the R package *glmmTMB* 1.0.0 (Brooks et al. 2017). Because our dataset based on relative abundance included many zeros, we tested our GLMMs for zero inflation compared to a Poisson distribution (Zuur et al. 2010; Bolker 2020) using the R package DHARMa 0.2.7 (Hartig 2020). We relied on quantile–quantile and goodness-of-fit plots using the same R package for verifying GLMM assumptions and detecting deviations from the expected distribution. Model selection was performed using the R package MuMIn 1.6 (Barton and Barton 2019) and based upon the adjusted Akaike's information criteria (AICc, Burnham and Anderson 2002). We considered models having $\Delta AIC_c \leq 2$ as equivalent for explaining variations of the presence or relative abundance of the species (Burnham and Anderson 2002; Arnold 2010). In order to assess the importance of model parameters, we relied on full-model averaging to estimate coefficients (β_i) of model parameters and their 85% confidence intervals (85% CI) (Burnham and Anderson 2002; Arnold 2010), counted as effect size (Galipaud et al. 2017). Model parameters that did not overlap zero were considered meaningful. Among the top ranked models (i.e., having $\Delta AIC_c \leq 2$), we selected the model including the meaningful parameters according to the full-model averaging procedure (Arnold 2010). We also relied upon the variable model-averaged absolute of the *t* statistics, which consists in dividing the absolute coefficient estimate of model parameter (β_i) with their standard deviation, so as to estimate the relative importance of each model parameter (Cade 2015; Galipaud et al. 2017). We also used the R package MuMIn 1.6 to calculate marginal R^2 (R^2_m) and conditional R^2 (R^2_c) values of the selected models, based on the delta method, to see to what extent fixed effect parameters and fixed effects combined with random effect parameters of models explained variation of the response, respectively (Nakagawa and Schielzeth 2013; Nakagawa et al. 2017). Based on presence/absence data, we also performed a McNemar change test in order to assess the ability of the CB method to detect pigeons when the AV method failed to do so. In order to assess the influence of call-broadcasting on the behaviour of WCPis, we assessed to what extent the circumstances of detection of individuals (circumtances of detection levels 1 and 2; Table 1) were dependent upon the method of detection used, using a Fisher's exact test and associated odds ratio.

Scaly-naped pigeon The survey strategy used in 2016 implied to broadcast the song of the two species at each count station. Therefore, we assessed whether the broadcasting order of calls impacted our ability to detect SNPis. To that end, we calculated the matched-pair rank biserial correlation coefficient, r_c (King et al. 2018), using the R package rcompanion 2.3.25 with the "none" zero method, which takes into account null pairs (Mangiafico 2020). Calculated effect size based on the relative abundance estimated when the intraspecific CB was played first vs. the opposite was compared based on 95% CI (Cumming and Finch 2005). The same procedure was performed for presence/absence data using the *Cohen's g* (C_g) as effect size (Cohen 1988).

Results aiming at comparing detection methods were obtained using the same procedures used for the WCPi. However, we did not include any habitat variable in GLMMs as the species was only found in tropical rainforests, such that only meteorological conditions and time of day interacting with detection method were considered. Besides, we assessed to what extent these variables could explain changes in abundance or the presence/absence of SNPis between count stations. In order to assess the influence of call-broadcasting on the behaviour of SNPis, we assessed to what extent the circumstances of detection of individuals (circumtances of detection level 1; Table 1) were dependent on the method of detection used, using a Fisher's exact test.

Comparison between species We performed interspecific comparisons in order to see whether the effectiveness of call-broadcasting differed between species and between interspecific and intraspecific call-broadcasting, by relying on the r_w and C_g effect sizes and their 95% CI. In order to assess interspecific attraction to calls, we relied on data obtained in 2016 for the WCPi, despite low statistical power resulting from weak abundance. We also compared the circumtances of detection level 1 (seen vs. heard; Table 1) between the two species using a Fisher's exact test and associated odds ratio. Habitat segregation between the two species was directly estimated from visual inspection of barplots as the observed pattern was obvious.

Results

We found significant and positive correlations between replications within each species and each detection method ($P < 0.05$ for all correlations, see Supplementary materials Table S3 for detailed results), indicating that the relative abundance at each count station was, on average, reproducible over our replications. For the two species, statistical power and number of counts per replication and detection method are presented in Supplementary materials Table S4.

Overall, we detected more individuals when using the CB method compared to when using the AV one for both species, with 134 vs. 59 WCPis and 118 vs. 67 SNPis, respectively. For both species, encounter rates (number of detections/min ± SD) were relatively low, but significantly higher for the SNPi for each detection method. We detected 0.054 ± 0.130 SNPis per min against 0.022 ± 0.087 WCPis per min using the AV method (Wilcoxon's test, $W = 72{,}033$; $P < 0.001$) and 0.098 ± 0.165 against 0.050 ± 0.155 using CB method, respectively ($W = 77{,}842$; $P < 0.001$). The proportion of birds heard or seen differed significantly between the two species (*P. squamosa*: heard = 155, seen = 30; *P. leucocephala*: heard = 91, seen = 102; Fisher's exact test, $P < 0.001$). In addition, the efficiency of the intraspecific CB method did not differ between the two species when assessing their presence/absence, whereas it was more effective for the SNPi when considering relative abundance (Fig. 2).

The vegetation pattern at point stations where SNPis were observed differed markedly from that of point stations where WCPis were observed (Fig. 3). SNPis were only detected at count stations dominated by tropical rainforests, located in the Basse-Terre island of the Guadeloupe archipelago, whereas the WCPi was found in the Grande-Terre and Basse-Terre island, at count stations characterized by more diverse vegetation types, including mangrove, swamp forests, dry forests and non-forest habitat.

Principal component regression reduced variation in vegetation cover to two principal components, PC1 and PC2, explaining 43.11% and 33.2% of the total variation, respectively (Table 2 and Supplementary materials Fig. S1). Larger values of PC1 represented count stations dominated by dry forest with large values of Hd, while lower values define count stations dominated by non-forest vegetation structure. For PC2, larger values represented count stations dominated by swamp forest, while lower values corresponded to those dominated by mangrove.

For all GLMMs, we included random effect parameters (i.e., replica nested within station ID) in order to explain variation in the presence/absence and the relative abundance of birds among and within count stations as the mere use of fixed effect parameters resulted in a poor R^2_m for the two species, contrary to R^2_c (i.e., $R^2_m < R^2_c$; Supplementary materials Table S5 and S7). Quantile–quantile plots and goodness-of-fit tests showed no evidence of problems with fit (Supplementary materials Figs. S2, S3 and S4).

White-crowned pigeon

The CB method improved significantly the detection of WCPis over the AV method (McNemar change test, $X^2 = 17$, df = 1, $P < 0.001$). We compared 17 logistic regression models to compare the efficiency of the detection method and the

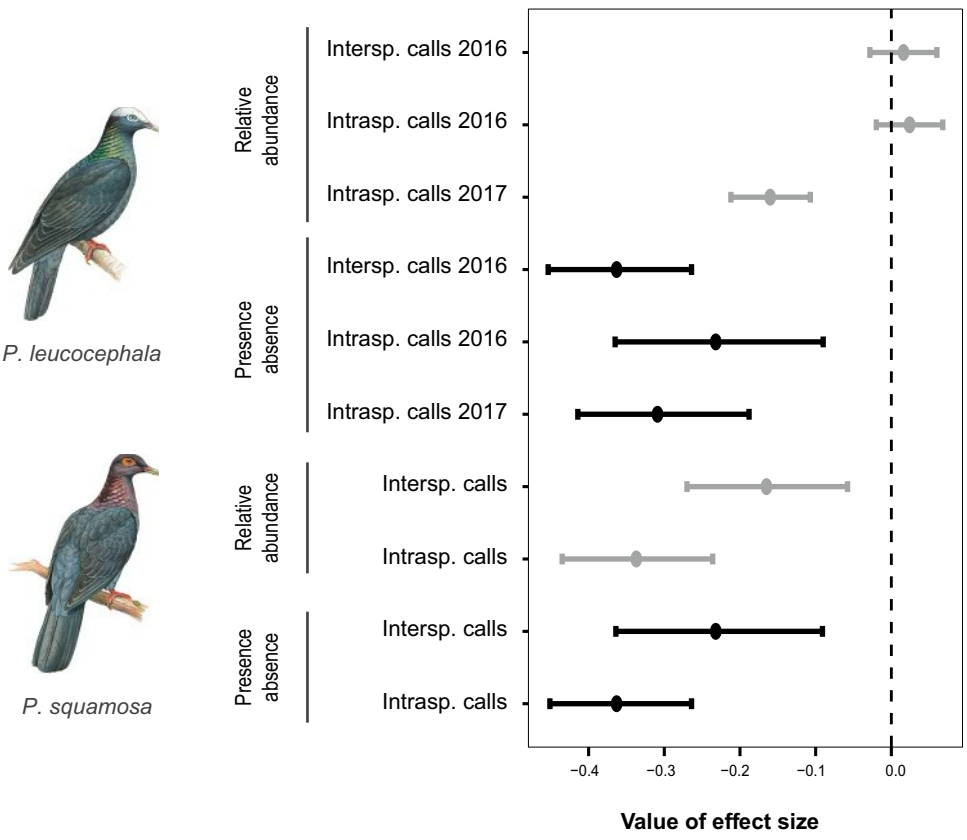


Fig. 2 Effect size values based upon the relative abundance (r_c in grey) and presence/absence (C_g in black) for both the WCPi and the SNPi. Error bars indicate the 95% CI. Significative negative values corresponds to AV < CB

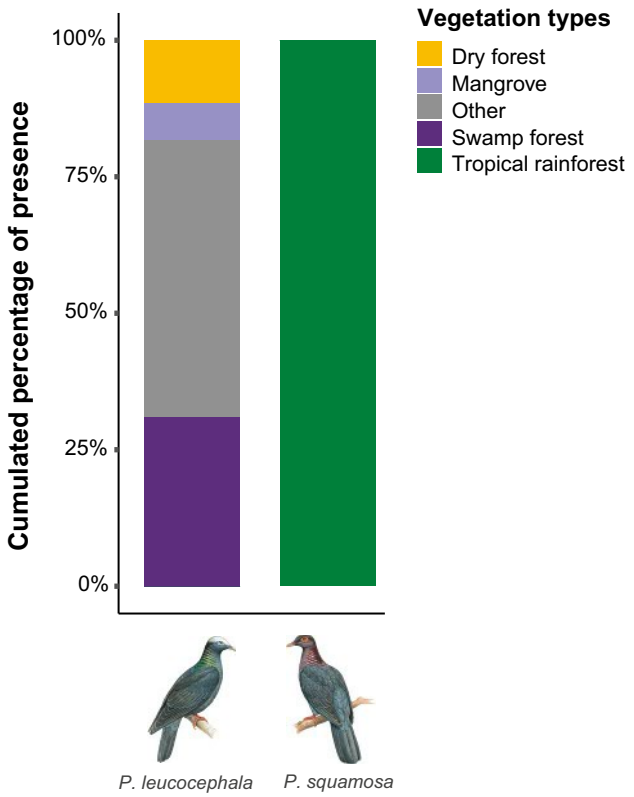


Fig. 3 General patterns of vegetation at count stations where SNPis and WCPis were detected in Guadeloupe during the survey

influence of several variables on the detection of the presence of the species (Table 3 (A)). The top ranked model included the detection method and its interaction with PC2, with the first variable having the largest influence on the detection of WCPis. As expected, the CB method was positively associated with the presence of the species ($\overline{\beta}_{CB}$ [85% CI] = 1.284 [0.678; 1.890]; Table 4 (A)). Besides, the estimated probability of detection was significantly higher when using the CB method than when using the AV one (AV [85% CI] = 0.010 [0.004; 0.021], CB [85% CI] = 0.028 [0.014; 0.054]; Fig. 4A).

Table 2 Factor loadings of the four habitats on the components 1 (PC 1) and 2 (PC 2). Bold values refer to loading values larger than |0.50| for each principal component. See Supplementary materials Fig. S1 for PCA plots

Habitats	PC 1	PC 2
Other	**−0.938**	−0.123
Mangrove	0.491	**−0.665**
Swamp forest	0.231	**0.905**
Dry forest	**0.666**	−0.013
Hd	**0.888**	0.035
Eigenvalues	2.406	1.333
Cumulative variance %	43.107	74.770

The presence of pigeons was also positively associated, but to a lesser extent, to the interaction between the detection method and PC2 ($\overline{\beta}_{AV:PC2}$ [85% CI] = 0.445 [0.246; 0.776] and $\overline{\beta}_{CB:PC2}$ [85% CI] = 0.267 [0.049; 0.564]; Table 4 (A)). Regardless of the method used, the probability of detection tended to be higher at count stations dominated by swamp forest compared to those dominated by mangrove, where it dropped to about zero (Supplementary materials Fig. S6).

Similarly, when comparing the 17 models based upon relative abundance, the detection method was included in the three top ranked models (Table 3 (A)). The full-model averaging procedure retained the model including the detection method, the interaction between detection method and PC2 and the interaction between detection method and meteorological conditions (Table 4 (A)). As expected, the use of the CB method was positively associated with relative abundance ($\overline{\beta}_{CB}$ [85% CI] = 0.862 [0.506; 1.218]; Table 4 (A)), with a higher averaged predicted relative abundance when using the CB method compared to when using the AV one (AV [85% CI] = 0.018 [0.010; 0.033]; CB [85% CI] = 0.040 [0.023; 0.071]; Fig. 4B). The interaction between detection method and PC2 was also positively related to the predicted relative abundance ($\overline{\beta}_{AV:PC2}$ [85% CI] = 0.243 [0.141; 0.561] and $\overline{\beta}_{CB:PC2}$ [85% CI] = 0.222 [0.120; 0.522]; Table 4 (A)), suggesting to be higher at count stations dominated by swamp forests and dropping to almost zero in habitats dominated by mangrove, regardless of the detection method considered (Supplementary materials Fig. S6). Meteorological conditions had also an influence on our ability to detect the species using the CB method, with a higher relative abundance on sunny days compared to overcast days (Fig. 5A).

Overall, the proportion of pigeons seen or heard was independent of the detection method used (Fisher's test, $P = 0.100$, odds ratio [95% CI] = 1.603 [0.309; 1.166]; Fig. 6A). However, the proportion of WCPis detected according to "circumstances of detection level 2" (see Table 1) was found to vary with the detection method (Fisher's test, $P = 0.020$; Supplementary materials Fig. S7). Besides, scanning flights were only observed when using the CB method (Fisher's test, $P < 0.001$; Supplementary materials Fig. S7). Finally, there was no significant difference in terms of response between interspecific (i.e., SNPi) and intraspecific calls when comparing count data in 2016 (r_c), and when comparing presence/absence (C_g) data in 2016 and 2017 (Fig. 2).

When comparing the 47 models consisting in assessing variation in the relative abundance or presence of WCPis, obtained with the CB method, in relation to environmental conditions at count stations and sessions, top ranked models included PC1, PC2, meteorological conditions, and time of day (Table 5A). However, only PC2 was meaningful and positively associated to both the presence and the relative abundance of the species, according to the full-model averaging procedure ($\overline{\beta}_{PC2}$ [85% CI] = 0.188 [0.040; 0.525] and

Table 3 Model selection results for comparing detection methods, for (A) the WCPi and (B) the SNPi. No habitat parameters are used for *P. squamosa* because all transects are in homogeneous habitat. Only models having ΔAICc < 2 are listed. Other models are in Supplementary materials Table S5

	Response	Models	*k*	−2lnL	ΔAICc	w_i
(A)	PA	DM + DM:PC2[a]	6	−273.204	0.000	0.436
		Full	12	−271.283	8.372	0.007
		Null	3	−283.860	15.256	~0.000
	RA	DM + DM:PC2[b]	6	−405.638	0.000	0.265
		DM + DM:PC2 + DM:W	8	−404.175	1.129	0.151
		DM	4	−408.613	1.909	0.102
		Full	12	−402.576	6.090	0.013
		Null	3	−423.577	29.821	~0.000
(B)	PA	DM[c]	4	−237.655	0.000	0.577
		DM + DM:W	6	−236.415	1.613	0.257
		Full	8	−236.150	5.213	0.043
		Null	3	−248.586	19.829	~0.000
	RA	DM + DM:Tod[d]	6	−342.999	0.000	0.304
		DM + DM:W	6	−343.082	0.166	0.280
		DM	4	−345.140	0.189	0.277
		Full	8	−341.726	1.582	0.138
		Null	3	−352.926	13.727	~0.000

Full models were (A) DM + PC1:DM + PC2:DM + W:DM + Tod:DM and (B) DM + W:DM + Tod

RA relative abundance, *PA* presence/absence, *DM* detection method (AV vs. CB), *PC1* principal component 1, *PC2* principal component 2 (see Table 2), *W* meteorological conditions (sunny/cloudy vs. overcast/scattered rains), *Tod* time of day (i.e., AM vs. PM), *−2lnL* the 2log likelihood, *k* number of model parameters, w_i Akaike weight

[a] $AIC_c = 558.486$

[b] $AIC_c = 823.355$

[c] $AIC_c = 483.394$

[d] $AIC_c = 698.175$

$\overline{\beta}_{PC2}$ [85% CI] = 0.220 [0.080; 0.481], respectively; Supplementary materials Table S8).

Scaly-naped pigeon

The order of presentation of intraspecific calls did not affect response to stimulus (intraspecific broadcasting played first: r_w [95% CI] = −0.203 [−0.379; −0.023], played second: r_w [95% CI] = −0.310 [−0.502; −0.107]). Therefore, subsequent analyses were performed without including the order of intraspecific call-broadcasting during the 2016 sampling scheme.

As for the WCPi, the CB method significantly improved the detection of pigeons over the AV one (McNemar change test, $X^2 = 24$, df = 1, $P < 0.001$). The influence of detection method and that of other variables was further assessed through the comparison of five models (Table 3 (B)). Based on the presence of SNPis at count stations, top ranked models included detection method and its interaction with meteorological conditions as model parameters. However, the full-averaged model procedure showed that detection method was the only meaningful parameter (Table 4 (B)). For this species as well, the CB method was positively associated with the presence of the species at count stations ($\overline{\beta}_{CB}$ [85% CI] = 1.564 [0.604; 2.525]; Table 4 (B)). Indeed, the probability of detection was higher when using the CB method than the AV one (AV [85% CI] = 0.096 [0.061; 0.149]; CB [85% CI] = 0.267 [0.193; 0.363]; Fig. 4C). When restricting the analysis to data obtained through the CB method, meteorological conditions and time of day failed to explain variations in the presence of the species across and within counts station (Table 5 (B) and Supplementary materials Table S8).

Based upon relative abundance, the detection method was the most important parameter, with its interaction with meteorological conditions and its interaction with time of day (Table 3 (B)). However, the time of day parameter was not meaningful according to the full-averaged model procedure, what led us to only consider the detection method and its interaction with meteorological conditions. These two parameters were in fact positively associated with the relative abundance at count stations ($\overline{\beta}_{CB}$ [85% CI] = 0.915 [0.182; 1.647] and $\overline{\beta}_{AV:W}$ [85% CI] = 0.370 [0.108; 1.663]; Table 4 (B)). The use of the CB method resulted in a higher relative abundance of SNPis compared to the AV one (AV [85% CI] = 0.149 [0.109; 0.203]; CB [85% CI] = 0.270 [0.203; 0.360]; Fig. 4D). The averaged predicted relative abundance was significantly higher in sunny conditions when using the AV method, whereas there was no such difference

when using the CB method (Fig. 5B). In addition, the effectiveness of the CB method was meaningful regardless of meteorological conditions. GLMMs aiming at assessing to what extent relative abundance could be associated with environmental features did not reveal meaningful parameters (Table 5 (B) and Supplementary materials Table S8).

The proportion of SNPis seen or heard was independent of the detection method (Fisher's test, $P = 1.000$, odds ratio [95% CI] = 1.023 [0.409; 2.456]), even when the interspecific CB was used (Fisher's test, $P = 0.700$; Fig. 6B). In addition, there was no difference in the efficiency of the CB method between intraspecific and interspecific calls (i.e., WCPi calls), in terms of presence data. However, the use of the CB method resulted in a significantly higher estimate of abundance when using intraspecific calls compared to when using interspecific calls (Fig. 2).

Discussion

Information on both the WCPi and the SNPi is scarce, and all studied populations so far are restricted to the Florida Keys, the Greater-Antilles or the US Virgin Islands (e.g., Strong et al. 1994; Rivera-Milán 1996; McNair 2008; Rivera-Milán et al. 2014, 2016). No study so far had been conducted on populations located in the southern part of the distribution areas of these species. In the Lesser Antilles, the two species are exposed to hunting pressure, with little or no regulation (Hay 2008). In addition, population sizes or trends of the two species are not monitored and no standardized methods have been developed so far to that end (Hay 2008; Florida Fish and Wildlife Conservation Commission 2013). The CB method is of particular interest to improve the ability to detect birds when population density is low, and when dealing with shy and secretive species (Sutherland 2006; Fuller et al. 2012). Although numerous studies have pointed out its effectiveness for various bird species, such as waterbirds (Allen et al. 2004; Conway and Nadeau 2005; Conway and Gibbs 2005; Rehm and Baldassarre 2007), partridges (Kasprzykowski and Goławski 2009; Jakob et al. 2010; Warren et al. 2018;

Fig. 4 Averaged probability of detection and relative abundance for **A**, **B** WCPis and **C**, **D** SNPis, respectively, predicted from the selected GLMMs, including all significative parameters for both relative abundance and presence/absence (Table 3). Error bars corresponds to the 85% CI

Table 4 Averaged coefficient estimates ($\bar{\beta}_i$), their standard error (SE) and the relative importance of parameters (RI) determined from the full-averaged model procedure performed upon models aiming at comparing detection methods (Table 3). (A) is for the WCPi and (B) for the SNPi. Due to the number of parameters, we listed parameters having RI > 0.30. Supplementary materials Table S6 presented all of them. In bold are significative effect (95% CI not overlaping zero), and see Table 3 for abbreviations

	Responses	Parameters *i*	β_i [95% CI]	SE	RI
(A)	PA	**Intercept**	−5.035 [−6.067; −4.004]	0.716	
		DM	1.284 [0.678; 1.890]	0.421	3.048
		DM(A/V):PC2	0.445 [0.246; 0.776]	0.243	1.836
		DM(C-B):PC2	0.267 [0.049; 0.564]	0.196	1.364
		DM(C-B):PC1	−0.145 [−1.082; 0.017]	0.309	0.468
		DM(A/V):PC1	−0.093 [−0.951; 0.264]	0.268	0.349
		DM(A/V):Tod	0.090 [−0.175; 1.009]	0.256	0.349
	RA	**Intercept**	−4.337 [−5.111; −3.562]	0.538	
		DM	0.862 [0.506; 1.218]	0.247	3.487
		DM(A/V):PC2	0.243 [0.141; 0.561]	0.203	1.198
		DM(C-B):PC2	0.222 [0.120; 0.522]	0.188	1.176
		DM(A/V):W	−0.171 [−0.918; −0.013]	0.294	0.581
		DM(C-B):W	−0.087 [−0.546; 0.075]	0.173	0.500
		DM(C-B):PC1	−0.128 [−0.917; 0.006]	0.266	0.480
		DM(A/V):PC1	−0.106 [−0.867; 0.111]	0.247	0.428
		DM(C-B):Tod	0.030 [−0.097; 0.403]	0.098	0.306
(B)	PA	**Intercept**	−0.915 [−1.496; −0.335]	0.403	
		DM	− .564 [−2.525; −0.604]	0.667	2.346
		DM(A/V):W	0.234 [−0.203; 1.764]	0.517	0.453
		DM(CB):W	−0.121 [−1.137; 0.328]	0.334	0.363
	RA	**Intercept**	−1.412 [−1.805; −1.020]	0.272	
		DM	−0.915 [−1.647; −0.182]	0.509	1.798
		DM(A/V):W	0.370 [0.108; 1.663]	0.559	0.663
		DM(A/V):Tod	0.165 [−0.001; 0.747]	0.253	0.651
		DM(CB):Tod	0.104 [−0.037; 0.506]	0.171	0.607

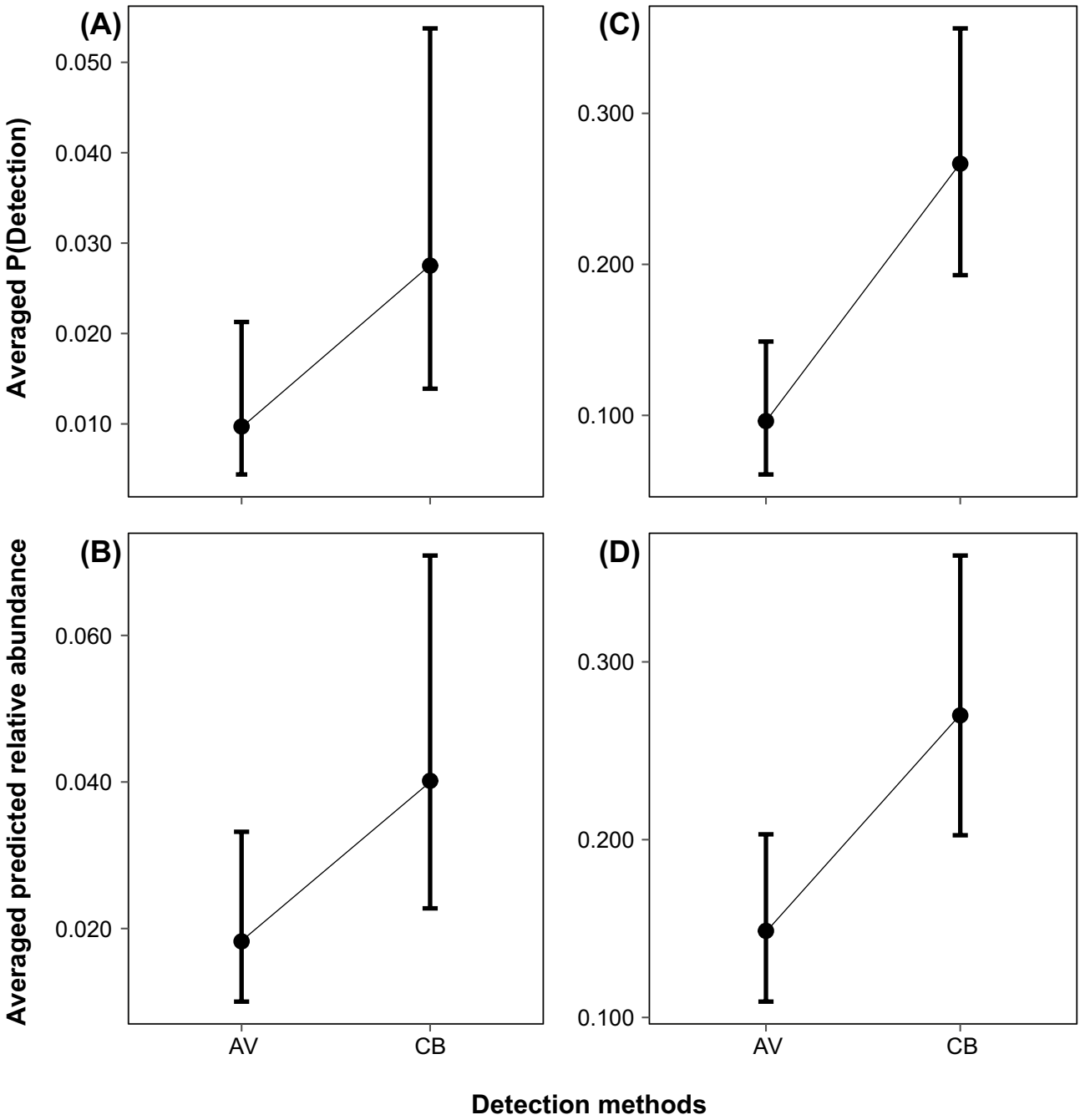
Variables are sorted by the relative importance



P. leucocephala



P. squamosa



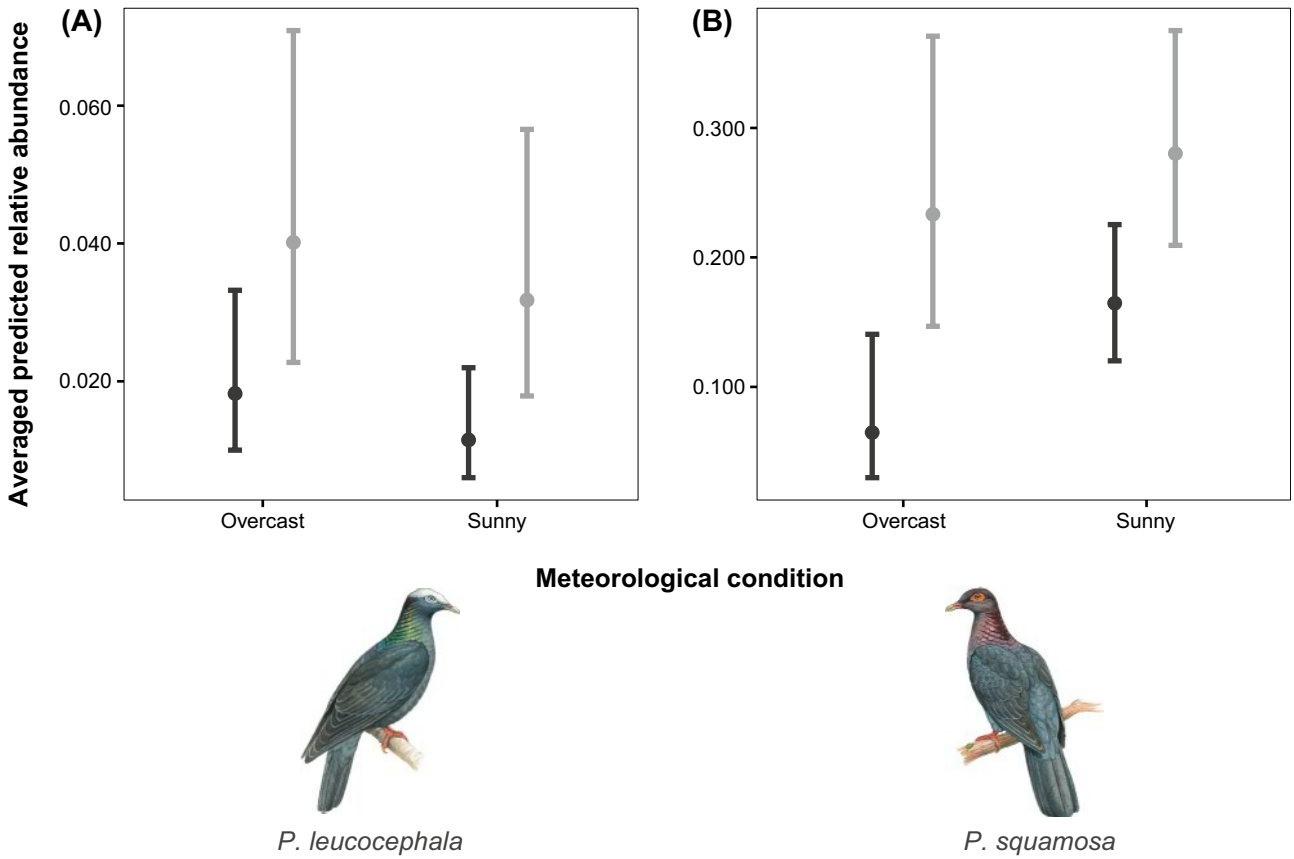


Fig. 5 Averaged relative abundance in relation meteorological conditions for **A** the WCPi and **B** the SNPi, when using AV (black dots and error bars) and CB (grey dots and error bars). GLMMs used for estimating predicted values included all significative parameters (Table 3). Error bars corresponds to the 85% CI

Chiatante et al. 2020), birds of prey (Barnes et al. 2012; Clewley et al. 2016; van der Horst et al. 2019), passerine species (Boscolo et al. 2006; McNeil et al. 2014; Lewis et al. 2017; Broughton et al. 2018) and other forest birds species (Boscolo et al. 2006; Frieze et al. 2012; Figarski 2017), only one study concerned columbids so far (*P. fasciata fasciata*; Kirkpatrick et al. 2007). The present study is then the second to confirm the benefit of the use of this detection method for the monitoring of *Patagioenas* species. Indeed, our results showed that the CB method improved the probability of detection (as defined by Marsh and Sinclair 1989) through both reducing the problem of false absence and allowing to detect more individuals. This suggests that, for a similar amount of sampling effort, the mere use of the AV method may result in an underestimation of the relative abundance and spatial distribution of the WCPi and SNPi (Duren et al. 2011).

However, some cues of presence induced by the CB method may result in biased population estimates. For instance, during our study, some behavioural responses to CB, especially in WCPis (e.g., coming to perch or performing a scanning flight), did not occur, or more rarely, when using the AV method (e.g., St. Clair et al. 1998; Bélisle and Desrochers 2002; Summers and Buckland 2011). Such induced behaviours are not compatible with some methods of population size estimation, such as distance sampling which requires that individuals are detected at their initial location (Buckland et al. 2015; but see Summers and Buckland 2011; Fuller et al. 2012). They were not observed in the SNPi, possibly because the species was only detected in dense rain forests and rarely below the canopy, where its behaviour is more difficult to observe, which may also explain why this species was more often heard than seen, contrary to the WCPi (Brewster and Simons 2009). Therefore, further investigations on behavioural responses to broadcasted calls by SNPis should be carried out before using the method in conjunction with distance sampling to see whether similar behaviours to those exhibited by WCPi occur in this species.

In addition, the CB method may induce sex-biased estimates. Indeed, it is supposed to preferentially induce responses by males (Sutherland 2006). In the absence of both marked sexual dimorphism in both species and knowledge of population sex ratio, we were unable to check this assumption in the field. However, it is likely that both males and females reacted to the broadcasted calls. Indeed, there is

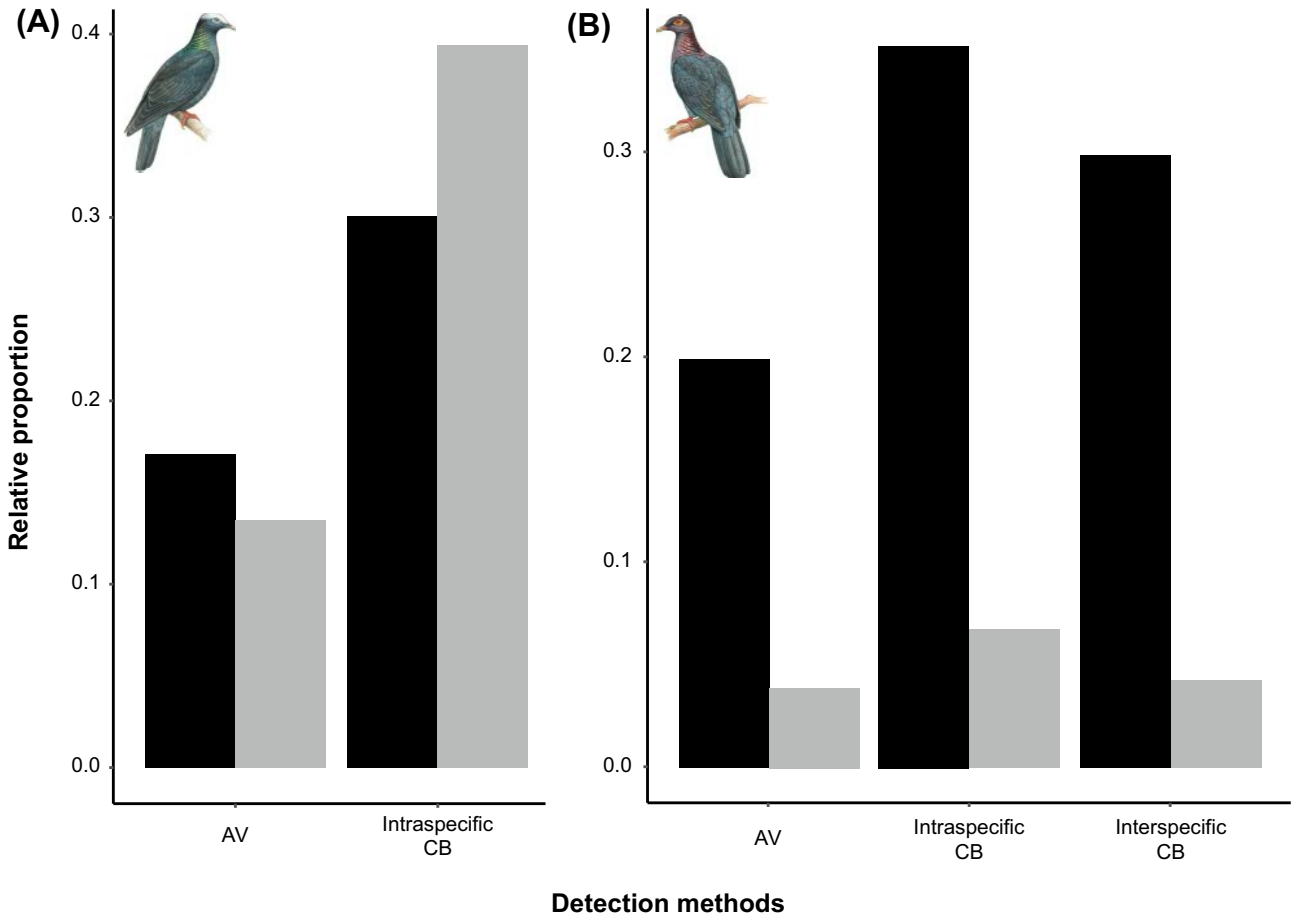


Fig. 6 Relative proportion of **A** WCPis and **B** SNPis according to whether pigeons were detected by visual (grey bars) or hearing cues (black bars), and according to the detection method

evidence that males of both species perform callings at the beginning of the reproductive season to signal their presence to females and attract them (Bancroft et al. 2020; Baptista et al. 2020), suggesting that females usually react to conspecific calls. In addition, female pigeons may response to playback if they take an active part in defense of the pair territory, irrespective of the sex of intruders, as has been observed in other columbid species (Quinard and Cézilly 2012). In this respect, breeding status may also influence the effectiveness of the call-broadcast method. Response rates to broadcasted calls may be related to the timing of surveys within the breeding season (Kirkpatrick et al. 2005, 2007; Rehm and Baldassarre 2007). For the closely related *P. fasciata fasciata*, Kirkpatrick et al. (2007) suggested that the number of unmated males, which decreased over time during the reproduction season, might be positively correlated to the probability that one individual performed coo-calls in response to conspecific calls in the population. In the present study, we observed significative correlations between each pair of replications based upon the relative abundance of white-crowned and scaly-naped pigeons using the CB method, suggesting that pigeons responded equally to call-broadcasting over time, possibly because the study period coincided with the first half of the breeding season. Indeed, one WCPi fledgling was observed in the swamp forest habitat during our survey when using CB. This confirms previous anecdotal observations by local ornithologists and confirms that the reproduction of this species, although rare, does occur in Guadeloupe (Delcroix et al. 2016).

For the two species, we recommend the systematic use of the CB method for monitoring population trends at several point count transects over years using the number of detections as an index of population density, especially in islands where they are at low density. The surveys could be performed in the morning, starting at sunrise, in the evening, finishing at sunset or both, since time of day had no significant influence in our surveys. The interspecific CB tended to be as effective as intraspecific CB, especially in terms of detection of species presence. This might be explained by the fact that the two species have evolved in sympatry in some islands, encouraging the recognition of interspecific calls in context of competition, mutual helping against predators or simply because they

Table 5 Model selection results to see in what extent the presence and relative abundance were associated with count stations features, for (A) the WCPis and (B) the SNPis. Models are based on data collected during intraspecific call-broadcast methods for both the relative abundance (RA) and the presence/absence (PA). Only models having ΔAICc < 2 are listed. Other models are in Supplementary materials Table S7. See Table 3 for abbreviations

	Response	Models	*k*	−2lnL	ΔAICc	w_i
(A)	PA	PC2[a]	4	−176.549	0.000	0.113
		PC1 + PC2	5	−175.672	0.282	0.099
		PC1	4	−176.986	0.873	0.073
		PC2 + W	5	−176.290	1.518	0.053
		Tod + PC2	5	−176.356	1.652	0.050
		PC1 + PC2 + W	6	−175.411	1.806	0.046
		Tod + PC1 + PC2	6	−175.479	1.941	0.043
		Full	11	−174.556	10.439	0.001
		Null	3	−178.175	1.221	0.062
	RA	PC2[b]	4	−272.788	0.000	0.085
		PC2 + W	5	−271.856	0.174	0.078
		PC1 + PC2	5	−272.094	0.649	0.061
		PC1 + PC2 + W	6	−271.157	0.820	0.056
		Tod + PC2	5	−272.414	1.291	0.044
		Tod + PC2 + W	6	−271.443	1.393	0.042
		Tod + PC1 + PC2	6	−271.720	1.948	0.032
		Full	11	−269.218	7.286	0.002
		Null	3	−274.909	2.213	0.028
(B)	PA	Null[c]	3	−149.227	0.000	0.462
		W	4	−148.821	1.255	0.246
	RA	Null[d]	3	−216.252	0.000	0.373
		Tod	4	−215.420	0.404	0.305
		W	4	−216.012	1.588	0.168

Full models were PC1 + PC2 + W + Tod + PC1:Tod + PC2:Tod + PC1:W + PC2:W for (A) and W + Tod + W:Tod for (B)

[a] $AIC_c = 361.174$

[b] $AIC_c = 533.650$

[c] $AIC_c = 304.556$

[d] $AIC_c = 438.606$

confuse their calls (Møller 1992), which are very similar. For instance, the two species share same nest sites in US Virgin Islands (McNair 2008). However, we recommend to broadcast intraspecific song to monitor population trends. In addition, in order to document spatial or temporal variation in relative abundance, environmental conditions during count events should be taken into account, by considering meteorological conditions and determining vegetation patterns at each count station between each year of survey, especially where natural vegetation landcover is subject to rapid changes due to human activities. To do so, it is possible to follow the same methodology used in this study for characterising vegetation patterns of each count stations, by using Google Earth platform, which regularly updates their aerial photographies. The second option would require more skills in remote sensing technique and would consist in using these methods in addition to geographical information system (GIS) and Google Earth Engine platform to define landcover and analyse changes over years, as explained by Sidhu et al. (2018). Such additional variables might be important as our study showed that the effectiveness of the CB method may be affected by vegetation type and meteorological conditions, suggesting that a change in terms of number of detections might be the result of a decrease of CB effectiveness due to a change of vegetation in count stations or different meteorological condition over surveys, but not of a change of population density. Indeed, although such effects appeared to be weak in our results, they suggested a lower efficiency of the CB method in swamp forests and in overcast/scattered rain meteorological condition for the WCPi. This might be explained by the fact that swamp forests are essentially used by pigeons as a corridor connecting the nesting (i.e., mangroves or islets off the coast) and foraging habitats (i.e., humid evergreen and semi-deciduous hardwood dry forests; Gibbs et al. 2001: Bancroft et al. 2020). In addition, both the propagation of calls (from loudspeaker to birds and from birds to the observer, Schieck 1997) and the activity of individuals might be reduced under overcat/scattered rain conditions (Robbins 1981b; Buckland et al. 2005; Overton et al. 2005).

As our methodology was initially designed to compare two detection methods, the relevance of our data to habitat selectivity and the spatial distribution of the two species in Guadeloupe is limited. Nevertheless, the two species were never observed together at a given count station, suggesting that they do not occupy the same places in Guadeloupe, at least for the period of the survey. In addition, vegetation patterns at the count stations associated to each species were in accordance with what has been observed in other Caribbean islands. The WCPi was associated with coastal areas: coastal flooded forests and islets off the coast dominated by mangrove and swamp forest, which are usually used as breeding sites, and humid evergreen and semi-deciduous hardwood dry forests in mangrove hinterland, usually used for foraging (Wiley and Wiley 1979; Gibbs et al. 2001; Bancroft et al. 2020). In contrast, the SNPi is more of an upland species, occupying primary and secondary rainforests (Gibbs et al. 2001; Baptista et al. 2020). However, the present study did not report local co-occurence of the two species in Guadeloupe, contrary to other places where they can occasionally be observed together (Wiley and Wiley 1979; Gibbs et al. 2001; Bancroft et al. 2020).

Management implications

The present study suggests that both species occur at low density in Guadeloupe, compared to what is observed in the Greater Antilles (Rivera-Milán et al. 2014, 2016), even though the SNPi seems to occur at higher density than the WCPi. In addition, we showed that the CB method provides an easy-to-use and standardized tool to estimate population trends across the spatial distribution area of the two species, which might be of use in the future for the monitoring of relative abundance of the two species through time, especially in relation to threats faced by the two species such as habitat degradation, local hunting pressure, climate change and the impact of invasive predators, parasites or competitors (Baptista et al. 2020; Bancroft et al. 2020). However, both species are considered vagrant, easily moving across their distribution area (Wiley 1979; Wiley and Wiley 1979; Rivera-Milán 1992; Bancroft et al. 2000, 2020; Gibbs et al. 2001; Strong and Johnson 2001; Florida Fish and Wildlife Conservation Commission 2013; Baptista et al. 2020). In addition, recent genetic evidence suggests that the SNPi consists in a single panmictic population (Cambrone et al. 2021), whereas corresponding information is still missing for the WCPi. Therefore, local fluctuations in the relative abundance of both species could be a consequence of local demographic processes or irregular movements of individuals between islands, induced by different factors, such as a sudden drop in resources availability, particularly following extreme climatic events such as hurricanes. Indeed, Perdomo-Velázquez et al. (2017) have shown that species feeding on plant products are more impacted by hurricanes than more generalist or insectivorous ones; hence, SNPis and WCPis may be more prone to move both within and between islands following natural perturbations (Wunderle 1995; Campos-Cerqueira and Aide 2021). Therefore, further investigations on movements of individuals within and between islands are important to better ascertain population trends and identify factors influencing population size and movements. Such investigations, combining population genetics (e.g., Cambrone et al. 2021 for the SNPis) and satellite tracking of individuals would also directly contribute to determine the more suitable management units for the two species.

More broadly, although columbid species are particularly threatened (Devenish-Nelson et al. 2019), especially those living in insular areas (Walker 2007), this group is one of the least studied avian group in ecology and conservation biology. In general, columbid species play however an important ecological role in island ecosystems through their capacity to disperse seeds over long distances (Shanahan et al. 2001; Bucher and Bocco 2009). The relevance of the CB method to other species of pigeons and doves therefore deserves further attention, especially to document population trends and habitat use in elusive species of conservation interest.

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Author contribution F.C., E.B. and C.C. conceived the project, design and experiments. C.C. performed the experiments. C.C. and F.C. analysed the data. F.C., E.B. and C.C. wrote and edited the paper.

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Data availability The datasets analysed during the current study are available from the corresponding author on reasonable request.

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ARTICLE 3

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Obtaining DNA samples from sensitive and endangered bird species: a comparison of saliva and blood samples

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Short Communications

OBTAINING DNA SAMPLES FROM SENSITIVE AND ENDANGERED BIRD SPECIES: A COMPARISON OF SALIVA AND BLOOD SAMPLES

OBTENCIÓN DE MUESTRAS DE ADN DE ESPECIES DE AVES SENSIBLES Y EN PELIGRO: COMPARACIÓN DE MUESTRAS DE SALIVA Y SANGRE

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Summary.—Methods used to collect biological samples from birds for genetic analyses should allow high-quality DNA to be obtained in sufficient quantities, while limiting negative effects on sampled individuals. In this context, we assessed the potential use of saliva sampling (using buccal swabs) as an alternative to blood sampling (supposedly more stressful) in a near-threatened Caribbean-endemic, the White-crowned Pigeon *Patagioenas leucocephala*, a bird known to be highly sensitive to capture and handling, based on samples collected from 28 adults captured in the wild. We quantitatively and qualitatively compared DNA extracts, amplifications of two mitochondrial genes (~430 bp and 1040 bp), and molecular sexing between saliva and blood samples. As expected, blood samples provided larger amounts of DNA of heavy molecular weight than buccal swabs. However, buccal swabs were as reliable as blood samples as a source of genetic material to sequence mtDNA. On the other hand, buccal swab samples might require an improved PCR protocol to sex all individuals successfully. We discuss the use of buccal swabs vs. blood sampling as a way to obtain DNA in relation to research objectives and minimising stress and harmful effects.—Cambrone, C., Motreuil, S., Reyes, F.O., Landestroy, M.A.,

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Key words: buccal swabs, mtDNA, non-destructive sampling, non-passerine, *Patagioenas leucocephala*, White-crowned Pigeon.

RESUMEN.—Los métodos utilizados para recolectar muestras biológicas de aves para análisis genéticos deberían permitir la obtención de ADN de alta calidad en cantidades suficientes, limitando al mismo tiempo los efectos negativos a los individuos muestreados. En este contexto, evaluamos el uso potencial del muestreo de saliva (usando hisopos bucales) como una alternativa al muestreo de sangre (supuestamente más estresante) en la paloma coronita *Patagioenas leucocephala*, una especie endémica del Caribe, casi amenazada, y conocida por ser altamente sensible a la captura y manejo. En el estudio se usaron muestras recolectadas de 28 aves adultas capturadas en la naturaleza. Comparamos cuantitativa y cualitativamente extractos de ADN, la amplificación de dos genes mitocondriales (~430 pb y 1040 pb) y el sexado molecular entre la saliva y la sangre. Como era de esperar, las muestras de sangre produjeron mayores cantidades de ADN y con mayor peso molecular que los hisopos bucales. Sin embargo, los hisopos bucales resultaron ser tan confiables como las muestras de sangre como fuente de material genético para secuenciar el ADNmt. Por otro lado, las muestras de frotis bucales pueden requerir un protocolo de PCR mejorado para sexar a todos los individuos. Discutimos el uso de hisopos bucales frente a la toma de muestras de sangre como una forma de obtener ADN en relación con los objetivos de la investigación y la minimización del estrés y los efectos nocivos.—Cambrone, C., Motreuil, S., Reyes, F.O., Landestroy, M.A., Cézilly, F. y Bezault, E. (2022). Obtención de muestras de ADN de especies de aves sensibles y en peligro: comparación de muestras de saliva y sangre. *Ardeola*, 69: XX-XX.

Palabras clave: ADN mitocondrial, aves no Paseriformes, hisopos bucales, muestreo no destructivo, paloma coronita, *Patagioenas leucocephala*.

INTRODUCTION

Securing reasonably large amounts of high-quality DNA from rare or threatened taxa is of prime importance in avian conservation biology. Indeed, individual DNA samples can be required for various investigations, such as sex determination in sexually monomorphic species (Underwood *et al*., 2002; Patiño *et al*., 2013; Niemc *et al*., 2018), parentage analyses and pedigree reconstruction (Pemberton, 2008; Le Gouar *et al*., 2011; Ferrie *et al*., 2013), assessing levels of inbreeding and gene flow among populations (Cortes-Rodriguez *et al*., 2019; Davidović *et al*., 2020; Cambrone *et al*., 2021; Li *et al*., 2021) or estimating effective population size (Olah *et al*., 2021). To that end, the sampling method should achieve an optimal balance between the necessity of acquiring sufficient amounts of high-quality DNA and the need for minimising invasiveness or harm to the individual, especially in the case of rare species or those particularly sensitive to capture and handling (Wilson & McMahon, 2006; McMahon *et al*., 2012; Zemanova, 2017, 2020).

Blood, usually obtained through puncturing the brachial vein, has been the most common tissue collected from bird species for genetic analyses (Owen, 2011). In addition, the presence of nucleated red blood cells in birds ensures the obtention of sufficient amounts of high-quality DNA. However, some concerns have been raised about the negative consequences of blood sampling on survival, breeding success, dispersal or behaviour (Sheldon *et al*., 2008; Brown & Brown, 2009; Voss *et al*., 2010; but see Angelier *et al*., 2011; Redmond & Murphy,

2011; Smith *et al*., 2017). Although plucked feathers have been used with some success as an alternative source of DNA in avian studies (Taberlet & Bouvet, 1991; Dubiec & Zagalska-Neubauer, 2006; Harvey *et al*., 2006), that method has also been criticised for having a negative impact on some species (McDonald & Griffith, 2011), while providing very limited genetic material.

More recently, buccal cell sampling has been proposed as a less invasive and alternative technique to blood and feather sampling (Handel *et al*., 2006; Vilstrup *et al*., 2018). The method has been used with some success to obtain DNA in a few avian species so far (Yannic *et al*., 2011; Wellbrock *et al*., 2012; Dai *et al*., 2015), although its ability to provide an adequate amount and quality of DNA and a high amplification success rate while maintaining low contamination risk and sequencing errors (Taberlet *et al*., 1999) deserves further consideration. Still, the method seems particularly suited for threatened species or those that are very sensitive to capture and handling (Zemanova, 2020). Also, compared to blood sampling, it is easier to implement (Vilstrup *et al*., 2018).

We assessed the relative performance of blood sampling and buccal swabs as sources of DNA in the Caribbean-endemic and near-threatened White-crowned Pigeon, *Patagioenas leucocephala* (BirdLife International, 2020). Although the species is exposed to strong hunting pressure and habitat destruction, reliable demographic and genetic data are scarce (Wiley, 1979; Strong *et al*., 1991, 1994; Strong & Bancroft, 1994; Rivera-Milán *et al*., 2016), hence the interest in obtaining DNA samples from that species. However, the White-crowned Pigeon is extremely sensitive to capture and handling (Meyer & Wilmers, 2007), sometimes displaying tonic immobility, an innate defensive state characterised by intense immobility, analgesia and lack of responsiveness (Hohtola, 1981; Mills & Faure, 1991; Gallup & Rager, 1996). A supposedly less stressful DNA sampling method, such as buccal cell sampling, could then be an appropriate alternative to blood sampling for that species. We therefore compared DNA extraction efficiency, amplification success and sequencing quality based on two mitochondrial genes of different sizes, D-Loop (~430 bp) and ND2 (~1040 bp), and assessed success in sex identification using each DNA source, as the White-crowned Pigeon is sexually monomorphic.

Methods

Capture and sampling

We captured 28 White-crowned Pigeons on an islet in Oviedo Lagoon, in the Dominican Republic (17.740575, −71.365890) in July 2019 by mounting three 3m-high mist nets of different lengths (3m, 6m and 12m). We continually monitored mist nets in order to minimise capture stress by promptly extracting captured pigeons from the nets. Immediately after, birds were placed into 50 × 50 cm opaque tents for at least ten minutes before ringing them and collecting blood and saliva samples. In order to minimise feather loss due to stress (Møller *et al*., 2006; Awasthy, 2010) and contact with wet hands, experimenters wore laboratory gloves during capture and handling. We collected blood from the brachial vein, as described by Owen (2011) and buccal cells using a swab, from each captured pigeon. Buccal swabs consisted of a foam tip on wood (VWR Int., United States, ref. 82030-594) that was gently rotated inside the mouth for ten seconds, taking care to avoid the tongue. The whole process, from capture to blood and saliva sampling lasted about thirteen minutes per bird. About 50-80µL of blood were collected and stored in absolute ethanol. Buccal swabs were air-dried and dry-stored in sterile collection tubes. Both samples were stored at room

temperature during the field period (ten days) and then at −20°C for up to 18 months until DNA extraction.

DNA extraction and amplification

DNA extraction was performed using the DNeasy Blood & Tissue commercial kit (Qiagen Inc., Valencia, CA, USA). For blood samples, we collected a piece of the blood clot weighing about 20.82 ± 9.15mg (mean ± S.D.) with a sterile spatula, since blood precipitates in contact with absolute ethanol. We amplified and sequenced mitochondrial genes D-loop (DLP, ~430 bp) and NADH dehydrogenase 2 (ND2, ~1040 bp). The same PCR protocol was used for DNA samples from both blood and buccal swabs for each mtDNA gene. To compare sex identification, we relied on universal primers 2550F and 2718R. According to sex identification performed on other columbid species (e.g., Monceau *et al*., 2013; Ayadi *et al*., 2016), two bands are expected for females and a single one for males (females being ZW and males ZZ; see detailed protocol in Supplementary Material, Appendix 1).

Statistical analyses

We relied on a two-tailed paired Wilcoxon signed-rank test to compare DNA concentrations obtained from blood and buccal samples. Using the two mtDNA genes, we compared amplification success, forward and reverse sequence quality, and sequencing success between the two tissue types. Amplification success was assessed as the percentage presence of correct band size for either blood or buccal swab samples based on agarose gel electrophoresis. The assessment of sequence quality relied on the Phred quality scores (Ewing & Green, 1998). Forward and reverse sequences were analysed independently, as sequencing performance may differ between primers. Sequencing success corresponded to the percentage of final sequence produced after the alignment of the forward and reverse sequences, and after manual correction for inconsistent base-calls using a reference sequence. We relied on Fisher Exact tests to assess to what extent the quality of sequenced bases, amplification success and sequencing success were dependent on the type of tissue used (see detailed protocol in Supplementary Material, Appendix 1). All statistical analyses were performed using R software 4.1.0 (R Core Team, 2021), with a significance level set at 0.05.

RESULTS

We successfully extracted DNA from both blood and buccal swab samples from every studied individual (N = 28). However, we extracted more DNA from blood than from buccal cells (Table 1). DNA quality was also higher in blood samples, with DNA fragments superior to 10kb present in all of them (Figure 1). The quality of DNA extracted from buccal swabs was more heterogeneous, with more fragmented DNA molecules. For nine buccal swab samples, nothing was visible on agarose gel due to the low DNA concentration and/or the presence of highly fragmented DNA molecules (7.31 ± 3.75ng/µL; mean ± S.D.). Five samples presented pale smears, suggesting that DNA extracts were very degraded and/or contained a low amount of DNA (12.12 ± 3.21ng/µL; mean ± S.D.). Eight samples had more marked smears with DNA molecules between 1kb to 10kb long, suggesting that DNA molecules were less degraded (13.22 ± 3.85ng/µL; mean ± S.D.). Finally, although also fragmented, six samples presented DNA molecules longer than 10kb (28.71 ± 10.12ng/µL; mean ± S.D.; Figure 1, Supplementary Material, Appendix 2, Figure B1).

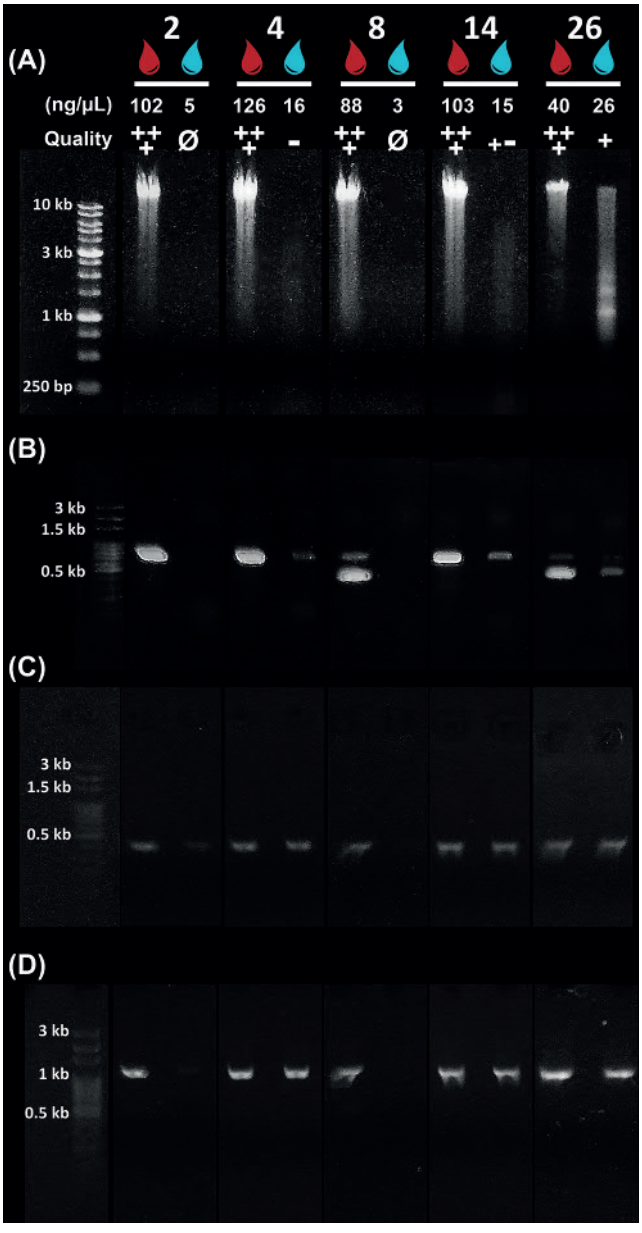
Although amplification failure only occurred with DNA extracted from buccal swabs, irrespective of the mtDNA gene used, amplification success was statistically not dependent on the sample source (Table 1). Moreover, we managed to improve the amplification of samples that failed during the one-shot PCR by conducting a second PCR

TABLE 1

Statistical summary comparing buccal swabs with blood samples. R and F indicate sequences obtained from reverse and forward primers, respectively. Percentages of HQ, MQ and LQ respectively are the mean percentages of bases of high, medium and low quality among each reverse and forward sequence, for each mtDNA gene. DNA concentrations correspond to the mean calculated from either all blood samples or all buccal samples. Dispersions around means were estimated through standard deviation. Outputs of electrophoresis gels used for scoring amplification successes are shown in Supplementary Material, Appendix 2, Figure B2 for D-loop gene (DLP) amplification, B6 for NADH dehydrogenase 2 gene (ND2) amplification and B7 for molecular sexing.
[Resumen estadístico para comparar los hisopos bucales con las muestras de sangre para su uso como muestra genética fiable. En esta tabla, R y F indican las secuencias obtenidas a partir de los cebadores inverso y directo, respectivamente. Los porcentajes de HQ, MQ y LQ, respectivamente, son el porcentaje medio de bases de alta, media y baja calidad entre cada secuencia inversa y directa, para cada uno de los genes de ADNmt. Las concentraciones de ADN corresponden a la media calculada a partir de todas las muestras de sangre o de todas las muestras bucales. La dispersión en torno a las medias se estimó mediante la desviación estándar. Los geles de electroforesis utilizados para puntuar los éxitos de amplificación se encuentran en el Apéndice 2 del Material Suplementario, Figura B2, para la amplificación del gen D-Loop (DLP), B6 para la amplificación del gen de la NADH deshidrogenasa 2 (ND2) y B7 para el sexado molecular, respectivamente.]

Steps	Blood	Buccal Swabs	Statistics
DNA concentration (ng/μL)	91.54 ± 27.49	14.19 ± 9.76	**Paired Wilcoxon test: V = 459, P < 0.001**
Amplification (success/fail)	DLP: 28/0	DLP: 27/1	Fisher exact test: NS McNemar test: $\chi^2 = 0$; NS
	ND2: 28/0	ND2: 25/3	Fisher exact test: NS McNemar test: $\chi^2 = 1.33$; NS
Sequence quality (% bases HQ/MQ/LQ)	DLP (F): 52/13/35	DLP (F): 67/6/27	Fisher exact test: NS
	DLP (R): 41/13/46	DLP (R): 66/5/29	**Fisher exact test: P < 0.01**
	ND2 (F): 72/9/19	ND2 (F): 64/13/23	Fisher exact test: NS
	ND2 (R): 52/16/32	ND2 (R): 61/14/25	Fisher exact test: NS
Sequencing success (success/fail)	DLP: 21/7	DLP: 21/7	Fisher exact test: NS McNemar test: $\chi^2 = 0$; NS
	ND2: 23/5	ND2: 20/8	Fisher exact test: NS McNemar test: $\chi^2 = 0.57$; NS
Molecular sexing (success/fail)	28/0	19/9	Fisher exact test, P < 0.001

FIG. 1.—Example of electrophoresis gel outputs based on five samples for (A) assessing DNA quality, (B) molecular sexing, and amplification of (C) D-loop and (D) ND2 mtDNA genes. Grey beads (red in the colour version of the figure) correspond to blood samples and white beads (blue in the colour version) to buccal swab samples. Entire electrophoresis gels are shown in Supplementary Material, Appendix 2, Figures B1, B2, B6 and B7. Symbols Key. **+++** DNA extracts of good quality, with many long DNA molecules (> 10kb); **+** DNA extracts contain long DNA molecules (> 10kb) but also many degraded molecules; **+–** DNA molecules shorter than 10kb and very degraded; **–** DNA molecules very degraded, shorter or equal to 3kb; **Ø** blank result. In the same line of "(ng/µL)", whole numbers correspond DNA concentration of samples.



[Ejemplo de los resultados de gel de electroforesis basados en cinco muestras para evaluar (A) la calidad del ADN, (B) el sexado molecular y la amplificación de (C) los genes de ADNmt D-loop y (D) ND2. Las cuentas grises (rojo en la versión en color de la figura) corresponden a muestras de sangre y las blancas (azul en la versión en color) a muestras de hisopos bucales. Los geles de electroforesis completos se encuentran en el Material Suplementario, Apéndice 2, Figuras B1, B2, B6 y B7. En cuanto a los símbolos utilizados para la calidad del ADN, (+++) significa que los extractos de ADN eran de buena calidad, con muchas moléculas de ADN largas (> 10kb); (+) significa que los extractos de ADN contienen moléculas de ADN largas (> 10kb), pero también tienen muchas moléculas degradadas; (+–) significa que las moléculas de ADN tienen un tamaño inferior a 10kb y están muy degradadas; (–) significa que las moléculas de ADN están muy degradadas, con tamaños de molécula inferiores o iguales a 3kb; (Ø) significa que no se veía nada en el gel de agarosa. En la misma línea de "(ng/µL)", los números enteros corresponden a la concentración del ADN de las muestras.]

increasing DNA quantity (30ng) and BSA concentration (1.50µg/µL). Regarding sequence quality, the percentages of sequenced bases/nucleotides of high, medium and low quality were dependent on sample type for the reverse sequence of the D-loop mtDNA gene (Table 1), with the percentage of high-quality bases being higher in sequences obtained from buccal swab samples and the percentage of medium and low-quality bases being lower compared to sequences obtained from blood samples. For the other sequences, no difference was found between sample types (Table 1). Sequencing success did not differ between blood and buccal samples, irrespective of the mtDNA gene considered (Table 1). When aligning final sequences produced from blood and buccal swab samples, sequences were identical.

Regarding sex identification, 100% (N = 28) of blood samples allowed sexing of individuals, whereas only 66% (18/28) of buccal samples did so (Fisher's exact test, $P < 0.001$). However, after optimising the PCR protocol for buccal samples, either by increasing DNA quantity (20ng to 60 ± 16ng) or by adding 4% of DMSO in PCR reactions (Supplementary Material, Appendix 2, Figure B2-B4), sexing rate reached 75% (21/28), which was still lower than for blood samples (Fisher exact test, $P < 0.05$). No mismatch was found between the two sample types for sex identification. In total, our samples consisted of 17 females and 11 males, of which 14 females and 7 males could be identified using buccal swabs. Although CHD1-Z (750 bp) was more difficult to amplify from buccal swab samples in females (heterozygous ZW) compared to CHD1-W (450 bp) (see Supplementary Material, Appendix 2, Figures B2-B5 and Tables B1-B2), the observed sex ratio was independent of the type of tissue used for DNA extraction (Fisher exact test, *NS*). However, low-quality DNA samples more frequently failed to sex individuals than higher-quality DNA samples (Fisher exact test, $P < 0.05$). In contrast, the concentration of DNA extract did not differ between buccal samples that failed and those that succeeded to sex individuals (Mann-Whitney-Wilcoxon test, $W = 69$, *N.S.*; (mean ± S.D.) = 13.84 ± 9.65ng/µL vs. 15.26 ± 10.78ng/µL, respectively).

Discussion

As expected, our results confirm that blood is a tissue of prime choice for genetic analyses, as it allows extracting large amounts of DNA of heavy molecular weight. However, although extraction from buccal swabs resulted in smaller amounts and a lower quality of DNA compared to blood, buccal cells appear to be a reliable source of DNA, allowing the sequencing of mtDNA genes up to 1040 bp. Interestingly, they even produced DLP sequences of higher quality than obtained from blood, probably because whole blood contains several inhibitors affecting DNA polymerase activity, as shown with human haemoglobin and immunoglobulin (Abu Al-Soud & Rådström, 2001; Sidstedt *et al.,* 2018), blood proteins found in all vertebrates (Hawkey *et al.,* 1991). Failure to observe any difference with the ND2 gene was probably related to the poorer DNA quality obtained with buccal cells, which may have limited amplifications of the longest sequences, thus hiding the effect of blood inhibitors. Although we did not assess microsatellite markers, other studies have showed that buccal samples can be used to amplify microsatellites (Yannic *et al*., 2011; Dai *et al*., 2015), even from saliva samples left behind by birds in their environment (Monge *et al*., 2020). In addition, the amount of DNA obtained from buccal swabs in this study was greater than what is usually obtained from plucked or shed feathers (e.g., Harvey *et al*., 2006; Peters *et al*., 2019; C. Cambrone, unpublished results), and of the same order of magnitude,

or greater, than usually obtained from buccal cell samples (Yannic *et al.,* 2011; Vilstrup *et al.,* 2018).

When considering molecular sexing, a significant difference was found between buccal and blood samples, with 75% and 100% successful identifications respectively. Furthermore, there was 100% consistency in sex identification from both blood and saliva for a given individual, although the success rate obtained with buccal cells accorded with results obtained by Asawakarn *et al.* (2018; success rate = 74%). Overall, the CHD1-W allele (450 bp) was relatively easier to amplify than the CHD1-Z allele (750 bp). This resulted in the absence of the Z-band on electrophoresis gels for most females when using buccal samples, and a less pronounced Z-band, compared to the W-band, with blood samples. Thus, most females were identified from the sole presence of CHD1-W fragment when using buccal swab samples, rather than their heterozygous ZW genotype, which does not cause any ambiguity since the W-chromosome is the sex determiner in birds (Fridolfsson & Ellegren, 1999). This difference of amplification might be explained by the lower quantity and quality of DNA samples obtained through buccal swabs, inducing stronger competition among annealing sites. The use of alternative primers, such as those developed by Griffiths *et al.* (1998) or Kahn *et al.* (1998) that produce smaller amplicons (Z = 282 bp, W = 287 bp, and Z = 224 bp, W = 252 bp, respectively), could improve the amplification of degraded DNA. Several studies have shown that these primers perform equally well when sexing birds using buccal samples (e.g., Bush *et al.*, 2005; Handel *et al.*, 2006; Wellbrock *et al.*, 2012; Yannic *et al.*, 2016), including *Columba* (Dijkstra *et al.*, 2010) and *Streptopelia* species, although for the latter, a combination of Kahn's forward and Griffiths' reverse primers seems to be more efficient (Secondi *et al.*, 2002; den Hartog *et al.*, 2010).

The harmful effects of blood sampling and feather plucking may vary according to the species and age of individuals. It may also depend, to a certain extent, on climatic conditions, with a higher risk of infection following vein puncturing in tropical or subtropical environments (Brown & Brown, 2006; Sheldon *et al.*, 2008; Voss *et al.*, 2010). On the other hand, recent information suggests that venepuncture in itself does not add stress to capture and handling (Bonnet *et al.*, 2020; Huber *et al.*, 2021). In any case, however, the method inherently increases handling time, potentially resulting in an increased level of stress (Wilson & McMahon, 2006; Duarte, 2013), especially with highly sensitive species such as the White-crowned Pigeon. In that respect, non-destructive buccal cell sampling may be a valuable alternative to blood sampling and feather plucking in the case of the White-crowned Pigeon, particularly when sampling chicks or juveniles. Although not free of discomfort, the use of buccal swabs may reduce accumulated stress, by reducing handling time and the number of steps needed to collect DNA samples. In addition, it also avoids the injury risk inherent to blood sampling.

From a practical point of view, the quantity and quality of DNA contained in buccal swabs may be an important issue in the future in relation to Next-Generation Sequencing (NGS) technologies. Such techniques offer new avenues for wildlife genetic studies (Hudson, 2008; Ekblom & Galindo, 2011; Kumar & Kocour, 2017; Carroll *et al.*, 2018) but require high-quality and quantity DNA (Kumar & Kocour, 2017; Carroll *et al.*, 2018). However, DNA extraction cannot always be performed rapidly nor may samples be frozen immediately upon collection under field conditions, either or both of which could limit subsequent genetic analysis significantly. It has been shown that DNA extracted from amphibian fresh buccal swab samples or that has been promptly frozen at −18°C

contains more DNA than samples stored at room temperature for nine weeks (Pidancier *et al*., 2003). The situation may be even worse when fieldwork lasts over several days in hot and moist field areas as humidity, warmth and sunlight exposure can increase DNA degradation (Baus *et al*., 2019). Given such constraints we opted for an easy and inexpensive sampling method to collect buccal cells that would be accessible to a majority of researchers, including those from developing countries who do not necessarily have access to laboratory grade buffers and/or long-term reliable low temperature storage equipment. In addition, transport of dry samples does not conflict with commercial airflight regulations, unlike samples kept in ethanol, a highly flammable product, which may not be carried either in checked-in or carry-on baggage. However, in the absence of studies comparing the effect of storage conditions of buccal swab samples, whether or not preserved in buffer solutions, we suggest that DNA extractions or sample storage at −18°C, should soon follow collection, whenever possible, in order to preserve DNA integrity and obtain maximum DNA yield. Relying on buffer storage is an efficient way to preserve DNA integrity (Seutin *et al.,* 1990; Kilpatrick, 2002) but may limit DNA yield after extraction, as buccal cells are likely to come loose from swabs and be suspended in the solution. As an alternative to buffer preservation, freezing samples becomes more and more feasible, even during isolated field work, thanks to the availability of portable freezers with rechargeable batteries that allow several hours of autonomy.

Additional information, such as infection with various pathogens known to infect columbid species (Lennon *et al*., 2013; Panella *et al*., 2013; Stockdale *et al*., 2015) could be obtained from saliva samples by using PCR techniques (quantitative, reverse-transcriptase or classical PCR). These include in particular the West Nile Virus (Komar *et al*., 2002), columbid herpesvirus (Phalen *et al*., 2017), avian influenza (Hall *et al*., 2013) or avian trichomoniasis (Lennon *et al*., 2013). The last of these is particularly relevant in the cases of the White-crowned Pigeon and other Caribbean columbids, such as the strongly invasive Eurasian Collared-dove, *Streptopelia decaocto*, a potential host of such pathogens (Lennon *et al*., 2013; Panella *et al*., 2013) suspected of transmitting *Trichomonas* spp. to the native avifauna in Saint Kitts and Nevis islands (Stimmelmayr *et al*., 2012). However, if buccal cells seem to be as effective as blood for classical genetic analyses, their use to investigate questions on avian parasite load, physiology, ecology and biology remains limited compared to blood. Indeed, given appropriate storage conditions, blood samples can provide information on hormonal levels (e.g., stress or reproductive hormones), metabolism, diet and movements (through using stable isotopes) and immunological parameters (Owen, 2011; Albano, 2012). Such data are also relevant for the conservation of species and/or populations but would be more difficult to obtain from saliva samples. For instance, a recent work showed that mesotocin (a hormone analogous to oxytocin in mammals and involved in avian social behaviour) can be quantified from saliva in Common Ravens *Corvus corax*, but with a low success rate (Stocker *et al*., 2021). Further technical improvements in collection and analysis methods may however allow the measurement of some hormones from bird saliva. However, collecting blood samples will remain necessary to investigate the prevalence of blood parasites (e.g. avian malaria), an important issue in ecology, population biology and conservation of bird species (LaPointe *et al*., 2012; Ricklefs *et al*., 2016). Therefore, the decision to rely on blood samples or to switch to buccal swabs as a way to collect biological samples should be made through assessing the balance between the need to minimise handling stress

and the benefits of extracting as much information as possible from samples, taking into account storage conditions and the interval between collection and DNA extraction. One possibility, where a large enough number of individuals are available for sampling in a population, could be to collect saliva from every individual handled systematically but to collect blood from only a randomly chosen subset of all captured individuals.

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Appendix 1: Detailed protocol for DNA extraction and amplification, and statistical analyses. *[Protocolo detallado para la extracción y amplificación del ADN, y de los análisis estadísticos.]*

Figure A1. Additional steps to extract remaining lysis solution from swab tips.
[Pasos adicionales para extraer el remanente de la disolución de los hisopos bucales.]

Appendix 2: Assessment of DNA quality and concentration, and molecular sexing.
[Evaluación de la calidad y concentración del ADN, y del sexado molecular.]

Figures B1-B7. Electrophoresis gel outputs used to visually assess DNA quality, identify sex, and gene amplifications.
[Geles de electroforesis para evaluar visualmente la calidad del ADN, la identificación del sexo y la amplificación de los genes.]

Table B1. Summary of DNA quality score, DNA concentration and molecular sexing for blood samples.
[Resumen de la calidad y concentración del ADN, y del sexado molecular para las muestras de sangre.]

Table B2. Summary of DNA quality score, DNA concentration and molecular sexing for buccal swab samples.
[Resumen de la calidad y concentración del ADN, y del sexado molecular para las muestras de sangre.]

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CHAPTER 3

An attempt of GPS track to visualise current movement pattern of the WCPi within the Caribbean region

Introduction

The observation *in natura* of Animals' movements, defined as a change in position of an animal in time, have always fascinated biologists (Focardi & Cagnacci 2013), and particularly behavioural ecologists (Liedvogel et al. 2013). The first way to observe animals' movements is to perform direct observations. The second consists in using individual marks to infer travel performed by animals from the locality of capture and of that of recapture. These two methods are simple and handy, but for the first, the presence of the observer can potentially alter the natural behaviour of the observed animals, and, for the second method, movements performed between the two stages of capture are totally unknown. In the course of technological advances, refined methods have been progressively developed to track more or less accurately animals' movements. Starting in the 1960s, VHF (very high frequency) devices have been increasingly used to track animals in their natural environments (Rongstad & Tester 1969; Craighead 1979; Crofoot et al. 2008). A few years later, the use of stable isotopes was included in the ecologist's tool kit to study various aspects of species ecology, including migration (Rubenstein & Hobson 2004; Fry 2006; Michener & Lajtha 2008; Newton 2016). Like for the capture-mark -recapture method, these valuable methods are however unable to provide an exhaustive spatial and temporal record of animals' movements. Then, programmable devices using ARGOS or GPS technologies, have been developed to track movements of animals in real time and in high resolution. First versions required the subsequent recapture of animals to retrieve movement data. However, more recent versions allow the collection of data remotely by using satellites, cell phone network, or using mobile or fixed antennas. Nowadays, such devices can also be combined with sensors, also called biologgers, that measure environmental variables (e.g., temperature, luminosity, noise), or physiological and behavioural features of animals (e.g., accelerometers, body temperature, heart rate; Liechti et al. 2018; Williams et al. 2020).

Thanks to these tracking movement devices, it is now possible to address fundamental questions regarding the behaviour of animals (Nathan et al. 2008; López-López 2016). In addition, they are important tools for conservations studies as they can provide information regarding life histories of species, ecosystem services performed by tracked species, habitat and landscape use, and ecophysiology of target species (Jeltsch et al. 2013; Kays et al. 2015; Allen & Singh 2016). Such information can be crucial for wildlife management, particularly to

adjust management plans at different scales, from species to ecosystems/landscapes (Jeltsch et al. 2013; Kays et al. 2015; Allen & Singh 2016; Jeltsch & Grimm 2020). In this context, we deployed seven GPS tags on WCPis in order to document better the spatial dynamic of the species, a information that is currently lacking to support efficient management plan at the Caribbean region scale. These seven tracked pigeons were a preliminary attempt with the hope of deploying more GPS tags on different Lesser Antilles islands and some in the Greater Antilles. Regarding the Greater Antilles, the Avian Research Conservation Institute, domiciled in Florida (U.S.A.), is currently performing monitoring of WCPi's movements, all captured in Greater Antilles. However, they rely on the ARGOS technology to document exclusively inter-island movement in the Greater Antilles, while we used GPS, which is more accurate in location estimation of animals, so as to analyse the movements of WCPis at both regional (i.e., inter-island) and local (i.e., habitat use) scale (Costa et al. 2010; Witt et al. 2010).

Methods, results and discussion

To that end, we sought to develop a method to capture WCPis as the species is usually observed flying or perched at 10 to 50-m high in the Lesser Antilles, what makes mist nets useless in such areas. We therefore tried to attract WCPi to lower strata by using call-broadcast (see Chap. 2 Article 1), WCPi decoys and berries usually eaten by the species, all placed on a wooden structure positioned about 1.20m above the ground. We spent about 24 hours in Morne-à-l'eau (97111, Guadeloupe F.W.I.) to observe how WCPis reacted to presence of the apparatus. Although WCPis were interested by the platform, no one came lower than tree tops.

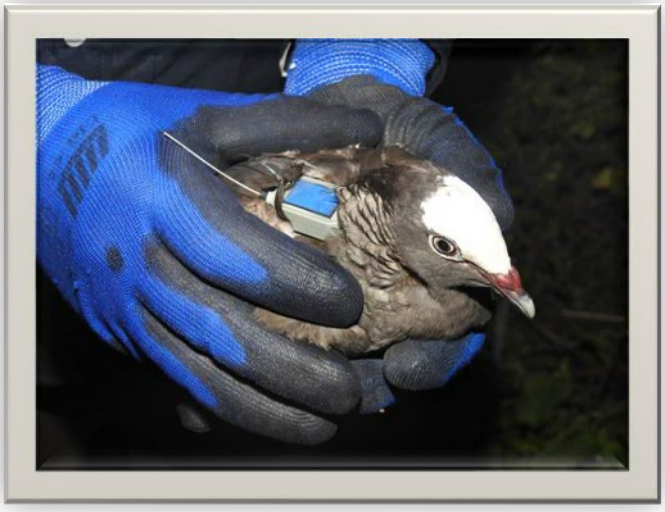


Photo 3 : A WCPi equipped with GPS tags, fixed with a back-pack harness in Teflon (Hill et al. 1999)

Consequently, we looked for locations where WCPi can be observed flying at lower altitude, and went to the Dominican Republic for ten days (12th – 22nd July, 2019) to capture WCPis with mist nets. After obtaining the relevant

authorizations from local authorities (Appendix 1), captures took place on an island in Oviedo Lagoon (17.740575, –71.365890, Figure 15) by mounting three 3m-high mist nets of different lengths (3, 6 and 12m). In total 29 WCPis were captured and banded with alphanumerical bands provided by the CRBPO (*Centre de Recherches sur la Biologie des Populations d'Oiseaux, Muséum National d'Histoire Naturelle*). Detailed capture methods and biological sampling appear in Chapter 2 – Article 2). Among captured pigeons, the seven larger individuals were equipped with a GPS tag (Lotek Wireless Inc., PTT PinPoint GPS Argos Solar 6 grams; Photo 3). The protocol to attach the harness followed the method of Hill et al. (1999). Since captured pigeons weighed between 224 g and 268g, the system did not exceed 4% of each pigeon mass, which is the range of what is usually accepted for birds (Gaunt et al. 1997; Bridge et al. 2011; Kays et al. 2015). Every two days, GPS tags were programmed to locate pigeons every two hours between 6am and 6pm, and every other day, at 7am, 12pm and 6pm. In addition, one position was recorded every night; at midnight. Once deployed on pigeons, the device began to collect birds locations the morning following the capture. Thus, we expected to collect data for at least one year, athough theorically, battery devices should have lasted between two and three years with our programmation.

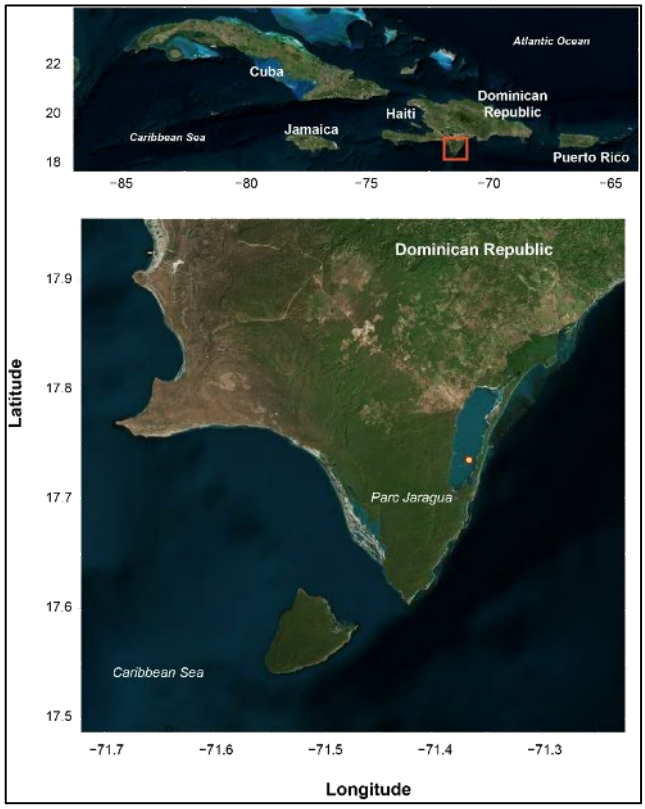


Figure 15: Aerial photograph of the capture location of WCPis in the Oviedo Lagoon, in Dominican Republic. Above is an aerial photograph of the Greater Antilles, in which is regionally located the Oviedo Lagoon.

However, we failed to reach our objectives as all GPS tags did not function for one year as expected (Table 4). We therefore investigated to what extent technical, biological or environmental reasons could explain such a failure by analysing the few data we collected. To that end, we used the R software and the R package *move* to conduct statistical analyses of movement data. GPS devices transmitted data during between one and 204 days, by providing between four and 1096 locations per WCPi (Table 4). When visualizing recorded locations on a map for PCB19-067, we can see that the ten days of data transmission were all located on an islet

right next to the islet where captures were performed (Figure 16). A second case of rapid loss of transmitting data were observed for PCB19-063, where the GPS device worked only for one day. The pigeon seems to have stayed on the islet after capture as showed by the first fix recorded the morning following the capture. However, other fixes were located in the water up to reach the coast for the last fix (Figure 16). These two individuals probably died soon after being equipped with the GPS device, suggesting they did not support GPS tags, the harness and/or the handling, or stress associated with the operation.

Table 4 : Information about WCPi equipped with a GPS tags. SD means standard deviation. Fix or fixes correspond to recorded location.

#WCPi	Capture date	Weight (g)	Wings length (cm)		Number of reception days	Number of fixes	Cumulated distance (km)
			Right	Left			
PCB19-041	2019/07/16	259	19.15	19.15	5	32	18.24
PCB19-044	2019/07/16	268	18.20	18.05	2	8	4.49
PCB19-054	2018/07/18	258	18.65	18.65	204	1096	1465.02
PCB19-063	2019/07/20	250	18.55	18.75	1	4	1.90
PCB19-064	2019/07/20	251	18.35	18.45	18	131	137.44
PCB19-067	2019/07/21	224	18.65	18.55	10	68	17.89
PCB19-068	2019/07/21	230	18.85	18.85	48	205	277.25
Min		224	18.20	18.05	1	4	1.90
Max		268	19.15	19.15	204	1096	1465.02
Mean (±SD)		248.57 (±15.98)	18.63 (±0.31)	18.64 (±0.34)	41.14 (±73.63)	220.57 (±392.76)	274.6 (±534.61)

In contrast, loss of signals for other pigeons was less sudden, such that failure might not be related to the rapid death of individuals caused by the tracking system, but rather a technical problem or particular event such as hunting (Photo 4). Indeed, flight distances between two successive fixes were stable over time, except for PCB19-054 and PCB19-044 for which flight distances tended to decrease over time (Figure 17 & 18). Regarding PCB19-044, the GPS device transmitted data for two days and recorded four fixes. The cumulated flight distance between fixes, estimated at 4.49km, was low (Table 4). When visualizing fixes on a map (Figure 19), we can see that the pigeon moved from the capture islet on the morning following the capture and moved towards the east shore of the Oviedo Lagoon. The last fix was recorded on November 22, 2019 at 9:25am, suggesting that the pigeon did not move from this area, or that fixes were only recorded when the pigeon was in this area. On Figure 17, the confidence interval of the coefficient of correlation rho is large due to the low number

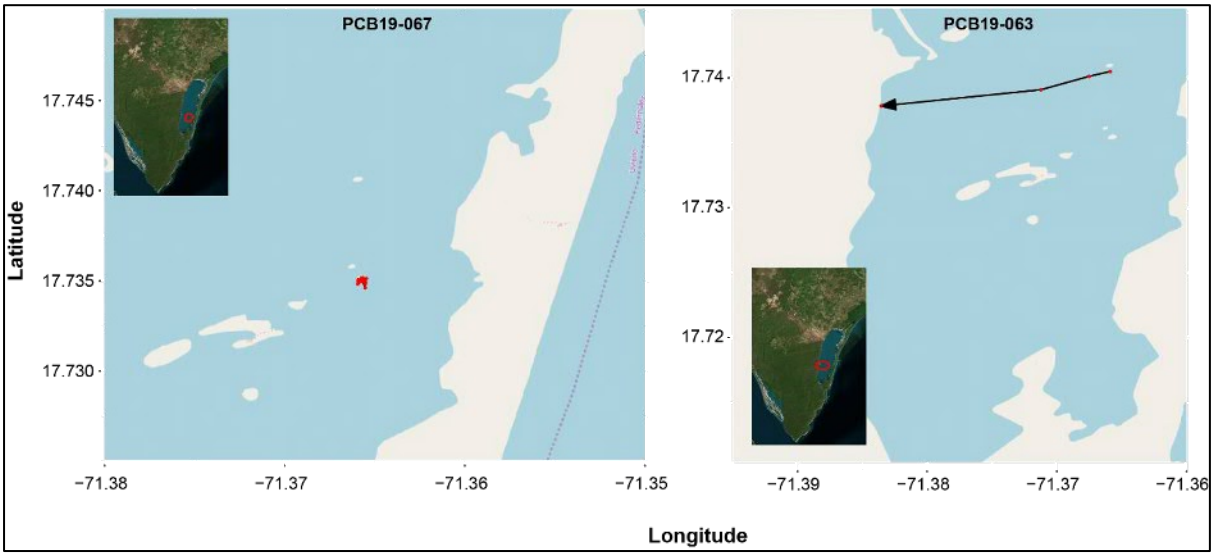


Figure 16 : Visualisation of locations recorded by GPS devices for PCB19-067 and PCB19-063. Framed maps represent the place at a wider scale. Red circles show broadly the distribution of fixes.

of fixes. In addition, the variability in fixes accuracy was also in part caused by the accuracy of devices (± 40m). In contrast, the PCB19-054 was the equipped pigeon for which data transmission lasted for the longest, with 1096 fixes recorded (Table 4). For this pigeon, a decrease of distance between two successive fixes were evidenced when considering all recorded fixes. However, data shown in Figure 18 indicate two distinct periods during which the activity of the individual differs. Indeed, the progression slope of these two parts are

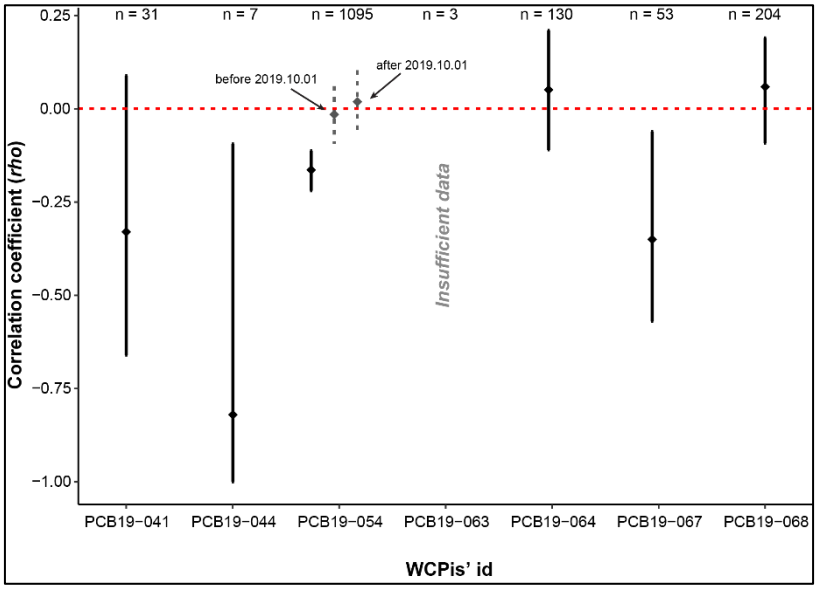


Figure 17 : Coefficient values of Spearman's correlation (rho) testing whether the distance between two successive GPS fixes remained stable over time. Error bars are 95% confidence intervals. When rho > 0, the distance increases over time and inversely when rho < 0. In contrast, when rho is not significantly different to zero, the distance over time is stable. "n" refers to the number of distance measures used. Regarding PCB19-054, three Spearman's correlation tests have been performed. In black is when considering all fixes, and in grey with dashed error bars is when considering dates indicated on the plot. See Figure 4 for visualizing the difference between these two periods.

different, suggesting that distances between the two successive points were longer before october 1st, 2019 than after (Figure 18). This was confirmed with a Wilcoxon's test (W = 117167, P < 0.0001; before (±SD)= 1.953 ± 2.855 km, after (±SD) = 0.705 ± 1.223 km). This is also visible on the map representing fixes (Figure 19). The pigeon seems to have occupied three distinct locations over the 204 days of GPS tracking. Among fixes recorded before the 1st october, the pigeon stayed around the Oviedo lagoon, venturing from time to time more into the mainland. At the time, the pigeon appears moving over longer distances. The same pattern is visible for PCB19-068 (Figure 20). After the 1st october, this individual spent more time in the mainland, but performed shorter distance flights (Figure 19). For the same individual, the acquisition of location data remained contant over time until october 2019. After this time, interruptions in data transmission started occurring. The same pattern of interruption in data transmission was observed in PCB19-068 (Figure 18). GPS devices then ultimately ceased to collect data, suggesting potential major technical issues.

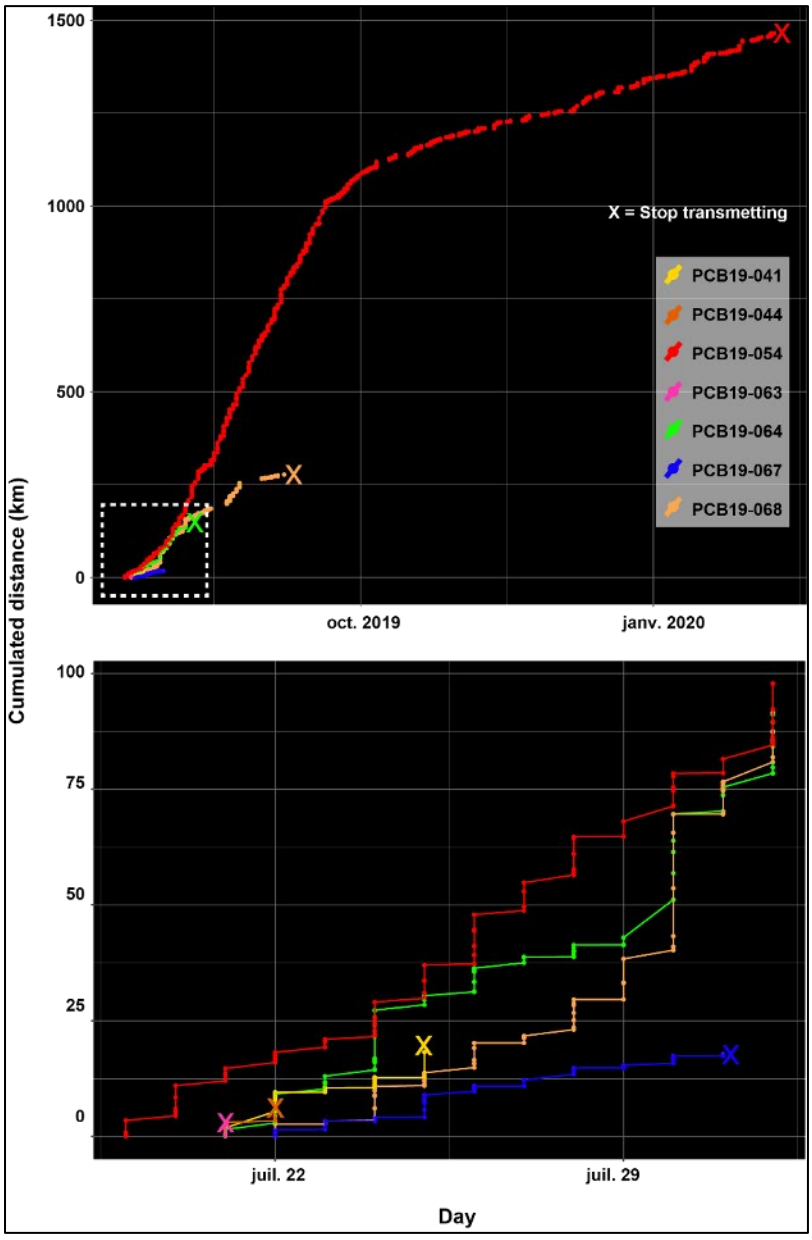


Figure 18 : Cumulated distance over time for all equipped pigeons. The plot below is the enlarged visualisation of curves located within the dashed frame in the top graph.

The case of the PCB19-068 is particular. Indeed, this pigeon was shot by a local hunter in April 2021 and we were able to recover the alphanumerical band and the GPS device (Photo 5). Interestingly, this GPS device stopped transmitting data since the 7th septembre 2019, that is one year and a half before the pigeon was shot. Moreover, when



Photo 4 : Hunting chart for one morning of hunting in Dominican Republic close to the Lagoon of Oviedo. The photo was taken just after having deployed GPS devices.

directly connecting the device into a computer, tentatives of data collection seem to have occurred until the 27th september 2020, that is more than one year after the last data transmission and seven months before the pigeon was shot. This case strongly supports the hypothesis that both hunting and technical issues are responsible to the loss of signals of our GPS device.

Although there are contradicting studies regarding the impact of such tags on the fitness of birds in long term, it is admitted that cumulated acts may induce, at least at short term, a decrease of fitness (Barron et al. 2010). For instance, the Blomberg et al. (2018) showed in a review on game birds that 65% of studies using satellite or radio tracking devices used threshold indicators to consider or not whether an equipped bird is dead so as to exclude it from their movement analyses when appropriate. In addition, in the Ruffed Grouse (*Bonasa umbellus*), they showed that mortality risk was higher during the first week following the release than afterward and that this mortality risk could be decreased by refining methods (i.e., capture, handling, birds retention…) and limiting cumulated stress in species (e.g., Nicolás Francisco et al. 2022). Similarly, high risk of mortality following capture, banding or tagging was observed in other game birds such as sand grouses (Casas et al. 2015), pyrenean western capercaillies (*Tetrao urogallus aquitanicus*, Nicolás Francisco et al. 2022), red-legged partridges (*Alectoris rufa*, Höfle et al. 2004) or Little Bustards (*Tetrax tetrax*, Marco et al. 2006). Regarding the WCPi, location data recorded with GPS devices deployed on the PCB19-063 and PCB19-067 suggest either pigeons did not support handling or the tracking equipment, or long-lastig effect of handling and processing. The WCPi is indeed extremely sensitive to capture and handling (Meyer & Wilmers 2007), sometimes displaying tonic immobility.

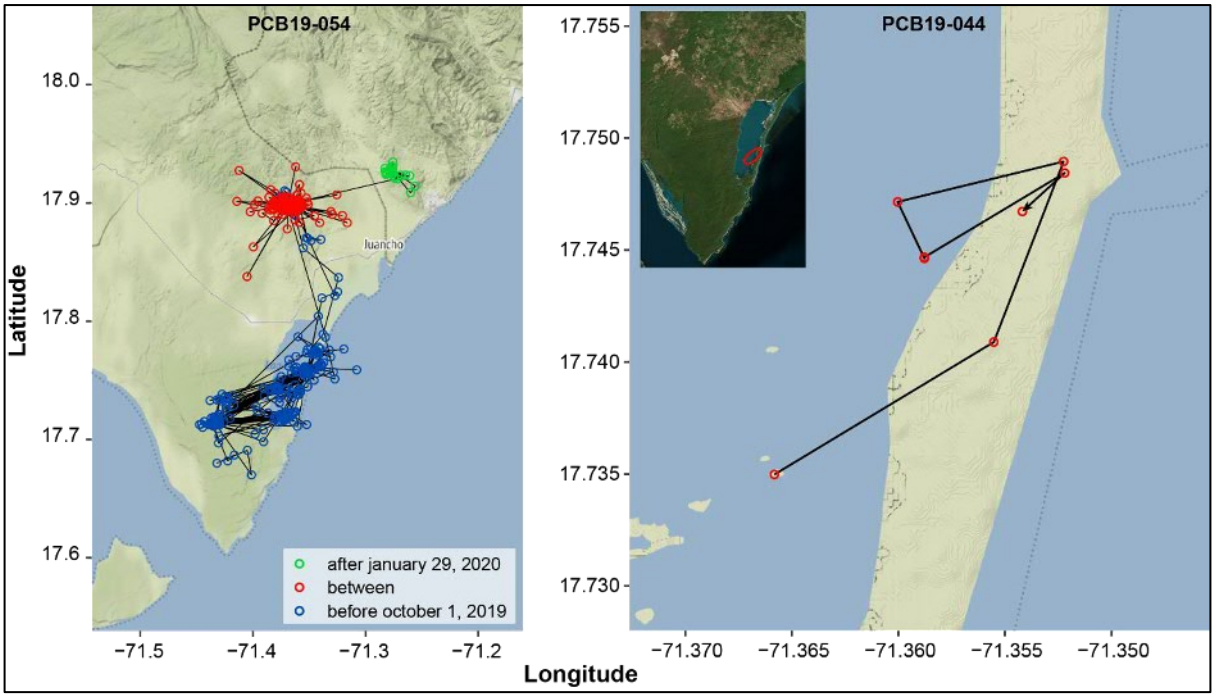


Figure 19 : Visualisation of locations recorded by GPS devices for PCB19-054 and PCB19-044. Framed maps represent the place at a wider scale. Red circles show broadly the distribution of fixes

Although other GPS devices, except for PCB19-054 and PCB19-068 that survived and transmitted data for several weeks, ceased to transmit data relatively rapidly, we inclined to think that this did not result from the rapid death of individuals as they initially performed movements quite similar to PCB19-054 and PCB19-068, suggesting they were not particularly handicaped by the appparatus. Moreover, a study on feral pigeons (*Columba livia domesticus*) showed that a tag weighing less than 6% of pigeons mass did not impacted the global activity of pigeons and their ability to fly (Tian et al. 2020), suggesting that the WCPi may support GPS tags we used. However, LeTourneux et al. (2022) showed that the act of marking birds and hunting activity acted synergistically in the risk of mortality of greater snow geese (*Anser caerulenscens atlantica*). Besides, in the Mourning Dove (*Zenaida macroura*), hunting activity tended to be associated with higher level of corticosterone and specifically with a decrease in foraging activity and body weight (Roy & Woolf 2001). Hence, we cannot exclude a potential synergistic effect of hunting and handling stress in our study of WCPis.

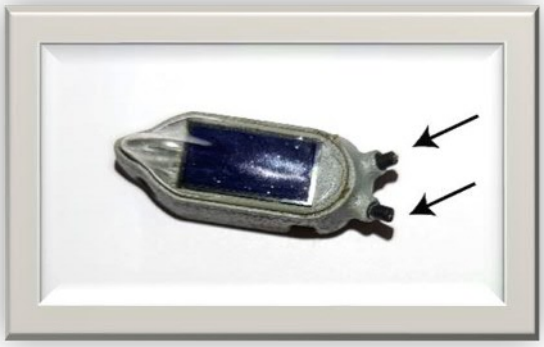


Photo 5 : The recovered GPS device that was deployed on the PCB19-068. Arrows indicate the location of the GPS and ARGOS antennas. They were probably cut by the hunter.

The loss of signals has often been reported in studies using satellite devices, with some never transmitting data after the release of individuals (*e.g.,* Bertram et al. 2016; Vardanis et al. 2016; Fuglei et al. 2017; Silva et al. 2017; Sullivan et al. 2018; Moore et al. 2019; Exo et al. 2019; Rühmann et al. 2019). For instance, Fuglei et al. (2017) reported 53% of dysfunctional Argos tags among the 29 deployed on rock ptarmigans, *Lagopus muta*. Out of 38 GPS-Argos tags (3.5g) deployed on three species of shorebirds weighing less than 300 g in Texas and New Jersay, data were obtained from only four tags (Scarpignato et al. 2016).

Some of authors suggest technical issues, either correspondong to mistakes during the preparation of devices or due to manufacturer defaults. Moreover, the loss of signals can be

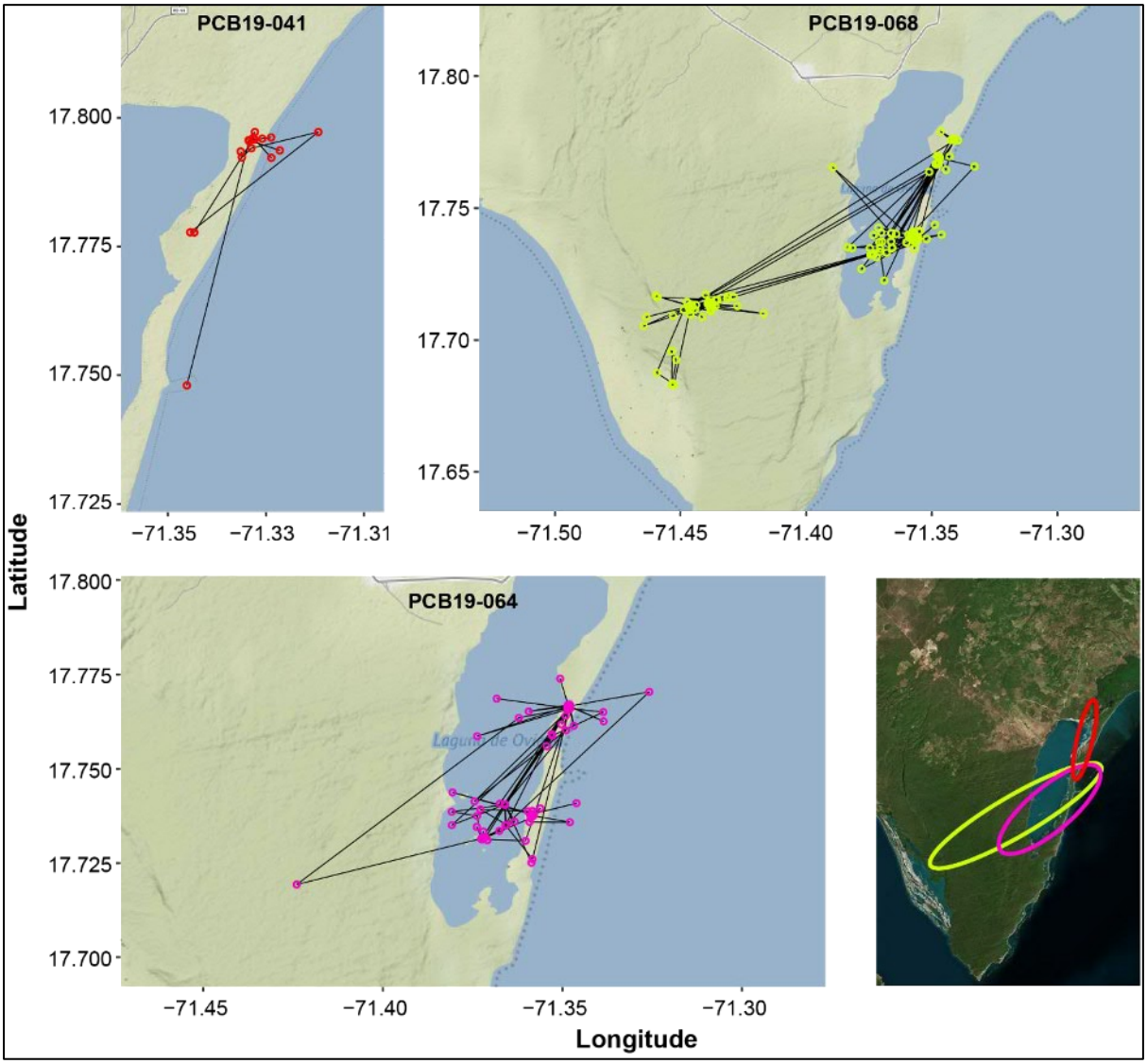


Figure 20 : Visualisation of locations recorded with GPS devices deployed on PCB19-041, PCB19-064 and PCB19-068. The aerial photograph, at the bottom right of the figure, represent the place at a wider scale. Circles show broadly the distribution of fixes for each individual according to the colour code.

also attributed to behavioural features of the studied species and its environement. For instance, arboreal species like the WCPi may induce failures in GPS tags due to the fact they spend considerable time under forest cover (Byrne et al. 2017; Hofman et al. 2019). This is particularly true when individuals are nesting as they do not move much, except for feeding. Under such conditons, the solar panel might not have been sufficiently exposed to the sun, and fix and data transmission may have rapidly drained the battery (Byrne et al. 2017; Hofman et al. 2019). In a review aiming at assessing the performance of satellite telemetry in wildlife, causes of failures were identified as, in order of importance : battery, mortality, electronics, mechanics or removal of tags by animals (Hofman et al. 2019). In addition, it appears that on average 1 out of 10 deployed tags ended prematurely for unknown or non-specified reasons. In the present study, thanks to the recovery of the device deployed on PCB19-068 and the feedback from Dominican colleagues, we could state that (i) GPS devices stopped working for unknown reasons, certainly due to technical issues, and (ii) that equipped pigeons were probably exposed to a high risk of mortality due to hunting activity in the region (Photo 4), although hunting is officially prohibited in the Dominican Republic. In accordance with this, the experience of the Avian Research and Conservation Institute (ARCI) succeeded in tracking movement of six WCPis in Florida, three in The Bahamas and Puerto-Rico, two in Cayman Islands and one in Jamaica (https://www.arcinst.org/tracking-studies/), islands where hunting is either well-regulated or strictly prohibited. They used ARGOS technology (less precise, Microwave Telemetry Inc.), instead of GPS like the present study, and had also signal loss with tags transmitting for 36 or 89 days, while other reached 1,800 days.

Therefore, in order to exclude any effects of GPS devices and harnesses on the behaviour of equipped WCPis, we planned an experimental test within aviaries of the breeding program managed by the Cayman Turtle Center in Cayman Islands. The aim was to score through videos particular behaviours like social behaviour, preening, foraging or any behaviour aiming at removing the GPS device or the harness. Unfortunately, the Covid crisis forced us to abandon this project for a while. However, before renewing the deployment of such satellite devices on WCPis, we would recommend performing a preliminary study in a controlled environment in order to be sure about behavioural responses of pigeons and refine methods to reduce potential negative effects on both equipped pigeons and collected data.

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A. Appendices

Appendix 1 – Study permit delivered by the Environment Ministry from Dominican Republic.

Ministerio de Medio Ambiente y Recursos Naturales
República Dominicana
"Año de la Innovación y la Competitividad"

26 ABR 2019

AUTORIZACIÓN

Por medio de la presente, El Ministerio de Medio Ambiente y Recursos Naturales autoriza a la UNIVERSIDAD DE LAS ANTILLAS, el desarrollo del proyecto de investigación denominado: "Estudio del Desplazamiento de la Paloma Coronita Blanca, *Patagioenas leucocephala*, en la Región del Caribe, Utilizando Herramientas Moleculares y Monitoreo de GPS-Argos".

La referida investigación será ejecutada en el municipio Oviedo, provincia Pedernales.

Esta investigación se desarrollará en el período comprendido entre mayo del 2019 y mayo del 2022.

Dada en la ciudad de Santo Domingo, Capital de la República Dominicana, a los veinte y dos (22) días del mes de abril del año dos mil diecinueve (2019).

Atentamente,

Ángel Estévez
Ministro de Medio Ambiente y Recursos Naturales

CHAPTER 4

Parasite status of the White-crowned Pigeon – Scanning for haemosporidians

ARTICLE 4

-

First estimates of prevalence and diversity of haemosporidian parasites in the Caribbean-endemic White-crowned Pigeon, *Patagioenas leucocephala*

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Draft manuscript

Introduction

Haemosporidians (or blood parasites) are intracellular protozoan parasites belonging to the order *Haemospororida*. They are transmitted by blood-sucking diptera to several terrestrial taxa, including birds (Atkinson et al. 1991; Valkiunas 2004). Haemosporidians are widespread and infect especially passerines, but also a broad range of other bird species (Atkinson 2008a, 2008b; Forrester & Greiner 2008). Birds can be infected by three genera of haemosporidians: *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (Valkiunas 2004). Recently, advanced genetic analyses have led to consider the genus *Haemoproteus* as polyphyletic with the identification the divergent *Parahaemoproteus* clade (Outlaw & Ricklefs 2014; Borner et al. 2016; Pacheco et al. 2018; Galen et al. 2018; Videvall 2019).

As often highlighted in host-parasite associations, haemosporidian infections can induce multiple fitness costs. For instance, several studies have evidenced a negative impact of haemosporidians on avian host reproductive performance, arising from a trade-off between energy allocation to reproduction (from sexual mate selection to parental care) and immune defences (Rätti et al. 1993; Hatchwell et al. 2001; Tomás et al. 2007; Knowles et al. 2009; Asghar et al. 2011; but see Zylberberg et al. 2015). In addition, although evidence for wild bird populations remains elusive, several studies provide evidence for a decrease in survival probability following infection with blood parasites (Martínez-de la Puente et al. 2010; Lachish et al. 2011). In particular circumstances, as in case of naïve populations or captive birds, mortality may be directly induced by the pathogenicity of haemosporidian parasites (Samuel et al. 2011; LaPointe et al. 2012; Meister et al. 2021). On the other hand, in most cases, mortality induced by haemosporidians is considered as indirect, for instance through making hosts less vigilant and/or less reactive when exposed to predators (Møller and Nielsen 2007, Garcia-Longoria et al. 2015)(Møller & Nielsen 2007; Garcia-Longoria et al. 2015).

The family *Columbidae* includes 369 pigeon and dove species that are particularly threatened by habitat degradation, invasive species and hunting (Winkler et al. 2020; BirdLife International 2021). As a result, of all columbid species which are listed in the IUCN Red List about 34% are considered as at risk (NT, VU, EN and CR; Winkler et al. 2020; Walker 2007; Cambrone et al. in prep.). However, there are very few studies on infection with blood parasites in wild columbid populations (see however Yanga et al. 2011; Schumm et al. 2021),

excepted for the feral pigeon (*Columba livia domestica*, e.g., Amaral et al. 2017; Nebel et al. 2020).

In particular, although a rich literature exists on avian blood parasites in the Caribbean region, data for Caribbean-endemic columbids remain limited or anecdotical (e.g., Maria et al. 2009; Ricklefs et al. 2016). In this context, we investigated the prevalence and the diversity of haemosporidian lineages in five different populations (Cuba, Jamaica, Dominican Republic, Guadeloupe and Martinique) of the Caribbean-endemic White-crowned Pigeon (henceforth WCPi, *Patagioenas leucocephala*). The WCPi, is considered as near-threatened in the IUCN Red List, with a global population size estimated at 550,000 mature individuals. The species is particularly threatened with habitat loss, invasive species, and intense hunting and poaching pressure (Hay 2008; Latta et al. 2010; Latta 2012; Rivera-Milán et al. 2016; Bancroft et al. 2020; BirdLife International 2020).

Material and methods

Species and study area

This WCPi is endemic to the Caribbean region, with its natural range extending from the Florida Keys (U.S.A.) and Bahamas to Saint Lucia, with some minor populations on the coast of Central America (Gibbs et al. 2001; Bancroft et al. 2020). In the Greater-Antilles, where populations are the most abundant, the species usually moves, forages and breeds in large flocks including more than 100 individuals, whereas in the Lesser-Antilles the species is often observed in smaller flocks, sometimes just in pairs (C. Cambrone personal observation, Bancroft et al. 2020). The species is considered as vagrant, moving among the different islands of its distribution area in response to resource availability and suitable environmental conditions (Wiley & Wiley 1979; Wiley 1979; Rivera-Milán 1992; Bancroft et al. 2000; Strong & Johnson 2001; Meyer & Zimmerman 2006; Florida Fish and Wildlife Conservation Commission 2013). This frugivorous, arboreal pigeon mainly inhabits coastal and lower-lying forests such as mangroves, swamp forests and dry evergreen forests (Gibbs et al. 2001, Bancroft et al. 2020). However, on some islands individuals can be observed in urban areas consuming fallen fruits on beaches or in private gardens, sometimes in mixed-species flocks with other common urban columbid species such as Zenaida doves (*Zenaida aurita*) or feral pigeons (*C. livia*; A. Levesque pers. com., Bancroft et al. 2020).

Samples collection and DNA extraction

Overall, we screened 171 WCPi biological samples collected between 2016 and 2019 on five different Caribbean islands (Fig 1). The majority of them consisted of post-mortem tissue samples (toe-clips), collected from local hunters on all sampled islands (Fig. 1). Tissue samples were stored in a buffer storage consisting of 70% ethanol and 30% Tris-EDTA. In addition, we collected 29 blood samples by puncturing the brachial vein of live WCPi (Owen 2011) captured in the Dominican Republic using mist nets (see Cambrone et al. 2022 for details). Blood samples were immediately stored in absolute ethanol.

Before DNA extraction, we transversally cut a 1mm-wide piece of toe clip that we dried using clean wipe to remove storage buffer. We then extracted DNA from toe clips using the Qiagen DNeasy Blood & Tissue commercial kit, following standard protocols for tissue with minor adjustments and an overnight initial lysis. For blood samples, we collected a piece of blood clot weighing about 20.82 ± 9.15 mg (mean ± S.D.) with a sterile spatula as blood precipitates in contact with absolute ethanol. Before performing the overnight lysis, we twice rinsed the blood clot with 500 µL of PBS in order to remove all storage ethanol. To do so, each time we added PBS, we vigorously vortexed the tube and discarded the supernatant after centrifugation for five minutes at high speed (13,500 rpm).

Parasite detection

We estimated the prevalence of haemosporidians in all samples by amplification of a 188-bp fragment of ribosomal RNA using R330F and R480RL primers (Bell et al. 2015). As a positive control, we used three DNA solutions extracted from blood collected on Zenaida doves (*Zenaida aurita*) containing haemosporidians checked by microscopy (F. Cézilly unpublished data). We also used two types of negative control to assess cross-contamination and false amplifications during the experiment: DNA extracted from WCPi buccal swabs and sterile water. The detection of parasites was performed twice, by randomizing samples positions in PCR plates. Before performing polymerase chain reactions, all material were sterilized in an UV chamber. PCR mixes were performed in 15 µL, including 30 ng of DNA, 1 µg/µL of Bovin Serum Albumin (BSA), 0.200 mM of each dNTP, 0.400 µM of each primer, 0.375 U of Taq DNA polymerase (Qiagen Inc.) and 1X of Qiagen PCR buffer. Temperature cycles began by an initial

denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 45 sec, 60°C for 45 sec, 72°C for 30 sec, and ended by a final extension at 72°C for 10 min. The presence or absence of haemosporidians were visualised after electrophoresis on a 2% agarose gel.

Lineages identification

To determine the lineage of haemosporidians infecting WCPis, we amplified a region of the cytochrome b gene (cyt b) through a nested PCRs as described by Hellgren et al. (2004). The first PCR reaction was performed using the primer pair HaemNF1 and HaemNR3, and two nested PCR were performed using HaemNF1/HaemNR3 and HaemF/HaemR2 to respectively amplify the cyt b region of (*Para*-)*Haemoproteus*/*Plasmodium* and *Leucocytozoon* genus (Hellgren et al. 2004). All PCR reactions included 0.500 µg/µL of BSA, 0.200 mM of each dNTP, 0.600 µM of the primer, 0.025 U/µL of Taq DNA polymerase (Qiagen Inc.) and 1X of Qiagen PCR buffer. We performed the first PCR reactions with 30 ng of DNA in a final volume of 15 µL. We then used 1µL of the first PCR for the second round of the nested PCR process, in a final volume of 28 µL. The thermal profile of the first PCR corresponded to an initial denaturation at 95°C for 5 min, followed by 25 cycles of 95°C for 45 sec, 50°C for 45 sec, 72°C for 45 sec, and ended the process by a final extension at 72°C for 10 min. The nested PCRs included 35 cycles and were performed using the same temperature cycles. In addition, we performed another nested PCR using HaemNF1/HaemNR3 and HaemFL/HaemR2L following the same protocol to amplify cyt b sequences of the genus *Leucocytozoon*. We assessed the quality of amplifications on a 2% agarose electrophoresis gel. Well-amplified samples were externally sequenced by Eurofins Genomics (Germany) according to the BigDye Terminator v3.1 cycle sequencing kit protocol from ABI (Applied Biosystems 3730x).

Electropherograms were analysed using Geneious Prime (Kearse et al. 2012) and, forward and reverse sequences were aligned with a reference sequence of *Haemoproteus columbae* found in MalAvi database (COLIV03; Bensch et al. 2009) and GenBank (accession code: MT802175). We relied on BLASTN 2.3.0+ (Zhang et al. 2000) program to see to what extent our sequences matched with known haemosporidian lineages deposited in MalAvi database. We considered sequences with at least one nucleotide difference against sequences deposited in MalAvi database as new haemosporidian lineages and deposited them in GenBank.

Phylogenetic and statistical analyses

We relied on a lineage network using the minimum spanning network method implemented in PopART 1.7 (Leigh & Bryant 2015) to see to what extent lineages are spatially distributed. In addition, we calculated Φst using Arlequin 3.5, with 50,000 permutations and 5% nominal level that was adjusted for multiple comparison with Benjamini-Yuketieli step up procedure (Excoffier & Lischer 2010), to estimate molecular distance between populations based on the number of differences between lineages (analogous to Fst).

We constructed a bayesian phylogenetic tree using BEAST 1.8.4 (Drummond et al. 2012) to ascertain phylogenetic relationships between the new lineages found in this study and other known lineages. To that end, we selected the best-fit model of DNA sequence evolution using the program implemented in MEGA version X ((Tamura et al. 2011), which lead us to consider the General Time Reversible model including invariable sites and variations among sites (GTR+I+G; Gu et al. 1995) as the best model according to the Akaike information criterion. We used the program BEAUTi 1.8.4, associated to BEAST, to define tree priors with strict clock and a Yule speciation process (Yule 1925; Gernhard 2008). We set the Markov chain Monte Carlo simulations with 10,000,000 generations and one tree was recorded every 1000 generations.

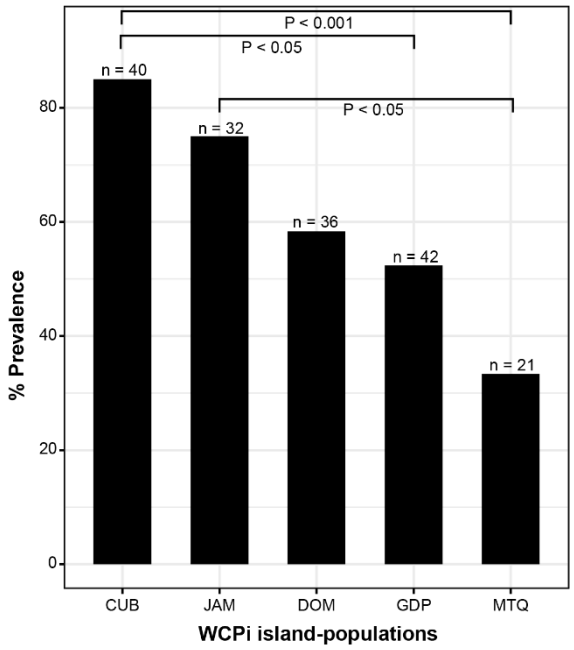


Figure 21 : Within-island population prevalence of haemosporidian. P values correspond to significant pairwise fisher' exact test.

Results

On the 171 WCPi screened, we detected haemosporidians using both blood and tissue samples, without any difference in term of detection rate (Fisher's Exact test, P = 0.205, Odd-ratio [95%CI] = 1.765 [0.725; 4.286]). The overall prevalence, regardless of island populations, was estimated at 63.16% (108 positives vs. 63 negatives). At the scale of island populations of WCPi, the prevalence of haemosporidians was 85.00% in CUB, 75.00% in JAM, 58.33% in DOM, 52.38% in GDP and 33.33% in MTQ, with a consistent decrease along a north-south gradient (Figure 21). In addition, the within-populations prevalence was positively correlated with the within-islands number of columbid species (n= 14, 12, 11, 10 and 9 different species of Columbidae in CUB, JAM, DOM, GDP and MTQ according to Birdlife International; Kendall's correlation test, T = 10, P = 0.0167).

Among positive samples, we were able to sequence the cytochrome b gene of parasites of 47 samples from CUB, JAM, DOM and GDP (Table 5), corresponding to 43.52% of positive samples. The success rate in producing a *Cyt b* sequence from positive samples was significantly higher for blood samples (42.31%, n=26) than for tissue samples (27.91%, n= 129; Mc Nemar's test, $\chi^2 = 64.650$, $P < 0.0001$). However, in the DOM population, the sequencing success did not differ between blood (37.93%, n = 29) and tissue positive samples (28.57% n = 7; Fisher's exact test, P = 1). In addition, the sequencing success from positive samples did not differ among populations (Chi² test, $\chi^2 = 1.925$, P = 0.588; GDP = 61.91%, CUB = 50.00%, DOM = 61.91% and JAM = 29.17%).

Table 5 : Occurrences and prevalence of haemosporidians per lineage within sampled island-population of WCPi. Lineage beginning by PATLEU were evidenced during this study. Diversity refers to the diversity of genetic lineages (similar to haplotype diversity).

Lineages	GDP	DOM	CUB	JAM	Total/lineage
PATLEU01	10 (100%)	9 (69.23%)	10 (58.82%)	1 (14.29%)	30 (63.83%)
PATLEU02		1 (7.69%)		2 (28.57%)	3 (6.38%)
PATLEU03			1 (5.88%)		1 (2.13%)
PATLEU04			1 (5.88%)		1 (2.13%)
PATLEU05		1 (7.69%)	5 (29.41%)	3 (42.86%)	9 (19.15%)
ZEMAC17		2 (15.38%)			2 (4.26%)
PATLEU07				1 (14.29%)	1 (2.13%)
Diversity	0.000 ± 0.000	0.526 ± 0.153	0.596 ± 0.099	0.810 ± 0.130	0.870 ± 0.025
Total/island	10	13	17	7	47

Among the 47 sequences, we found seven different lineages of haemosporidians, including 6 new lineages (Table 5, Figure 22 and 23). According to blasting results and phylogenetic tree of haemosporidians built using sequences already known to infect columbid species (Figure 22), WCPi were infected by two *Plasmodium* lineages that had not been reported before in any avian species (PATLEU03 and 04), which were both related to a lineage previously found in another *Columbidae*, the White-tipped Dove, *Leptotila verreauxi,* in Brazil. We also discovered three new lineages of *Haemoproteus* (PATLEU01, 05 and 07). PATLEU01 and 05 belong to a monophyletic lineage related to lineages of *H. columbae*, also found in the congeneric Band-tailed Pigeon, *Patagioenas fasciata*, in Peru. In contrast, PATLEU07 forms a monophyletic group with *Haemoproteus* lineages already found in the Mourning Dove, *Zenaida macroura* (ZEMAC05) in Mexico, the Eared Dove, *Z. auriculata* (ZENAUR01, 02 and 03) in Argentina, and the Zenaida Dove, *Z. aurita* (ZEAUR05; unknown origin). ZEMAC05 was identified as *H. multipigmentatus*, suggesting that PATLEU07 is another lineage of this species. Finally, among our samples, WCPis were also infected by two *Parahaemoproteus* lineages (ZEMAC17 and PATLEU02). ZEMAC17 has been already identified in mourning doves, sampled in the U.S.A and in Argentina, and was identified as *Parahaemoproteus sacharovi*. With the new PATLEU02 and MODO1 (sampled in the U.S.A. in a Mourning Dove), they formed a monophyletic group of *Parahaemoproteus*, suggesting that PATLEU2 is a *Parahaemoproteus sacharovi*, like ZEMAC17.

Only a single lineage was found in GDP (PATLEU01), which seems to corresponds to *H. columbae* (Table 5 and Fig. 23). This lineage was also the most prevalent in other island populations of WCPis, excepted in the JAM population where it was the second most prevalent. In contrast to GDP, other sampled island populations of WCPi harboured each four different lineages, with lineage diversity values varying between 0.526 ± 0.153 and 0.810 ± 0.130. The lineage called PATLEU05, which also seems to be a *H. columbae,* was found in DOM, CUB and JAM, where it is among the least prevalent in DOM (7.69%), and among the most prevalent in CUB (29.41%) and in JAM (42.86%). In this respect, PATLEU01 and PATLEU05 were the most prevalent when considering all island populations of WCPi (63.86% and 19.15%, respectively). In contrast, PATLEU03 and PATLEU04 were exclusively found in the CUB population, and PATLEU07 and ZEMAC17 were exclusively found in JAM and DOM

populations, respectively (Table 5 and Figure 23). As a result, they were the least represented in overall prevalence (Table 5).

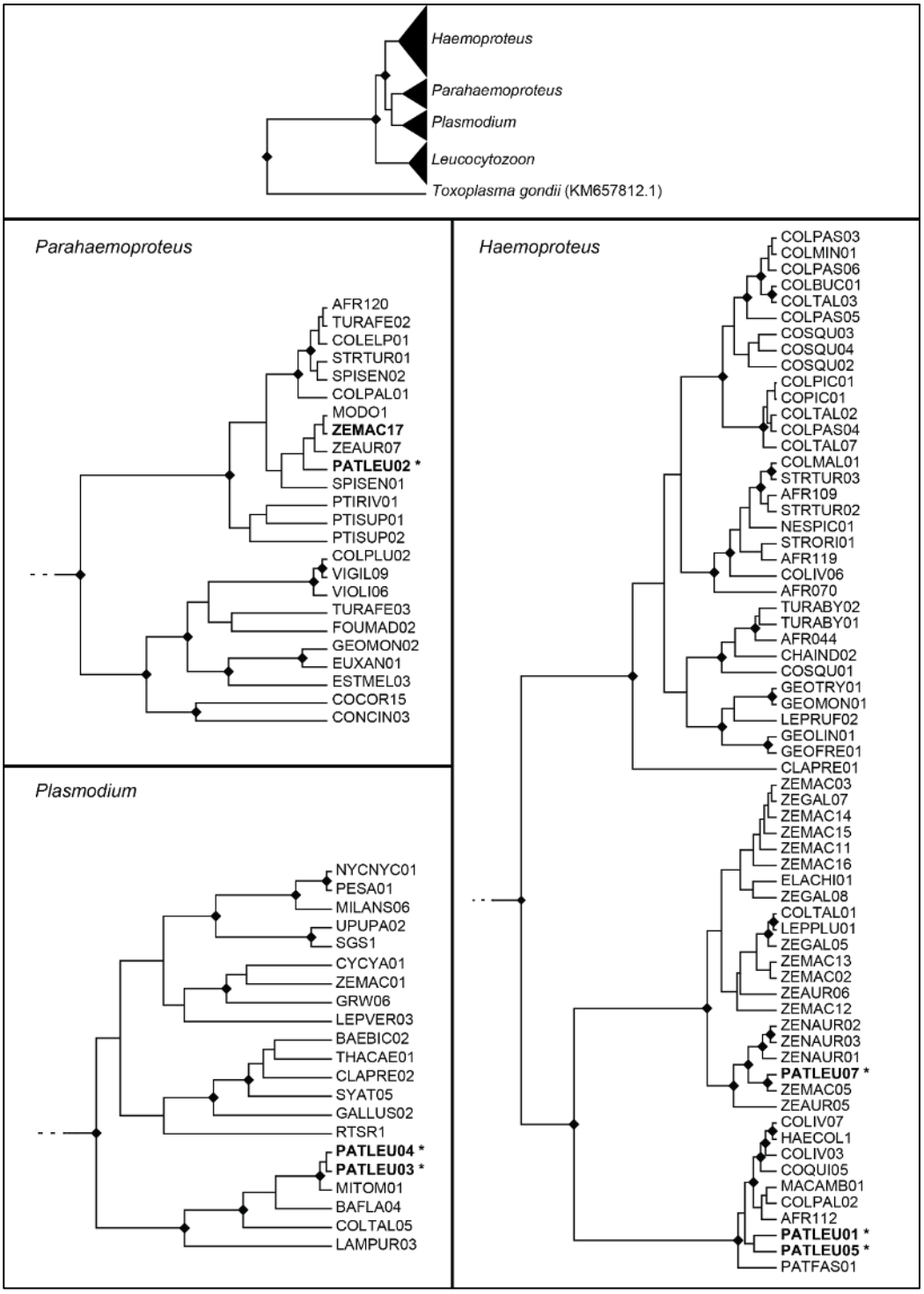


Figure 22 : Phylogeny of columbid haemosporidian based on cytochrome b gene lineages (476 bp) and bayesian phylogenetic tree construction method. In bold are lineage found in the present study. Asterisks indicate lineage considered as new. Node with black square were supported at ≥ 90%

Discussion

Our study is the first to document the prevalence and diversity of haemosporidians among populations of WCPis. We detected blood parasites through PCR using DNA from both blood samples, as commonly performed, and tissue samples obtained, for the first time for a bird species (to the best of our knowledge), from toe clipping. DNA obtained from toe clipping has however been previously used to identify apicomplexan blood parasites in amphibians (Seabra-Babo et al. 2015). The successful identification of haemosporidian parasites from toes of WCPi is not quite surprising given the high blood vascularisation of bird's feet used for regulating body temperature (Bernstein 1974; Midtgård 1982). Since our protocol included positive and negative controls and was performed twice regarding the detection of parasites, we are confident about the reliability of our results. Our findings therefore suggest that this type of tissue might be used to estimate prevalence of haemosporidians, especially from game species where such tissue samples are easy to collect from hunters. Regarding sequencing success, which is mainly affected by the quality and concentration of DNA extract (Freed & Cann 2006; Cambrone et al. 2022), results were more ambiguous. Considering samples of all island populations, we succeeded to sequence the Cyt b gene (~476 bp) significantly more times from infected DNA blood samples than infected DNA toe samples. However, when comparing the sequencing success rate among island populations, there were no significant differences, whereas the DOM population mainly consisted of blood samples (80.56%). Further investigations are necessary to compare the quality of the two types of samples, possibly by collecting both blood and toe samples on the same individuals, and performing paired statistical tests.

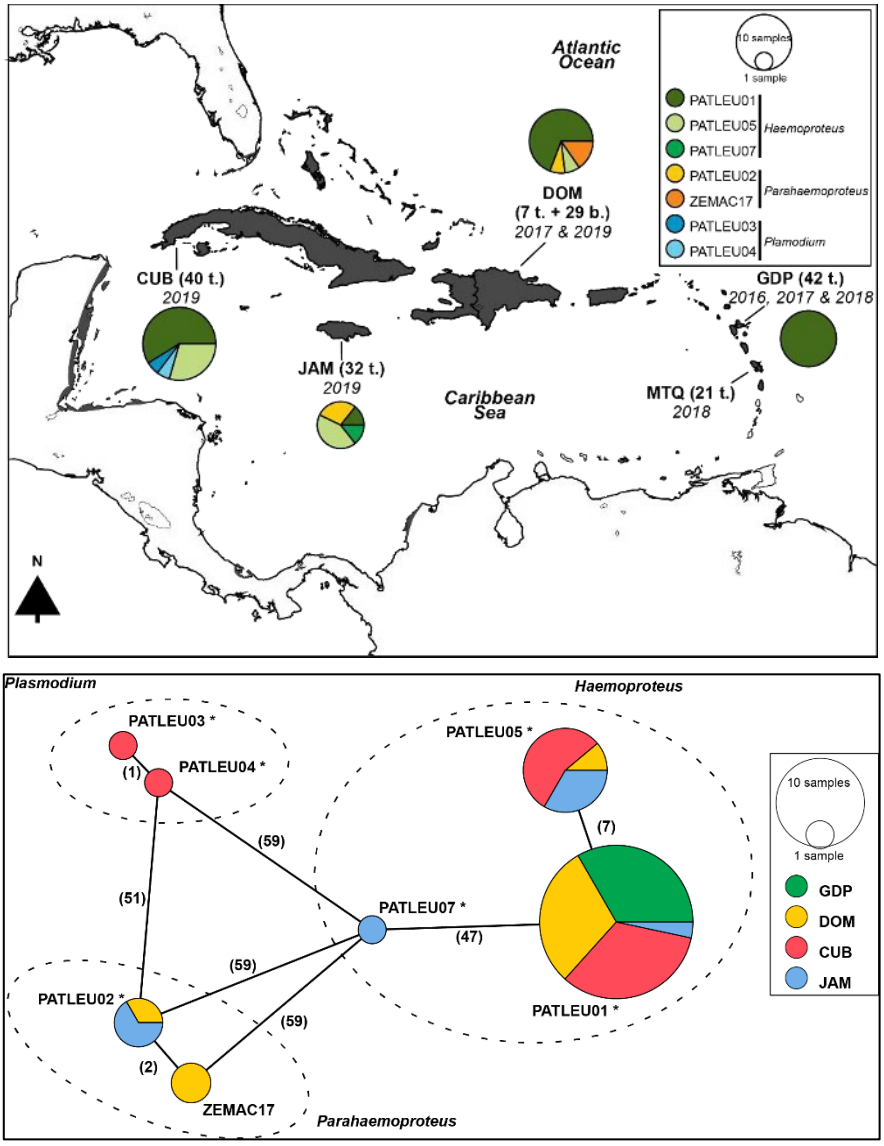


Figure 23 : Geographic distribution (above) and haplotype networks (below) of haemosporidian lineages found in sampled island populations of WCPis. Haplotype network is a median joining network constructed with the 47 Cyt b sequences of haemosporidians.

Both the overall prevalence of blood parasites and its variation among island populations are in accordance with previous studies that included columbid species in their sampled bird community. For instance, in the Caribbean region, the prevalence of infection with haemosporidians in the Common Ground Doves, *Columba passerina*, varied between 33% and 64% among Bahamian islands (Soares et al. 2020b), and reached 88% in the Dominican Republic (Latta & Ricklefs 2010). The prevalence was 74% on Socorro Island, compared to 92% in sympatric mourning doves (Carlson et al. 2013). In another archipelago context, the percentage of endemic Galapagos Doves, *Z. galapagoensis*, infected with blood parasites varied between 36% and 100% among the Galapagos islands (Santiago-Alarcon et

al. 2008). Interestingly, the within-island prevalence of haemosporidians in WCPi appeared to decrease along north-south gradient in the Antillean arc, with higher prevalence in the Greater Antilles (CUB, JAM and DOM) compared to the Lesser Antilles (GDP and MTQ). Several hypotheses can explain the geographical distribution of haemosporidians (e.g., Ricklefs et al. 2011; LaPointe et al. 2012; Cornuault et al. 2013; Padilla et al. 2017; Gutiérrez et al. 2019). Most of them consider factors influencing the probability of interactions between vectors (i.e., blood-sucking diptera) and birds. For instance, density of hosts and/or vectors, proximity to water (habitat of main vectors like mosquitoes), temperature and host immunocompetence are known to influence prevalence of haemosporidians in birds (LaPointe et al. 2012; Soares et al. 2020a). Other factors, more related to human activities, include habitat fragmentation and alteration, or agriculture, which may facilitate the geographic and demographic expansion of vectors, and hence increase the probability of transmission to birds (LaPointe et al. 2012; Laurance et al. 2013; Pérez-Rodríguez et al. 2018). In addition, although there are conflicting results according to the bird community studied, migratory birds tend to have a higher prevalence and diversity of blood parasites than resident or short-distance migratory species (Soares et al. 2020a; Schumm et al. 2021; but see Soares et al. 2020b). Regarding the WCPi, the species is more abundant in the Greater Antilles than in the Lesser Antilles. In addition, some WCPi populations might be nomadic as the species is seasonally present in the Lesser Antilles, whereas it is a year-round resident in the Greater Antilles (Delcroix et al. 2016; Cambrone et al. 2021). This is further suggested by observations of seasonal fluctuations in abundance in the Cayman Islands, where the species is present all year-round (Bancroft et al. 2020).

Spatial variation in the prevalence of infection with blood parasites in a given bird species might also be influenced by the number of potential host species present at the community level (Ricklefs et al. 2005). In addition, some bird species seem to be less subjected to infections by haemosporidians than others (Combes 1997; Fallon et al. 2005; Garcia-Longoria et al. 2019), and some haemosporidian lineages are more or less specific to some bird taxon (Silva-Iturriza et al. 2012; Garcia-Longoria et al. 2019, but see (Bensch et al. 2000; Ricklefs & Fallon 2002). In particular, *H. columbae*, *H. multipigmentatus* or *P. sacharovi*, are known to specifically infect columbid species (Valkiunas 2005; Santiago-Alarcon et al. 2010).

Interestingly, although the sample size is low (n = 5), we found that the within-population prevalence was positively correlated with the number of different columbid species recorded for the different sampled islands. Additional data form other Caribbean islands are needed to confirm this pattern.

In total, we identified seven different lineages, out of which, to the best of our knowledge, six had never been reported before. All lineages seemed to be more or less phylogenetically related to lineages already found in New World pigeons and doves, as defined by Johnson & Clayton (2000). Indeed, the columbid specific lineages of *H. columbae* were largely identified in all sampled populations of WCPi, corresponding to 82,98% of prevalence among sequenced samples. Then, the second most prevalent, with 10.64% of prevalence among sequenced samples, was a *P. sacharovi*, while 4.26% of sequenced samples were identified to be infected by *Plasmodium* lineages. This is not surprising as *Haemoproteus* and *Parahaemoproteus* genus are widespread within columbid species, whereas *Plasmodium* genus is often poorly represented (Križanauskienė et al. 2013; Carlson et al. 2013; Schumm et al. 2021; Salazar-Borunda et al. 2022).

The WCPi, considered as near-threatened or endangered in some islands (UICN et al. 2012; BirdLife International 2020), is particularly affected by massive habitat loss and alteration, invasive species and intense hunting and poaching pressure (Hay 2008; Latta et al. 2010; Latta 2012; Rivera-Milán et al. 2016; Bancroft et al. 2020). Like for most of Caribbean species, it is also facing threats induced by climate change (Courchamp et al. 2014). Such threats may affect infection with haemosporidians, either by making haemosporidians more prevalent and/or diversified within populations, and/or by reducing the fitness of infected individuals (Harvell et al. 2002; Laurance & Useche 2009; Garamszegi 2011). Indeed, habitat loss and alteration have been shown to increase the prevalence of haemosporidians in birds (Pérez-Rodríguez et al. 2018; Gao et al. 2019). In addition, climate change and invasive species (i.e., new hosts), especially invasive columbid species such as *Columba livia* or *Streptopelia decaocto* for the WCPi, may contribute to the spatial expansion of haemosporidian species and lineages and that of other pathogens (Lennon et al. 2013; Panella et al. 2013). Therefore, monitoring levels of infection with haemosporidians among Caribbean islands in the WCPi and other sympatric columbid species and assessing fitness and/or demographic consequences might prove useful in the future.

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END OF ARTICLE 4

CHAPTER 5

Populations genetics and gene flow in the White-crowned Pigeon & the Scaly-naped Pigeon

ARTICLE 5

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Levels of genetic differentiation and gene flow between four populations of the Scaly-naped Pigeon, *Patagioenas squamosa*: implications for conservation

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Levels of genetic differentiation and gene flow between four populations of the Scaly-naped Pigeon, *Patagioenas squamosa*: implications for conservation

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ABSTRACT

Island-endemic columbid species are particularly vulnerable to environmental degradation, extreme climatic events, and interactions with exotic species. The situation might be even more critical in the case of exploited species, where legal hunting and poaching can severely affect population dynamics. Here we document for the first time the genetic structure of the Scaly-naped Pigeon, *Patagioenas squamosa*, a Caribbean-endemic columbid species of cynegetic interest, over a large part of its range. Using both mitochondrial DNA and nuclear markers (microsatellites), we investigated gene flow, genetic diversity, and genetic structure among four islands populations originating from Puerto-Rico, Guadeloupe, Martinique and Barbados. We found evidence for a significant genetic differentiation only between the Barbados and the three other populations, consistent with the fact that the Barbados population originated from a few captive individuals escaped from a rooftop aviary in Bridgetown about 100 years ago. Given the absence of genetic differentiation between Puerto Rico and the French Antilles, our results suggest that, apart from Barbados, the species may mainly consist of a single large, homogeneous population. We discuss the relevance of our findings in relation to management and conservation issues.

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Introduction

It is now widely accepted that genetic diversity is crucial for the persistence of wildlife populations as low diversity results in both low individual fitness and reduced adaptability of populations in the face of environmental changes (Frankham 2005; Evans & Sheldon 2008). Accordingly, over the last 30 years, molecular and conceptual tools from population genetics and phylogeography have been increasingly used in conservation biology (Soulé & Mills 1998; Hedrick 2001; Russello et al. 2020). Population genetics and phylogeography are particularly helpful to determine the evolutionary and geographic boundaries of species, subspecies and populations (Haig et al. 2006, 2011; Garnett & Christidis 2007; Haig & D'Elia 2010). Defining such units is of prime importance to assess extinction risk, to prioritize conservation actions and identify the relevant spatial and temporal scales at which they should be conducted (Haig et al. 2011). This is especially true for species occurring in archipelagos, for which different populations may face different selection pressures because of contrasted anthropogenic, biotic or abiotic influences between islands (Clegg et al. 2002; Illera et al. 2007; Hoeck et al. 2010).

In addition, island endemic species are often characterized by restricted habitat range and smaller population sizes resulting in lower genetic diversity than mainland species, such that they are more exposed to inbreeding depression and, ultimately, face a higher risk of extinction (Frankham 1998, 2005; Brooks et al. 2002). Consequently, ecological disturbances and environment changes that are globally occurring, such as deforestation, urbanization, introduced pathogens, and competition and predation pressures from invasive species, particularly affect island endemic species (Walsh et al. 2012; Treglia et al. 2013; Ferrer-Sánchez & Rodríguez-Estrella 2014; Ortega et al. 2015; Dornburg et al. 2016; Palmas et al. 2017; Turvey et al. 2017). This is particularly true of bird species, with 80% of all avian species having become extinct since 1600 being island endemics (Manne et al. 1999). The

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situation might be even more critical in the case of exploited species, where legal and illegal hunting can severely affect population dynamics (Juillet et al. 2012; Carvalho et al. 2015). In that context, genetic information, including genetic diversity and genetic structure, is of prime importance for delimiting meaningful management units, and developing relevant conservation policies of island game bird species accordingly.

Endemic columbid species can play an important ecological role in island ecosystems through their capacity to disperse seeds over long distances (Shanahan et al. 2001; Bucher & Bocco 2009). However, they are particularly vulnerable to environmental degradation, extreme climatic events, predation, and hunting pressure (Walker 2007; Carvalho et al. 2015; Collar 2015; Stirnemann et al. 2018). Preservation and restoration of forest habitats, sustainability of hunting pressure and control of introduced predators are therefore the main priorities for the management of island columbid species (Walker 2007). However, at a regional scale, information about the degree of connectivity between different island populations is also necessary to understand population structure (Young & Allard 1997; Monceau et al. 2013), movements (Strong & Bancroft 1994), and the role of columbids in the intra- and inter-island transfer of seeds and ecosystem functioning (McConkey et al. 2004; Buelow et al. 2018).

Here we document the extent of genetic structure and gene flow in the Caribbean-endemic Scaly-naped Pigeon (*Patagioenas squamosa*) using both mitochondrial DNA and nuclear markers (microsatellites). The species' natural range includes both the Greater and the Lesser Antilles and extends to Florida in the north and to the islands off the coast of Venezuela in the south (Del Hoyo et al. 1997). Although the species is a year-round resident throughout much of its range (Nellis et al. 1984; Levesque et al. 2011; García–Quintas & Isada 2014; Rodríguez Batista et al. 2014; Madden et al. 2015), it occurs only seasonally on some islands (Paice & Speirs 2010) and is a vagrant on Jamaica. In addition, individuals can move between islands, particularly following extreme climatic events (Rivera-Milán 1995). The Scaly-naped Pigeon was introduced in Barbados in the early twentieth century, after about 25 individuals escaped from captivity from a rooftop aviary in Bridgetown (Buckley et al. 2009). This arboreal and frugivorous pigeon has a diverse diet and is supposed to play an important role in forest regeneration (Pérez-Rivera 1978). Although the species is currently considered of least concern in the IUCN red list, data on population size, demographic trends and movements of individuals between islands are scarce. Still, the species is exposed to intensive hunting pressure over a large part of its distribution area and is under the threat of ongoing habitat loss and fragmentation (Wiley 1985; Brooks et al. 2002; Acevedo & Restrepo 2008). According to Raffaele et al. (1998), the species has largely declined in the West Indies, except in Puerto-Rico thanks to the regeneration of secondary forests from abandoned agricultural lands (but see Case & Hughes 2011). In addition, climate change in the Caribbean, with a likely increase in extreme climatic events, such as hurricanes (O'Brien et al. 1992; Wiley & Wunderle 1993; Boose et al. 2004) and an overall rainfall reduction (Bhardwaj et al. 2018), may also put the species at risk in the near future. Therefore, using both mitochondrial and nuclear makers, we assess the extent of genetic differentiation and that of gene flow between four different islands spread over the geographical range of the species. In addition, we compare our results with previous estimates of the genetic structure of other columbid species of contrasted conservation status.

Materials and methods

Sample collection and DNA extraction

Biological samples were obtained between 2009 and 2012 from four different islands: Puerto-Rico (PR), Guadeloupe (GUA), Martinique (MAR) and Barbados (BAR) (Figure 1 and Table S1). Birds in GUA and MAR were collected only during the hunting season, such that birds were not necessarily local breeders. Depending on the island, various types of biological samples were taken from live individuals (blood, feathers) or from individuals shot by local hunters (liver, legs). Liver samples and blood were placed in a storage buffer (70% ethanol and 30% Tris-EDTA buffer pH 8), whereas legs were deep-frozen and feathers were stored in envelopes with silica-gel.

From liver and toe samples, DNA extraction was carried out from small pieces (about 1 cm2) of tissues. In the case of feathers samples, the calamus tip (2–3 mm in length) was used. For each blood sample, 100 µL were centrifuged at 4000 rpm, at 4°C, during one minute in order to separate the plasma (the supernatant) from the red blood cells. Then, all samples were overnight incubated with 200 µL of Queen Lysis Buffer (Tris 10 mM, EDTA 10 mM, NaCl 10 mM, n-lauroylsarcosine 1%; Seutin et al. 1991) and 5 µL of Proteinase K at 55°C. DNA was thereafter extracted according to a standard phenol-chloroform method, as described by Hillis et al. (1996).

Mitochondrial DNA amplification and sequencing

We amplified a mitochondrial DNA (mtDNA) gene coding for the Cytochrome c Oxidase subunit I (COI) from 111 scaly-naped pigeons, by using

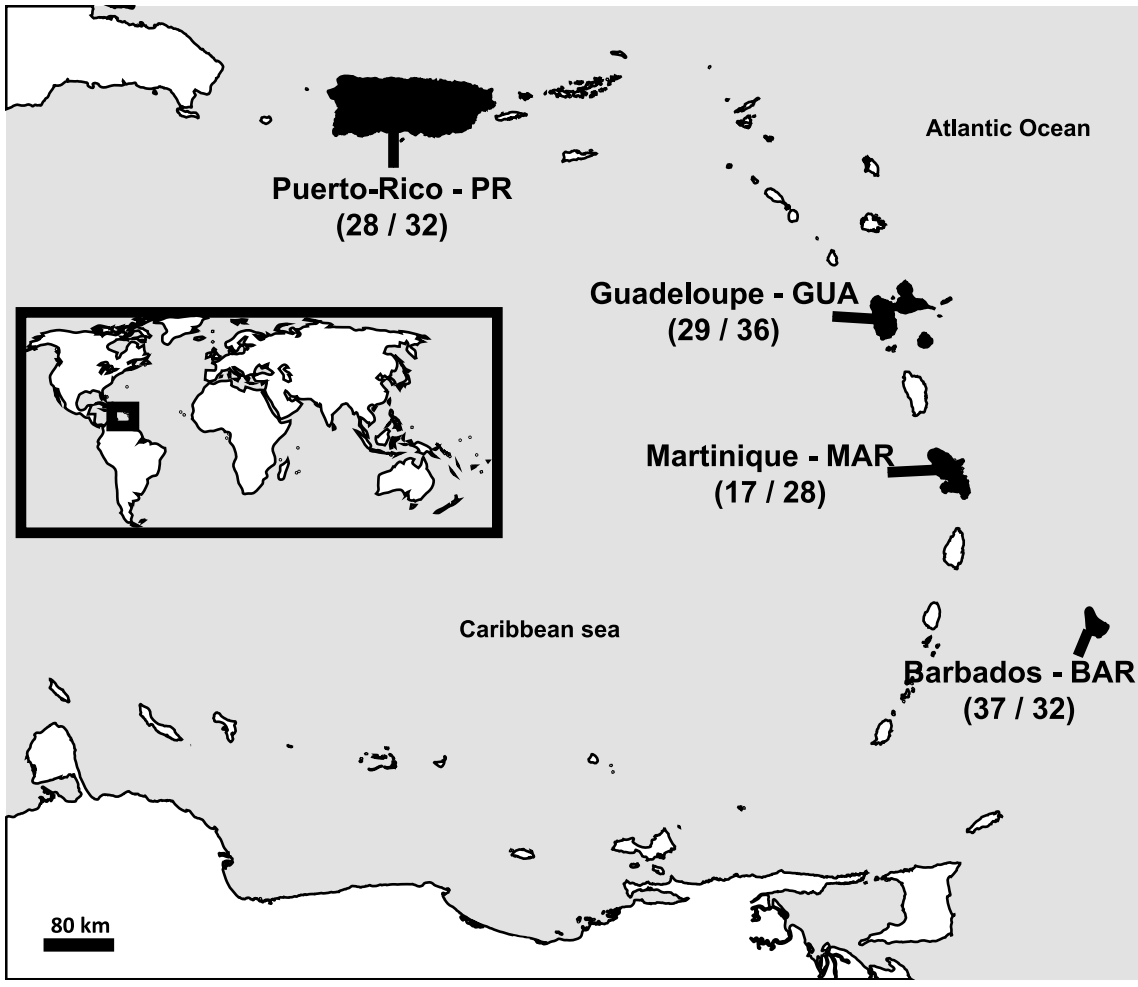


Figure 1. Map of the Caribbean archipelago, with the sampled islands in black. Island names are followed by their acronym used in the present study and parenthesis include sampling sizes for mtDNA and microsatellite analyses, respectively.

reverse and forward primers described by Kerr et al. (2007): BirdF1 (5′-TTCTCCAACCACAAAGACATT GGCAC-3′) and VertebrateR1 (5′-TAGACTTCTGG GTGGCCAAAGAATCA-3′). The 50 µL PCR mixture contained 200 µmol/L dNTP, 1X Buffer (HotMaster^{TM} Taq Buffer, 5 PRIME), 0.25 U Taq (HotMaster^{TM} Taq DNA Polymerase, 5 PRIME) and 200 nmol/L R and F primers. The PCR reaction, performed within a Biorad DNA Engine Peltier Thermal Cycler (Bio-Rad Laboratories, Inc.), began by an initial denaturating at 94°C for 1 min. 30 s, following by 34 thermal cycles consisted of: 94°C for 30 s 53°C for 45 s, and 65°C for 45 s, ended by a final extension at 65°C for 10 min. The quality of amplifications was assessed on 2% agarose electrophoresis gel. Thereafter, PCR products were purified by adding two enzymes: Exonuclease I (EXOI) at a final concentration equals to 2 U per PCR product, and Shrimp Alkaline Phosphatase (SAP) at a final concentration equals to 1 U per PCR product. These enzymes were incubated for one hour at 37°C to be activated, and were deactivated through an incubation at 80°C during 10 min. All samples were externally sequenced by Macrogen Inc. (Republic of Korea) on an Applied Biosystems 3730xl DNA Analyzer sequencer (Applied Biosystems), carried out according to a protocol of Big Dye Sequencing.

Microsatellite amplification and genotyping

A total of 128 individuals were genotyped at seven microsatellite loci developed specifically for *P. squamosa* (Barker et al. 2011): PsA130, PsC11, PsC101, PsC120, PsC128, PsD2 and PsD5. The 10 µL PCR mixture consisted of 200 µmol/L dNTP, 1X Buffer (HotMaster^{TM} Taq Buffer, 5 PRIME), 0.25 U Taq (HotMaster^{TM} Taq DNA Polymerase, 5 PRIME), 200 nmol/L R and F primers, and 200 nmol/L of

Fluochrome 700 or 800. The PCR thermal condition consisted of an initial denaturing period at 94°C for 5 min., following by 35 thermal cycles including: 94°C for 40 s, 53°C for 30 s, and 65°C for 1 min., ended by a final extension at 65°C for 5 min. Microsatellite alleles were visualized on 6.5% acrylamide 25 cm long and 0.25 mm thick gels on a LICOR 4000 L automated sequencer.

Mitochondrial data analysis

Sequences were manually aligned using MEGA 5 software (Tamura et al. 2011). Molecular diversity indices such as haplotype diversity (h), average number of nucleotide differences between haplotypes (π), number of polymorphic sites (S), Nucleotide diversity (k), and pairwise island differentiation (Φ_{ST}) were estimated with ARLEQUIN 3.5 software (Excoffier & Lischer 2010). Φ_{st} is a F_{ST} analogue for mtDNA sequences and corresponds to the molecular distance between populations based on the number of pairwise differences between haplotypes. These values were estimated with 50 000 permutations and 5% nominal level was adjusted for multiple comparisons with Benjamini-Yekutieli step up procedure (BY; Benjamini & Yekutieli 2001; Narum 2006), resulting in α_{BY} = 0.0204. A minimum spanning network was constructed through POPART 1.7 software (Bandelt et al. 1999; Leigh & Bryant 2015) based on pairwise absolute distances between haplotypes. Neutrality and demographic history tests such as Tajima's D test (Tajima 1989) and Fu's F_S test (Fu & Li 1993) were performed, as well as the more conservative square differences statistic (SSD, mismatch distribution), to compare the observed distribution of the number of nucleotide differences between haplotype pairs to that expected under the null hypothesis of sudden expansion, using 10 000 bootstraps (Rogers & Harpending 1992; Harpending et al. 1993; Fahey et al. 2012). These analyses were also performed with ARLEQUIN 3.5.

Microsatellite data analysis

MICROCHECKER 2.2.3 was used to identify potential genotyping errors due to null alleles, scoring errors due to stuttering, and large allelic dropout, using 10,000 iterations and Bonferroni correction (Van Oosterhout et al. 2004). The number of alleles per locus (N_a), observed heterozygosity (H_0), expected heterozygosity in Hardy-Weinberg equilibrium (H_E; Nei 1978) and the deviation from Hardy-Weinberg equilibrium (HWE; Guo & Thompson 1992) were performed with Arlequin 3.5, considering 1,000,000 Markov chain steps and 100,000 dememorisation steps. Inbreeding coefficient (F_{IS}) was calculated in *FSTAT* 2.9.3 (Goudet 1995) by independently taking each island and at the regional scale. Since some loci included null alleles (Table S2), islands paiwise F_{ST} value were first computed using FREENA (Chapuis & Estoup 2007). However, corrected values of pairwise F_{ST}, which excluded null alleles, were not significantly different than those including them (Table S3). Uncorrected values of pairwise F_{ST} were therefore used to visualize pairwise island differentiation, using *FSTAT* 2.9.3. Nominal significance level (5%) was adjusted with BY's correction.

Population structure was also visualized using an individual-based Bayesian clustering method, as implemented in *STRUCTURE* 2.2.3 (Pritchard et al. 2000), and using principal coordinate analysis (PCoA), as implemented in *GENALEX* 6.41 (Peakall & Smouse 2006). *STRUCTURE* assigns individuals into K optimal region groups according to their genetic similarity. To that end, and to determine the optimal number of genotype clusters (K), we ran simulations using the admixture and correlated allele frequencies models by programming ten independent runs for K values ranging from 1 to 6 and a burn-in length of 30,000 followed by 100,000 Markov chain Monte Carlo. Thereafter, the optimal number of genotype groups (K) was estimated using the *R* (version 3.6.2; R Core Team 2019) package *CORRSIEVE* (version 1.6-8; Campana et al. 2011) by following the ad hoc statistic ΔK method described by Evanno et al. (2005). Clusters were finally visualized using *DISTRUCT* 1.1 (Rosenberg 2004).

Scaly-naped pigeons were not historically present in Barbados but were imported by local pigeon fanciers (Buckley et al. 2009). Therefore, we used *BOTTLENECK* 1.2.02 (Piry et al. 1999) to detect distortions in the distribution of alleles frequencies due to a recent founder effect (Luikart et al. 1998), considering the single-step mutation model (SSM), known to be more appropriate for microsatellites loci (Di Rienzo et al. 1994; Piry et al. 1999).

Results

Mitochondrial polymorphism

We obtained 624 bp long COI sequences for the 111 samples of Scaly-naped Pigeon, revealing five haplotypes (H_1–H_5) associated with polymorphism at four single nucleotides (Tables 1 & Tables 2). Haplotype and nucleotide diversities at the local and regional scale are presented in Table 2. The haplotypic network (Figure

Table 1. Occurrences and percentage of COI haplotypes revealed in each island (H_R = total number of haplotypes per island; N = sample size). Sequences of haplotypes are available in GenBank database (Accession codes: MW411793-97).

	PR	GUA	MAR	BAR	All islands
H_1	28 (100%)	24 (82.76%)	13 (76.47%)	37 (100%)	**102 (91.89%)**
H_2	-	3 (10.34%)	3 (17.65%)	-	**6 (5,41%)**
H_3	-	1 (3.45%)	-	-	**1 (0.90%)**
H_4	-	1 (3.45%)	-	-	**1 (0.90%)**
H_5	-	-	1 (5.88%)	-	**1 (0.90%)**
H_R	1	4	3	1	**5**
N	28	29	17	37	**111**

2) presents the relationship between the five different mitochondrial haplotypes detected across the 111 studied pigeons. GUA had the highest haplotype richness (H_R = 4), followed by MAR (H_R = 3). PR and BAR presented only one haplotype (H_1), which was the most abundant at the local and regional scale (Tables 1 & Tables 2 and Figure 2). Exclusive haplotypes were found in both GUA (H_3 and H_4) and MAR (H_5). H_2, found in both GUA and MAR, was the second most frequent haplotype (Table 1 & Figure 2). Pairwise island mtDNA Φ_{ST} values (Table 3), revealed differentiation between BAR and both GUA and MAR, and between MAR and PR.

We first carried out independent demographic scenario tests for islands showing polymorphism (GUA and MAR), and thereafter carried out the same tests at the regional scale. Results from these tests were in accordance with a demographic stability for MAR (Tajima's D = −0.0697, P = 0.239; Fu's F_S = −0.6240, P = 0.224 and SSD = 0.00870, P = 0.046). For GUA, results from Fu's F_S test supported a within-island demographic expansion (Fu'F_S = −2.194, P = 0.021), whereas both Tajima's D and mismatch distribution tests were in agreement with demographic stability (Tajima's D = −1.350, P = 0.069 and SSD = 0.00228, P = 0.032). All of the demographic scenario tests clearly supported a demographic expansion at the regional scale (Tajima's D = −1.544, P = 0.015; Fu's F_S = −4.919, P = 0.001 and SSD = 0.000322, P = 0.101).

Microsatellite polymorphism

Genotyping of 128 individuals resulted in a total of 73 alleles (Mean ± SD: 10.429 ± 3.359 alleles per locus), with an average of observed heterozygosity equals to 0.731 ± 0.145 over all loci and at the regional scale (Table 2). We found no difference in terms of number of alleles (Friedman-χ^2 = 2.766, df = 3, P = 0.429), allelic richness (Friedman-χ^2 = 6.6, df = 3, P = 0.086) and observed heterozygosity between populations (Friedman-χ^2 = 3.514, df = 3, P = 0.319; Tables 2 & S4). Global F_{IS} was estimated at −0.0560. The basic summary statistics of genetic variation within different islands are presented in Supplementary Table S4.

All island pairwise F_{ST} values for BAR were significantly different from zero (F_{ST} ranging from 0.0496 to 0.0594), whereas all other values were not, suggesting a moderate level of differentiation between BAR and the three other islands (Table 3). This result was in accordance with results based on *STRUCTURE* analysis (Figure 3) and PCoA (Figure 4). In both analyses, GUA, MAR and PR seemed to form a homogenous group, whereas BAR appeared to be genetically isolated. PCoA result was based on the first and second axes that, respectively, explained 22.66% and 21.23% of the overall variance in microsatellite data.

Based on *BOTTLENECK* analysis, we found that BAR was significantly in deficiency of heterozygosity under the single-step mutations model (Wilcoxon rank test: P = 0.0391; Table 4). At the regional scale, no excess or deficiency of heterozygosity was highlighted.

Table 2. Genetic characteristics of sampled islands, based on the mtDNA COI sequence. See Figure 1 for island acronyms. Variables are: Sample size (N), Number of polymorphic sites (*S*), Haplotypic diversity (*h*), Average number of nucleotide differences between haplotypes (π), Nucleotide diversity (average over loci, *k*), ($\bar{N}_a$) average number of alleles per microsatellite loci, observed and expected heterozygosity (*Ho* and *He*, respectively) and the inbreeding coefficient (*Fis*); s.d. = standard deviation.

	PR	GUA	MAR	BAR	All islands
Mitochondrial polymorphism					
N	28	29	17	37	**111**
S	0	3	2	0	**4**
h* ± *s.d.	0	0.313 ± 0.106	0.404 ± 0.130	0	**0.154 ± 0.0456**
π* ± *s.d.	0	0.330 ± 0.341	0.426 ± 0.405	0	**0.157 ± 0.219**
***k* ± *s.d.* (x 10^{-3})**	0	0.529	0.683	0	**0.252**
Microsatellite polymorphism					
N	32	36	28	32	**128**
$\bar{N}_a$ ± *s.d.*	7.714 ± 3.352	8.286 ± 3.251	8.000 ± 2.380	7.143 ± 1.773	**10.429 ± 3.359**
Ho	0.766 ± 0.174	0.758 ± 0.200	0.718 ± 0.205	0.683 ± 0.135	**0.731 ± 0.145**
He	0.783 ± 0.134	0.777 ± 0.160	0.769 ± 0.173	0.695 ± 0.122	**0.774 ± 0.137**
Fis	0.0181	0.0162	0.0691	0.0244	**0.056**

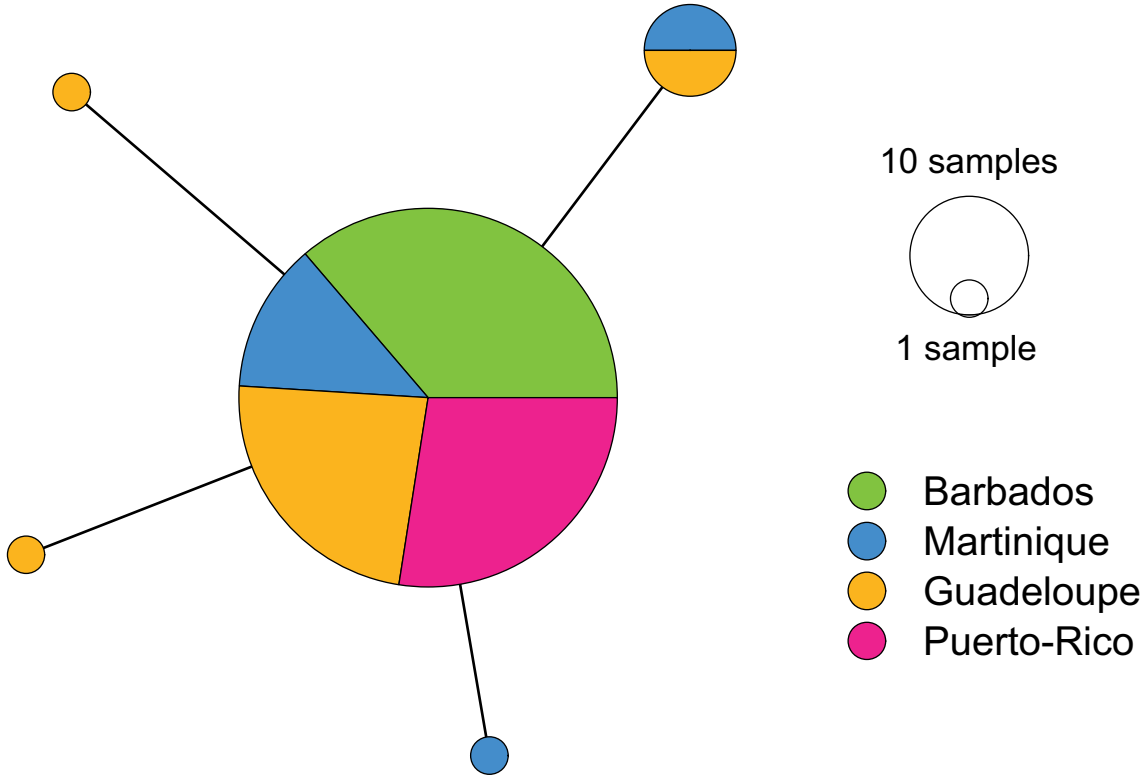


Figure 2. Minimum spanning network of 111 haplotypes for a 624 bp sequences of mtDNA COI. Each circle represents a haplotype and its size is proportional to the number of individuals sharing this haplotype. One continuous segment represents one nucleotidic difference. Colors indicate the origin of individuals and its proportion, the relative abundance of individuals sharing the same haplotype and originating from the same location.

Table 3. Pairwise island mtDNA *Φst* values (below diagonal) and microsatellite *Fst* values (above diagonal) of Scaly-naped Pigeon for four Caribbean islands. Significant values considering *BY's* correction (αBY = 0.0204) are in bold (NS = not significant; * = P < 0.0204; ** = P < 0.00408; *** = P < 0.000408).

	PR	GUA	MAR	BAR
PR	-	0.0124^{NS}	0.0165^{NS}	**0.0496***
GUA	0.0903^{NS}	-	0.0079^{NS}	**0.0586***
MAR	**0.201***	-0.0254^{NS}	-	**0.0594***
BAR	0.000^{NS}	**0.112****	**0.242****	-

Discussion

Genetic diversity

Based on mtDNA analysis, we found an overall relatively low haplotype (h = 0.154) diversity across the four Caribbean islands populations, compared to other island-endemic columbid species that was estimated using the control region gene. These species include the 'critically endangered' Red-headed Wood Pigeon Subspecies, *Columba janthina nitens* (h = 0.12; Ando et al. 2014), the 'endangered' Pink Pigeon *Nesoenas mayeri* (h = 0.45; Swinnerton et al. 2004), the two subspecies of the 'near threatened' Plain Pigeon (*P. inornata wetmorei* with h = 0.48 and *P. i. inornata* with h = 0.59; Young & Allard 1997), the 'near-threatened' New Zealand Pigeon *Hemiphaga novaeseelandiae* (h = 0.623; Goldberg et al. 2011) and the 'least-concern' Emerald Dove *Chalcophaps indica* (h = 0.94; Kirchman & Franklin 2007). The level of haplotype diversity across islands has been influenced by the absence of mtDNA polymorphism in Barbados and Puerto-Rico. Indeed, haplotype diversity values for Guadeloupe and Martinique was h = 0.313 and h = 0.404, respectively. These values are still low compared to what has been previously reported for other columbid species, but in accordance with the Caribbean-endemic and 'least concern' Zenaida Dove, *Zenaida aurita* (h = 0.404; calculated from Monceau et al. 2013) for which the same mtDNA gene was used. A similar pattern was also visible from the average nucleotide diversity across loci and the average number of nucleotides differences between haplotypes (Young & Allard 1997; Kirchman & Franklin 2007; Goldberg et al. 2011; Ando et al. 2014). The absence of haplotype polymorphism in Barbados was in accordance with what we expected (influence of founder effect, see below), but less so for Puerto-Rico, which is known to harbor a large population with more than 260,000 scaly-naped pigeons (Rivera-Milán et al. 2014). This lack of diversity across Puerto Rico samples might have been influenced by the fact that captures were conducted in

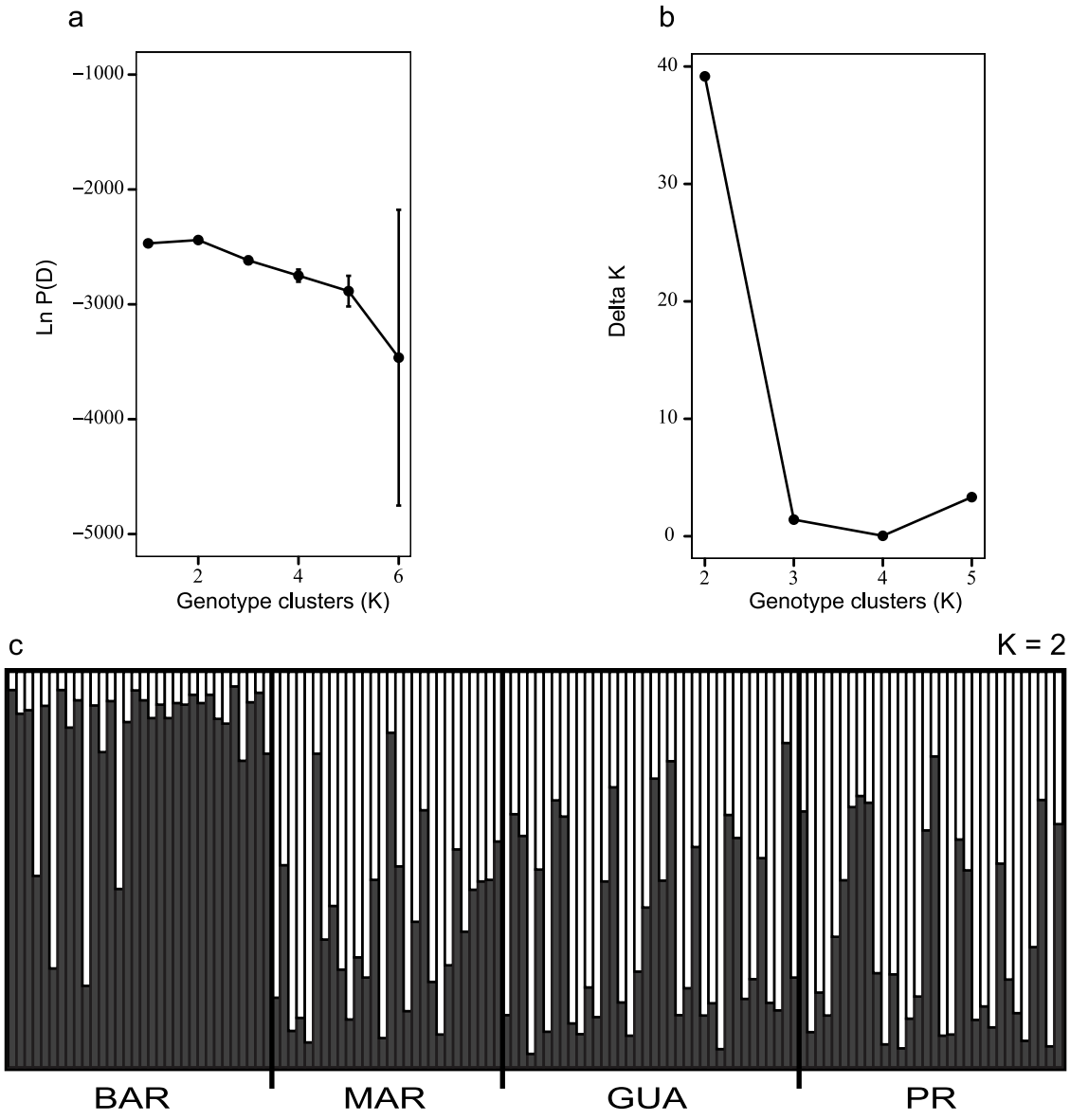


Figure 3. Genetic structure of populations of the Scaly-naped Pigeon using Bayesian inference through *STRUCTURE* analysis. (a) and (b) represent results from simulation to determine the optimal number of genotype clusters (K) according to the method describes by Evanno et al. (2005). Error bars represents standard deviations over ten runs. (c) represents results from *STRUCTURE* analysis carried out with maximum-likelihood centered at K = 2 genotype clusters. Each individual is represented by a single vertical bar, partitioned into K colored segments that represent its estimated population membership fractions. Individuals are grouped by population on the x-axis. Population codes are given in Figure 1.

a restricted area within the island (i.e. two separated sites distant from 8 km, Cidra and Comerio municipalities), thus increasing the probability of collecting related individuals, in the case of a geographically structured population.

Contrary to mtDNA diversity, genetic diversity based on the seven microsatellite markers was in accordance with what we expected. All islands showed similar values of observed heterozygosity, with Barbados showing the lowest value (PR: H_O = 0.77, GUA: H_O = 0.76, MAR: H_O = 0.72 and BAR: H_O = 0.68). In addition, the average observed heterozygosity across the four islands (0.731) was in accordance with its IUCN conservation status, since quite similar to the value reported for the Zenaida Dove (0.725; Monceau et al. 2013). In contrast, this value was clearly higher than the one found for the critically endangered red-headed Wood Pigeon (H_O = 0.04; Ando et al. 2014).

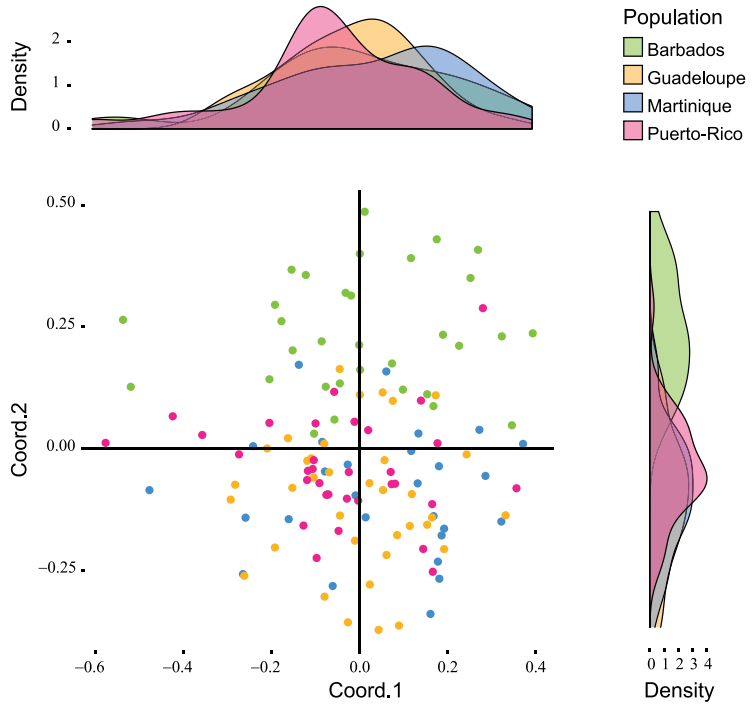


Figure 4. Principal coordinate analysis (PCoA) based on the genetic distance matrix constructed through seven microsatellite markers from 128 Scaly-naped Pigeons. Diagrams on the right side and at the top represent the density and the distribution of points according to the y-axis (Coord. 1) and the x-axis (Coord. 2), respectively.

Table 4. Wilcoxon signed rank test for mutation-drift equilibrium estimated based on seven microsatellite loci to assess a variation in term of heterozygosity (*H*) due to demographic events such as a bottleneck or a founder effect.

	SMM	
POPULATIONS	*H* deficiency	*H* excess
PR	0.961	0.0547
GUA	0.766	0.289
MAR	0.469	0.594
BAR	**0.0391***	0.973
ALL ISLANDS	0.148	0.945

Genetic structure

Genetic diversity metrics based on both mtDNA and nuclear markers were indicative of genetic depletion in Barbados compared to the three other studied populations, suggesting that this population experienced a recent historical demographic event such as a bottleneck or founder event, a more or less long-term geographical isolation or a combination of these phenomena. This hypothesis was supported by the *BOTTLENECK* analysis (conducted using the stepwise mutation model), which provided evidence for heterozygosity deficiency, suggestive in the present case, of a founder effect. This recent historical demography event broadly explained the observed genetic structure. Indeed, Barbados was strongly differentiated from Guadeloupe and Martinique based on haplotype frequencies (0.112 and 0.242, respectively). In contrast, Barbados was not differentiated from Puerto-Rico, since both populations exclusively consisted of the same haplotype (H_1). For the same reason, Puerto-Rico was differentiated from Martinique, whereas, using microsatellite markers, differentiation was only observed between Barbados and the other islands. This pattern was in accordance with results obtained with *STRUCTURE* and the PCoA analyses, from which two genetic groups were identified, separating individuals from Barbados from those from Martinique, Guadeloupe and Puerto-Rico. This genetic structure could be explained by: (i) the emergence of the Barbados population from a few individuals imported by local pigeon fanciers (i.e., founder effect), (ii) the geographic context of the Barbados island, which is relatively isolated compared to other islands from the Antillean Arc (i.e. restricted gene flow) and (iii) genetic drift, which may be intensified by the two previous phenomena.

In contrast, Martinique, Guadeloupe and Puerto-Rico were not differentiated or structured, what suggests that these islands could be inter-connected by effective migrants and regular movements. This pattern contrasts to some extent with what has been observed in two other Caribbean-endemic columbid species. In

the Zenaida Dove, limited gene flow was observed between eight Caribbean islands, even at very short distance like, for instance, between Martinique and Saint-Lucia (Monceau et al. 2013). Interestingly, the latter study also observed a strong divergence between Barbados and the other islands. In the congeneric Plain Pigeon, *P. inornata*, restricted gene flow was observed between Puerto-Rico and the Dominican Republic, although the extent of genetic differentiation between the two populations suggests that they may correspond to two sub-species (Young & Allard 1997). However, the observed pattern of genetic structure is in accordance with several other studies on columbids, which highlighted the peculiar aspect of the evolution of this family in a biogeographic context of insularity (Santiago-Alarcon et al. 2006; Kirchman & Franklin 2007; Seki et al. 2007; Goldberg et al. 2011; Ando et al. 2014). Indeed, most columbid species have conserved a strong flying ability contrary to most other island-endemic terrestrial birds (Bollmer et al. 2005; Petren et al. 2005; Kawakami et al. 2008). For instance, the White-crowned Pigeon, *P. leucocephala*, the phylogenetically closest species to the Scaly-naped Pigeon (Johnson et al. 2010), is known to perform daily movements between breeding areas and foraging sites by flying over 50 km, and can fly up to 150 km over water to reach another Caribbean island in order to track food resources and/or suitable environmental conditions (Wiley 1979; Wiley & Wiley 1979; Rivera-Milán 1992; Bancroft et al. 2000; Strong & Johnson 2001; Meyer & Zimmerman 2006; Florida Fish and Wildlife Conservation Commission 2013), such as for avoiding hurricanes (Wiley & Wunderle 1993; Ries et al. 2018). Hurricanes are known to affect avian community and population dynamics by mainly disturbing feeding and breeding habitats (Rittenhouse et al. 2010; Jenouvrier 2013). Due to the inherent strong ability to fly of most of Columbidae, the high frequency of hurricane events in the Caribbean region may induce movements of individuals, potentially homogenizing the allelic pool over the different island populations, as observed in the present study. Therefore, if movements of individuals between islands are frequent and philopatry is low, it is possible that the whole species actually consists of a single large population.

At the geological timescale, results from historical demographic analyses suggest a recent demographic expansion of the Scaly-naped Pigeon population at the regional scale. The Scaly-naped Pigeon might have had a restricted range during the glacial maxima, while population expanded after the Last Glacial Maximum. The same pattern of expansion was found for the New Zealand Pigeon (Wallis & Trewick 2009; Goldberg et al. 2011).

Implications for the management of the species

The Scaly-naped Pigeon is facing several threats such as habitat loss, legal and illegal hunting, exotic predators and climate change. There is a relatively high hunting pressure within its range of distribution, but monitoring data are lacking (Raffaele et al. 1998; Latta et al. 2010; Latta 2012; Rivera-Milán et al. 2014). In Puerto-Rico, where studies are particularly concentrated, it has been argued that the species requires the development of a sustainable harvest strategy at the local level (Case & Hughes 2011). However, given our results and the strong flying ability of Columbiformes, the insular Caribbean (to the possible exception of Barbados) could be considered as an evolutionarily significant unit (ESU) or management unit (MU; Moritz 1994; Paetkau 1999; Zink 2004), emphasizing the need for conservation effort to be undertaken at the regional rather than local scale. To that end, further sampling could be carried out in other Caribbean islands to confirm the genetic structure of the species at the regional scale evidenced in the present study. In addition, other methods could be used to distinguish between historic and contemporary gene flow, and to identify recently diverged population for an effective conservation management of the species (Paetkau 1999; Palsboll et al. 2006; Whitehead 2010; Esteban et al. 2016). For instance, Paetkau (1999) criticized the unique use of genetic data for MUs recognition and advised to combine it with movement data such as Mark-Recapture, telemetry, or GPS tracking data. Using such methods would indeed be valuable to detect contemporary migration/dispersal due to, for instance, current anthropogenic disruptions (Esteban et al. 2016).

Lay summary

- No genetic differentiation between Puerto-Rico, Guadeloupe and Martinique.
- Possibly one large population of Scaly-naped Pigeon at the Caribbean regional scale.
- Significant founder effect in the Barbados population, consistent with its introduced origin.

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Disclosure statement

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ARTICLE 6

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High genetic diversity and gene flow as evidence of past demographic fluctuations in the Caribbean-endemic White-crowned Pigeon

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Introduction

Genetic diversity, defined as the variation of alleles within and among individuals of a population (Frankham et al. 2002), is an essential component of biodiversity and contributes to species and population persistence (Humphries et al. 1995, Lande & Shannon 1996, Frankham 2010, Vitorino et al. 2019, Schreiber 2020). Indeed, the ability of species to adapt to environmental change depends to a large extant on the amount of genetic diversity, particularly in small populations (Westermeier et al. 1998, Frankham et al. 2002, Frankham 2005, Evans & Sheldon 2008). Measuring genetic diversity is therefore of major importance for assessing extinction risk in the long term (McNeely et al. 1990, Spielman et al. 2004, Evans & Sheldon 2008).

The relationship between low genetic diversity and high extinction risk is well evidenced in birds (Evans & Sheldon 2008), and particularly relevant to avian species living in hotspots of biodiversity, such as tropical islands (Myers et al. 2000, Russell & Kueffer 2019). Over the last 400 years, 81% of recorded extinctions have concerned islands species (Whittaker & Fernandez-Palacio 2007), and almost half of threatened bird species (as defined by the IUCN Red List) are endemic to islands (Birdlife International 2017). This situation is alarming as global change and other human-induced disturbances are severely affecting tropical islands (Taylor & Kumar 2016, Browning & Sawyer 2021). In addition, avian endemic species are particularly vulnerable to environmental disturbance due to their peculiar evolution history in more or less isolated conditions (Duncan & Blackburn 2004, Loope et al. 1988). In particular, habitat degradation and invasive species (including competitors, predators and pathogens) are negatively affecting insular bird species (Whittaker & Fernandez-Palacio 2007), eventually leading to reduced intra and interspecific diversity (Morris-Pocock et al. 2012, Davies et al. 2015, Major et al. 2021).

In particular, when an avian species' distribution extends to several islands, both geographical and ecological discontinuities can induce different selection pressures among populations that affect dispersal and gene flow (Clegg et al. 2002, Illera et al. 2007, Hoeck et al. 2010, Bertrand et al. 2014, Ando 2019, Martin et al. 2021). For instance, different threats, such as particular invasive species or pathogens, can be present only on some islands and absent on others (Jesse et al. 2017, Russell et al. 2017, Soares et al. 2017). This may ultimately result in allopatric divergence and diversification, leading to isolated populations, subspecies

or, in extreme cases, different species between islands (Brown et al. 2013, Mapel et al 2021). In this respect, the delimitation of evolutionarily significant units (ESUs; Ryder 1986, Moritz 1994, Waples 1991, Candrall et al. 2000), and management units (MUs), using appropriate genetic and molecular tools, is of prime importance for the conservation of such species. The definition of genetic MUs is particularly important for identifying population structure (i.e., units or islands) and assessing the extant of gene flow at the regional level (Ladle and Whittaker 2011). Such information allows in turn the prioritisation of conservation efforts, as recommended by most governmental and nongovernmental conservation agencies around the word (Phillimore & Owens 2006; Haig and D'Elia 2010). As argued by Crandall et al. (2000), such intraspecific units should be estimated from both genetic (*i.e.*, gene flow estimates) and ecological data (*i.e.*, related to differences of biotic and/or abiotic selection pressures existing among units). However, in widely distributed species, genetic data, using usually both mitochondrial DNA (mtDNA) and/or neutral nuclear markers (e.g., microsatellites or Single nucleotide polymorphism), are easier to obtain and still provide a useful first estimation for the delineation of intraspecific units (Haig et al. 2011).

Most avian species, with the exception of flightless ones, have a strong ability to disperse compared to other terrestrial vertebrates (Haig et al. 2006, Haig & D'Elia 2010, Ando 2019). As a result, the level of population genetic structure of insular avian species may vary, from closely reflecting the geographical distances between islands to showing no significant structuration across islands (Ando 2019). For instance, the Wattled honeyeater, *Foulehaio carunculatus*, a Polynesian archipelago endemic species consists of distinct genetic units at the level of either individual islands or group of islands (Mapel et al. 2021). On the other hand, the Purple-throated Carib (*Eulampis jugularis*) shows no obvious genetic structure across islands at the scale of the Lesser-Antilles (Khimoun et al 2016). Heterogeneity in species populations' genetic diversity and structure can actually be explained by several historical and contemporary factors, including colonization history and dynamics (e.g., in hummingbirds see McGuire et al. 2007), demographic patterns and evolutionary history of populations in response to different selection pressures, geographical barriers (Wallis & Trewick 2009), evolutionary history of species lineage (e.g., transmission of traits influencing flight abilities or dispersal/migration behaviour traits; Clobert et al. 2012, Sheard et al. 2020), and ecological features of species (e.g., habitat use or diets, Miller et al. 2021).

The family *Columbidae* includes 369 species distributed throughout the world, with almost 58% of them strictly occurring in islands (Gibbs et al. 2001, Birdlife International 2021) and about 34% being considered at risk in the IUCN Red List (Walker 2007; Winkler et al. 2020). The main threats to which pigeons and doves are exposed are habitat loss, invasive species and hunting (Walker 2007). The *Columbidae* family is highly diversified in terms of morphology, ecology and behaviour (Gibbs et al. 2001, Winkler et al. 2020), with marked variation in dispersal ability among species. Some species, although occurring in several islands, tend to be sedentary whereas other move between different localities either seasonally or in a more erratic way depending on resource availability (Gibbs et al. 2001, Birdlife international datazone 2021). This variation is reflected in the two extreme and iconic examples of extinct columbid species, the Dodo, *Raphus cucullatus*, a flightless species which only occurred on Mauritius Island and the Passenger Pigeon, *Ectopistes migratorius*, a highly nomadic species reported to move in flocks of millions of birds (Blockstein 2020).

In the Caribbean region, the second most important hotspot for columbid species (Walker 2007), population genetic structure has been investigated in only two endemic species. Evidence for genetic structure at the regional scale was first provided for the Zenaida dove, *Zenaida aurita* (Monceau et al. 2013). Data from mitochondrial DNA, documenting the historical demographic events that have shaped the population of a species (Avise 1994), indicated that the species was genetically structured in two main groups of populations corresponding to Greater-Antilles and the Lesser-Antilles. In contrast, data from nuclear markers (microsatellites), that provide a better resolution than mitochondrial genes to assess population dynamics at a more recent timescale and are free of bias due to maternal inheritance (Avise 2000, Zhang & Hewitt 2003, Freeland 2005, Zink & Barrowclough 2008), provided evidence for genetic structure at the scale of each single sampled island. More recently, no obvious genetic structure was found between Puerto-Rico, Guadeloupe and Martinique in the Caribbean-endemic Scaly-naped Pigeon, *Patagioenas squamosa*, based on both mitochondrial and nuclear markers, whereas the Barbados population, originating from the recent introduction of a few individuals, differed from the three other sampled islands (Cambrone et al. 2021).

Here, we investigate the pattern of genetic structure and diversity of White-crowned Pigeon (WCPi - *P. leucocephala*) populations at the scale of the insular Caribbean. The WCPi

is considered to be endemic to the Caribbean basin, with its natural range extending from the Florida Keys and Bahamas to Saint Lucia, with some individuals reaching the coast of Central America (Gibbs et al. 2001, Bancroft et al. 2020). This arboreal and frugivorous species is regarded as an important seed disperser, given its flying ability (Pérez-Rivera 1978, Wiley 1979). It can indeed perform daily foraging flights exceeding 50 km, and can fly up to 150 km over water to reach another Caribbean island in order to track food resources and/or suitable environmental conditions (Wiley & Wiley 1979, Rivera- Milán 1992, Ware 1997, Bancroft et al. 2000, Strong & Johnson 2001, Meyer & Zimmerman 2006, Florida Fish and Wildlife Conservation Commission 2013, Bancroft et al. 2020). However, nothing is known about the amount of gene flow between islands or the spatial structuring of its genetic diversity. Still, the species is suspected to decline in population size and is considered as near-threatened in the IUCN Red List at the global scale (Birdlife international 2021). In the French West-indies, the species has been reclassified as endangered in Guadeloupe and vulnerable in Martinique by the French IUCN comity (UICN France et al. 2012, 2020). The WCPi is threatened by both habitat loss, invasive species and intensive hunting pressure (legal and illegal), as well as harvesting of squabs (Hay 2008, Latta et al. 2010, Latta 2012, Rivera-Milán et al. 2014, 2016; BirdLife International 2021). Still, information on population size and demographic trends remains scarce and does not cover the entire distribution range of the species. We therefore relied on both mitochondrial and neutral nuclear markers to assess gene flow between eight different island populations spread over the geographical range of the species. We particularly aimed at determining the number of genetic units, assessing the extent of connectivity between islands and intraspecific units, estimating genetic diversity and effective population size (i.e. the number of reproductive individuals within a given population). The latter is a key parameter in conservation biology, as it reflects loss of genetic variability due to inbreeding or genetic drift (Frankham et al. 1995, Kalinowski & Waples 2002; Charlesworth 2009) and be an early clue to population decline (Antao et al. 2011).

Materials

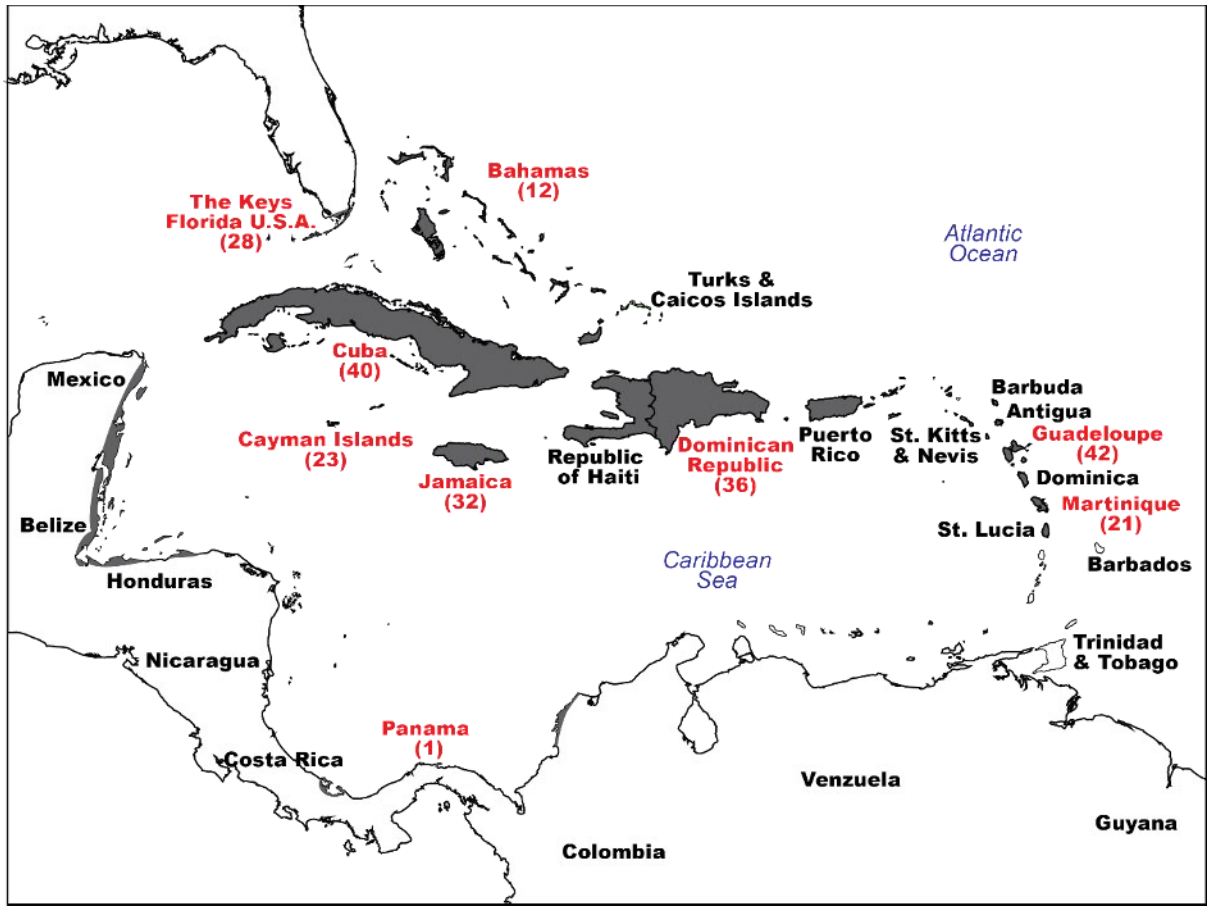


Figure 24 : Distribution area of the White-crowned Pigeon (in grey). In red are islands where samples of White-crowned pigeons were collected and in brackets are the number of samples collected in each island.

Ethical Statement

Bird trapping and blood sampling were carried out with all the necessary permits issued by the Ministry of Environment of the Dominican Republic. Permits to export or import biological samples were obtained from relevant authorities in each country.

Samples collections

Sample collection covered most of the known distribution area of the WCPi, except for Central America, which accounts for a minor part the distribution (Figure 24). Samples were obtained from captive flocks, using collections from natural history museums, by taking advantage of legal hunting, or by capturing individuals in the field (Table 6). Overall, 237 DNA samples from

various tissues were collected (Table 6). Feathers were collected from individuals in captive flocks or free-ranging individuals. Several contouring feathers were plucked and stored in envelop or like-Eppendorf tubes at room temperature or at 4°C until DNA extraction. Silica gel was added to prevent any contamination by fungi and mildew because of the moist tropical climate. Toes from individuals freshly killed by hunters were stored in buffer containing 70% of Ethanol and 30% of Tris-EDTA pH 8 at 4°C or -20°C. Blood samples were collected on birds captured in the field in the Dominican Republic by puncturing the brachial vein using sterile syringe (20G), as described by Owen (2011). We thereafter used sterile capillary tubes to collect about 50-80µL of bloods, which was conserved in absolute ethanol at room temperature during the field work and at -20°C once in the lab.

Table 6 : Tissue type, collection date, collection means and number of samples collected in each island.

Islands	Tissue	Collection date	N	Sampling
Bahamas (BAH)	Museum tissue	1999	1	Florida Museum of Natural History
Bahamas (BAH)	Museum tissue	2000	1	Florida Museum of Natural History
Bahamas (BAH)	Museum tissue	2002	2	Florida Museum of Natural History
Bahamas (BAH)	Museum tissue	2003	2	Florida Museum of Natural History
Bahamas (BAH)	Museum tissue	2004	1	Florida Museum of Natural History
Bahamas (BAH)	Museum tissue	2011	2	Florida Museum of Natural History
Bahamas (BAH)	Museum tissue	2012	3	Florida Museum of Natural History
Captive, California (UKN)	Museum tissue	2004	1	Florida Museum of Natural History
Cayman Islands (CAY)	Feathers	2017	23	Breeding program in the Cayman turtles center
Cuba (CUB)	Toe-clips	2019	40	Hunters
Dominican Republic (DOM)	Toe-clips	2017	7	Hunters
Dominican Republic (DOM)	Blood	2019	29	Trapping with mist nets
Florida (USA)	Museum tissue	2004	2	Florida Museum of Natural History
Florida (USA)	Museum tissue	2006	1	Florida Museum of Natural History
Florida (USA)	Museum tissue	2007	1	Florida Museum of Natural History
Florida (USA)	Museum tissue	2012	2	Florida Museum of Natural History
Florida (USA)	Museum tissue	2013	3	Florida Museum of Natural History
Florida (USA)	Museum tissue	2014	2	Florida Museum of Natural History
Florida (USA)	Museum tissue	2016	3	Florida Museum of Natural History
Florida (USA)	Museum tissue	2017	1	Florida Museum of Natural History
Florida (USA)	Feathers	2018	13	From the Avian research conservation institute
Guadeloupe (GDP)	Toe-clips	2016	39	Hunters
Guadeloupe (GDP)	Toe-clips	2017	1	Hunters
Guadeloupe (GDP)	Toe-clips	2018	2	Hunters
Jamaica (JAM)	Toe-clips	2019	32	Hunters
Martinique (MTQ)	Toe-clips	2018	21	Hunters
Panama (PAN)	Museum tissue	2007	1	Florida Museum of Natural History
Unknown (UKN)	Museum tissue	Unknown	1	Florida Museum of Natural History
		Total	**237**	

DNA extraction, amplifications and sequencing

DNA extraction

DNA extraction was performed following a commercial kit manual (Nucleospin® Tissue, Macherey-Nagel GmbH & Co, Düren, Germany) at the Université des Antilles in Guadeloupe (University of the French West-indies). We used the calamus tips and the blood clot and/or barbs from feathers, as described by Horvath et al. (2005) and Speller et al. (2011). Samples were thereafter digested overnight by adding 30µL of 1M dithiothreitol (DTT) to the lysis solution of the commercial kit, including Proteinase K. For blood samples, we collected a piece of the blood clot with a 1000 µL tips of pipette (2-4 mm^3) because blood clots in absolute alcohol. In order to remove as much as possible storage ethanol within clots, we performed two rinses with 500 µL of PBS. Each time we added PBS, we vigorously vortexed the tube and discarded the supernatant after centrifugation for 5 minutes at high speed (11,000 rpm). After the second round of rinses, we followed the standard protocol of the commercial kit. For the other tissues, a piece of 5 to 40 mg was digested between four hours to overnight and the standard protocol of DNA extraction was followed as well. DNA concentration were estimated using Qubit 4 (Thermo Fisher Scientific, Waltham, MA, United States) and Qubit dsDNA broad-range reagents (detection range 0.2 to 4,000ng/µL; Thermo Fisher Scientific), and when possible, they were adjusted to 10ng/µL to standardized PCR protocols.

Mitochondrial genes

We amplified three portions of mitochondrial genes, including 652 bp from the Cytochrome Oxidase subunit I (COI), 391 bp from the Control region (D-loop, hereafter DLP) and 1003 bp from the NADH dehydrogenase 2 (ND2). Pairs of primers are listed in Table 7.

Table 7 : Primers used to amplify mtDNA genes used in this study. For ambiguous nucleotides, R corresponds to A or G, and Y to T or C

Genes	Primers	Sequences (5'-3')	Reference
COI	BirdF1	TTCTCCAACCACAAAGACATTGGCAC	Kerr et al. 2007
	P.leuCOI-R	ACTTCTGGGTGGCCAAAGAATCAGAA	Modified from VertebrateR1, Kerr et al. 2007
DLP	PigDovF	TACCATATTCATRACCCYCATA	Developed for this study
	PAL-2-reverse	GGCCTGAAGCTAGTCGTGAT	Butkauskas et al. 2008
ND2	H6313	CTCTTATTTAAGGCTTTGAAGGC	Johnson and Sorenson 1998
	L5219	CCCATACCCCGAAAATGATG	Johnson and Sorenson 1998

PCR amplifications were performed in 30 µL with 5-10 ng of DNA, or less for feather samples. For COI and D-loop genes, PCR mixes included 200 nM of each primer, or 400 nM for feather samples, and 200 µM of each dNTP, 0.75 U of Taq DNA polymerase (Qiagen Inc.), 1X of Qiagen PCR buffer and 0.75 µg/µL of BSA, excepted for feathers DNA samples where BSA was up to 1.50 µg/µL. Temperature cycles began by an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 45 s, 50°C for 45 s, 72°C for 45 s, and 72°C for 10 min. For the ND2 gene, PCR mix was slightly different with the addition of 1X of the Q-solution® due to the presence of secondary structures, and concentration of primers were increased to 300 nM, even up to 500 nM for samples having low DNA concentration. Regarding the PCR cycles, hybridization was set at 58°C and elongation time was increased to 1 min 25s for 30 cycles. For all genes, 5 additional PCR cycles were performed for DNA samples from feathers due to the weak amount of DNA. PCR products were visualised on a 2% agarose gel to validate the amplification. They were thereafter sent to Eurofins Genomics (Germany) for sequencing according to the BigDye Terminator v3.1 cycle sequencing kit protocol from ABI (Applied Biosystems 3730x).

Electropherograms analyses and the alignment of the forward and reverse sequences were carried out with GENEIOUS Prime 2022.2.1 (https://www.geneious.com). We estimated molecular diversity indices such as haplotype diversity (h), average number of nucleotide differences between haplotypes (π), number of polymorphic site (S), Nucleotide diversity (k), and pairwise island differentiation (Φ_{ST}) with ARLEQUIN 3.5 software (Excoffier & Lischer 2010). Φ_{ST} is a F_{ST} analogue for mtDNA sequences and corresponds to the molecular distance between populations based on the number of pairwise differences between haplotypes. These values were estimated with 50,000 permutations and 5% nominal level was adjusted for multiple comparisons with Bonferroni's method. A median joining network was constructed through POPART 1.7 software (Bandelt et al. 1999, Leigh & Bryant 2015) with the three mtDNA genes. For the COI, we added to the analysis three sequences obtained on GenBank (Accession no: JQ175689, JQ175690) originated from Panama. Available data on WCPi suggest that the species is present year-round in GA in large numbers, whereas it occurs only seasonally in LA in smaller populations (Delcroix et al. 2016, Bancroft et al. 2020, Cambrone et al. 2021). Therefore, we performed an analysis of molecular variance (AMOVA; Weir & Cockerham 1984, Excoffier et al. 1992) to estimate hierarchical genetic structure at

the regional scale, and among the Lesser Antilles (LA) and the Greater Antilles (GA). In addition, AMOVA were performed according to the genetic structure evidenced by pairwise Φ_{st}. Significance of AMOVA were obtained after 10,000 permutations. We investigated demographic history relying on the neutrality and demographic history tests such as Tajima's *D* test (Tajima 1989) and Fu's F_S test (Fu & Li 1993), as well as the more conservative square differences statistic (SSD, mismatch distribution), to compare the observed distribution of the number of nucleotide differences between haplotype pairs to that expected under the null hypothesis of sudden expansion, using 10 000 bootstraps (Rogers & Harpending 1992, Harpending et al. 1993, Fahey et al. 2012). These analyses were also performed with ARLEQUIN 3.5. A Mantel test (Mantel 1967) were performed to see whether the genetic distance between populations were positively correlated with the geographic distance separating them. Genetic distance was calculated as $\Phi_{ST}/(1- \Phi_{ST})$, and a matrix of Euclidian geographic distance between islands was built using *GENALEX* 6.41 (Peakall & Smouse 2006), before performing Mantel tests using ARLEQUIN 3.5. These analyses were performed both for each mitochondrial gene separately and a sequence resulting from their concatenation. Sequences of all haplotypes will be downloaded in Genebank.

Microsatellite markers

Twenty microsatellite markers were developed by CD Genomics (New-York, United States or America; Table S1) and used to genotype 237 DNA samples of WCPi. The presence of potential genotyping errors due to null alleles or scoring errors was assessed using MICROCHECKER 2.2.3, with 10,000 iterations and Bonferonni correction (Van Oosterhout et al. 2004). We computed the number of alleles per locus (N_a), the observed heterozygosity (H_0), the expected heterozygosity in Hardy-Weinberg equilibrium (H_e; Nei 1978), and the deviation from Hardy-Weinberg equilibrium (HWE; Guo & Thompson 1992) with ARLEQUIN 3.5, considering 1,000,000 Markov chain steps and 100,000 dememorisation steps. Inbreeding coefficient (F_{IS}) was calculated in FSTAT 2.9.3 (Goudet 1995) by independently taking each island, considering separately islands from the Greater and the Lesser Antilles, and considering the entire distribution area of the sampling. We relied on F_{st} to test genetic differentiation among populations, as SPAGEDI v1.2 suggested that this metric was a better estimator of populations differentiation than R_{st}, which is an analog of F_{st} based on allele size

differences (stepwise-like mutation model; $R_{st} > F_{st}$, P = 0.806; Hardy et al. 2002). Pairwise F_{st} were therefore estimated using FSTAT 2.9.3 and P values were adjusted with Bonferroni's correction. The estimation of gene flow relying on the equation $N_m = 0.25 (1 - F_{st}) / F_{st}$ (Excoffier & Lischer 2010), which is the absolute number of migrants exchanged, per generation among between two populations. Just as mtDNA, we also performed AMOVAs based on F_{st} (Weir & Cockerham 1984, Excoffier et al. 1992) in order to estimate the hierarchical genetic structure at the regional scale and among GA and the Lesser Antilles LA. In addition, AMOVA were also performed according to genetic structure evidenced by pairwise F_{st} and STRUCTURE analysis. Significance of AMOVA were obtained after 10,000 permutations.

We visualised population structure of the species using an individual-based Bayesian clustering method, as implemented in STRUCTURE 2.2.3 (Pritchard et al. 2000), and using principal coordinate analysis (PCoA), as implemented in GENALEX 6.41 (Peakall & Smouse 2006). STRUCTURE assigns individuals into K optimal region groups according to their genetic similarity. To that end, and to determine the optimal number of genotype clusters (K), we ran simulations using admixture and correlated allele frequencies models by programming ten independent runs for K values ranging from 1 to 6 and a burn-in length of 30,000, followed by 100,000 Markov chain Monte Carlo. LOCPRIOR were also considered as prior for structure modelling in order to visualise even weak genetic clusters. Thereafter, the optimal number of genotype groups (K) was estimated using the R (version 3.6.2; R Core Team 2019) package CORRSIEVE (version 1.6-8; Campana et al. 2011) by following the ad hoc statistic ΔK method described by Evanno et al. (2005) and based on log likelihood as described in the user's manual of STRUCTURE. Clusters were finally visualized using CLUMPAK website (Kopelman et al. 2015). First generation migrants (F_0) were estimated using GENECLASS 2 (Piry et al. 2004) in order to visualise the dispersal pathway among sampled populations. To identify populations with excess or deficiency of heterozygosity suggesting bottleneck or founder effect, we relied on BOTTLENECK v1.2.02 (Piry et al. 1999). We considered IAM (Infinite Alleles Model), because as shown by SPAGEDI v1.2 (see above), this model seemed to be more appropriated than the Stepwise Mutation Model (SSM).

We relied on NeESTIMATOR v2.1 to estimate the effective population size (*Ne*) within each sampled island, at the scale of the Lesser and Greater Antilles, and considering all

locations. *Ne* calculations were based on linkage disequilibrium (LD) information (Waples & Do 2010, Do et al. 2014, Gilbert & Whitlock 2015). Although most of *Columbidae* are either strictly or seasonally monogamous (Winkler et al. 2020), the mating system of the WCPi remains uncertain (Wiley and Wiley 1979, Bancroft et al. 2020). Therefore, we run the program twice considering either random (N_{e_rand}) or monogamous (N_{e_mono}) mating system as an incorrect assumption regarding the mating system may lead to considerable bias (Waples 2006). In addition, we assumed a minor allele threshold of P_{Crit} = 0.05 to reduce bias (Waples & Do 2008). Confidence intervals at 95% were computed using the parametric method (Waples 2006). N_e can be considered as an estimate of the effective number of breeders since our samples is not age-structured (Waples & Do 2010). Divided by the census size (N_c), it provides an accurate estimation of population viability (Luikart et al. 2010).

Results

Mitochondrial polymorphism

From COI, DLP and ND2 sequences were obtained for all samples, excepted for JAM where one and two samples failed to produce good enough quality DNA for DLP and ND2 genes, respectively (Table 8). In total, we found 19, 37 and 62 different haplotypes for COI, ND2 and DLP mtDNA genes, respectively (Table 8, Figure S1-S3). The concatenation of these three genes resulted in 94 different haplotypes. Exclusive haplotypes were more frequently observed in GA compared to LA, with 52.63%, 62.16% and 66.13% of haplotypes exclusive to GA against 21.05%, 18.92% and 25.81% exclusive to LA for COI, ND2 and DLP genes, respectively. The same trend was found when using the concatenation of these genes, with 69.15% of haplotype being exclusive to GA whereas 23.40% were exclusive to LA. Moreover, although a minority of haplotypes was shared between LA and GA, they represented 86.64%, 70.69%, 54.55% and 30.13% of all samples for COI, ND2, DLP and concatenate genes, respectively. For both, each mtDNA gene and their concatenation, the USA sample had the highest haplotype diversity, followed by JAM, DOM and CUB for COI, DLP and ND2 respectively. At the regional scale, haplotype diversity was high for all mtDNA genes, with 0.946 ± 0.006 for COI, 0.976 ± 0.003 for ND2 and 0.977 ± 0.003 for DLP (Table 8 and Table S2), while at the scale of the GA and LA haplotype diversity was systematically significantly higher for GA (Table 8), whatever the mtDNA gene considered.

Based on haplotype networks constructed either with mtDNA genes separately or concatenated, no obvious genetic structure was found according to geographic location of samples (Figure 25 and Figure S1-S3). However, the high Φ_{st} values obtained [0.166 ; 0.261] suggest that the CAY population, which is a domestic population, was highly differentiated from all other island populations (Table 9). On the other hand, significant, but low Φ_{st} values [0.028;0.122] suggest that the DOM population was slightly differentiated from other GA islands, excepted BAH (Table 9). In addition, GDP population was also slightly differentiated from DOM and USA populations (Φ_{st} = 0.039; Table 9). Same results were obtained when considering mtDNA genes individually (Appendix Table S3 and S4). The absence of clear genetic structure, in addition to low values of Φ_{st}, suggests a more or less important gene flow among natural island-populations. In this respect, we found no relation between genetic differentiation and geographic distance among natural island-populations for mtDNA genes (Mantel's tests, r_{COI} = -0.246, P = 0.974; r_{ND2} = 0.111, P = 0.179; r_{DLP} = 0.111, P = 0.177; $r_{COI\text{-}ND2\text{-}DLP}$ = 0.012, P = 0.450). In addition, AMOVA indicates that genetic variation was more important within populations (> 97.37 %) than among populations (< 2.15 %) or between GA and LA (< 2.85 %), excluding or not the DOM population from GA (Table 10). This supports the weak genetic structure among island-populations of WCPi. However, although Φ-statistic was low (Φ_{CT} = 0.029), the AMOVA result indicated a significative differentiation among DOM, GA and/or LA (P = 0.029 ; Table 10), in accordance with Φ_{st} pairwise comparisons.

Neutrality tests (Tajima's D and Fu's Fs tests) and tests on the mismatch distribution of haplotypes (SSD and Harpending's raggedness index) supported demographic and spatial expansion of populations, especially at the scale of GA and LA, and at the regional scale (Table 8 and Table S2). Based on neutrality tests, both GDP and MTQ appear to have experienced demographic expansion (Table 8). For BAH, CUB and USA populations, demographic expansion was also supported using Fu's Fs, while Tajima's D values were not significantly different to zero. For the USA population, tests of mismatch distribution of haplotypes were significant, suggesting a stable spatial distribution over time, whereas Fu's Fs test supported demographic expansion (Table 8).

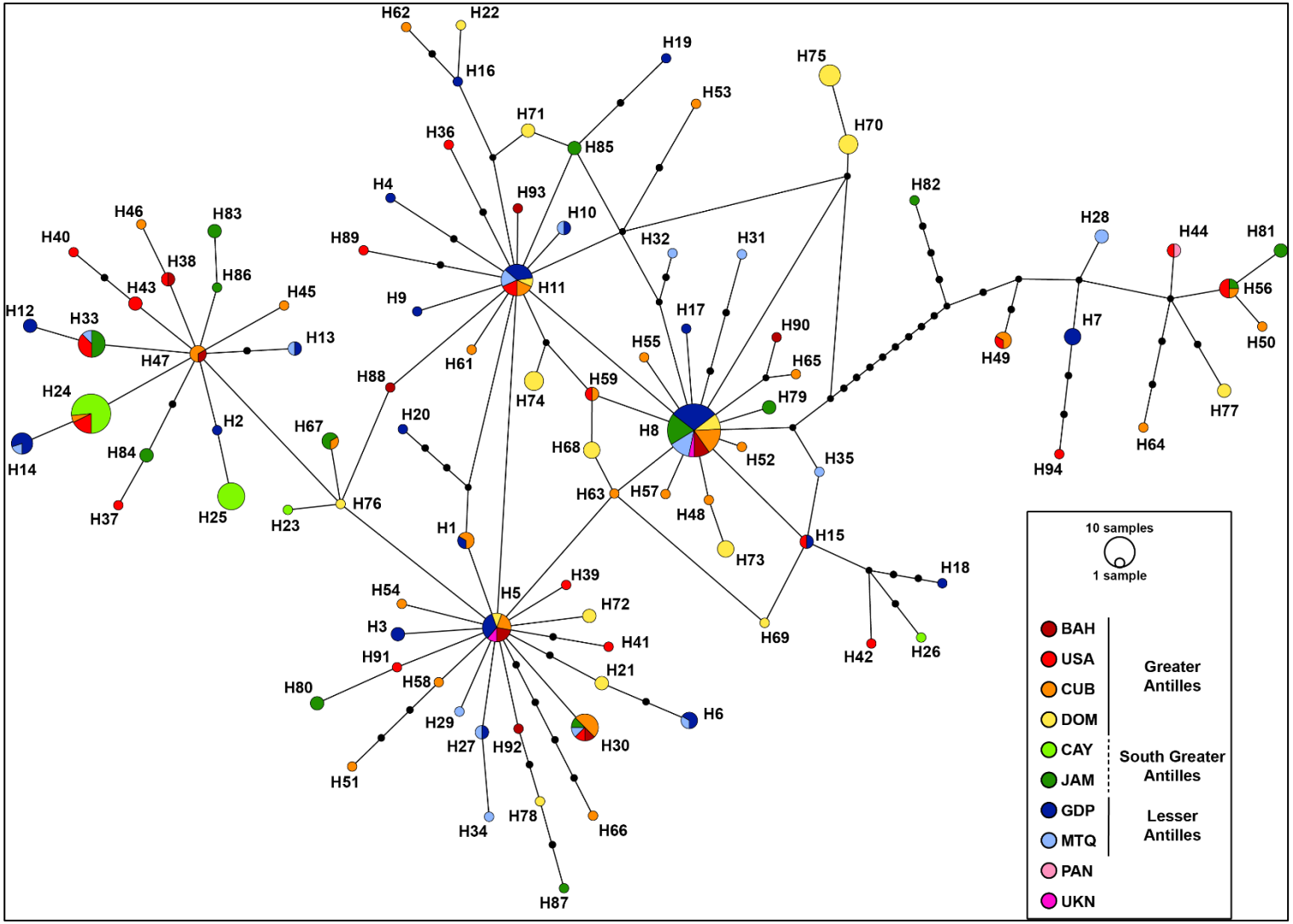


Figure 25 : Median joining haplotypes network constructed with the concatenation of COI, DLP and ND2 of *Patagioenas leucocephala*. Each circle represents a haplotype and its size is proportional to the number of individuals sharing this haplotype. Colors of circles indicate the geographic origin of individuals and the area within each circle is proportional to the number of individuals originating from the same geographic location. One continuous segment indicates one nucleotide difference. See Appendix Figure S1-S3 for haplotypes networks individually constructed for COI, DLP and ND2 mtDNA genes

Table 8 : Genetic characteristics of island-populations of white-crowned pigeons sampled for the study estimated from the result of the concatenation of COI, DLP and ND2 mtDNA genes, and from microsatellite markers. Based on mtDNA, neutrality statistics were also presented. For island acronyms, see Table 6. GA and LA means Greater Antilles and Lesser Antilles, respectively. GA gathers samples collected in island-populations of BAH, USA, CUB, DOM and JAM, and LA gathers samples of GDP and MTQ populations. CAY was not included in GA and All locations because samples were collected from a captive population. Variables are: Sample size (N), Number of polymorphic sites (S), Haplotypic diversity (h), Average number of nucleotide differences between haplotypes (π), Nucleotide diversity (average over loci, k), average number of alleles per microsatellite loci (N_a), observed and expected heterozygosity (H_O and H_e, respectively), the inbreeding coefficient (F_{is}) and effective population size (Ne) with confidence interval at 95% [95%CI]; s.d. = standard deviation. Nominal significance level was at 5%. The same table detailed for COI, ND and DLP is in Appendix Table S2.

	BAH	**USA**	**CUB**	**CAY**	**JAM**	**DOM**	**GDP**	**MTQ**	**GA**	**LA**	**All locations**
Concatenated genes											
N	12	28	40	23	29	36	42	20	116	62	233
N_h	9	20	29	4	14	16	21	15	74	36	131
S	10	44	51	11	39	34	46	39	80	54	112
h ± s.d.	0.939 ± 0.058	0.974 ± 0.017	0.974 ± 0.014	0.581 ± 0.072	0.931 ± 0.027	0.944 ± 0.016	0.934 ± 0.024	0.958 ± 0.033	0.990 ± 0.003	0.966 ± 0.012	0.990 ± 0.002
π ± s.d.	2.742 ± 1.562	9.029 ± 4.290	6.873 ± 3.303	2.261 ± 1.290	7.906 ± 3.786	5.498 ± 2.707	5.797 ± 2.829	6.653 ± 3.278	6.735 ± 3.198	6.006 ± 2.902	6.688 ± 3.167
k ± s.d. ($x10^{-3}$)	1.340 ± 0.860	4.413 ± 2.335	3.359 ± 1.793	1.105 ± 0.703	3.864 ± 2.060	2.687 ± 1.471	2.833 ± 1.536	3.252 ± 1.789	3.292 ± 1.731	2.936 ± 1.573	3.269 ± 1.712
SSD	0.026 (N.S.)	**0.071*****	0.009 (N.S.)	0.144 (N.S.)	0.015 (N.S.)	0.013 (N.S.)	0.009 (N.S.)	0.017 (N.S.)	0.008 (N.S.)	0.010 (N.S.)	0.006 (N.S.)
Harpending's Raggedness	0.117 (N.S.)	**0.169****	0.011 (N.S.)	0.580 (N.S.)	0.016 (N.S.)	0.034 (N.S.)	0.012 (N.S.)	0.039 (N.S.)	0.012 (N.S.)	0.017 (N.S.)	0.010 (N.S.)
Tajima's D	-0.707 (N.S.)	-0.789 (N.S.)	-1.522 (N.S.)	-0.826 (N.S.)	-0.754 (N.S.)	-1.164 (N.S.)	**-1.611***	**-1.571***	**-1.777***	**-1.611**	**-1.969****
Fu's Fs	**-4.386****	**-5.951***	**-16.377*****	2.427 (N.S.)	-0.625 (N.S.)	-2.739 (N.S.)	**-6.021***	**-4.806***	**-24.940*****	**-21.625*****	**-24.648*****
Microsatellite polymorphism											
N	12	28	40	23	32	36	42	21	116	63	237
N_a ± s.d.	6.500 ± 3.464	8.062 ± 5.105	9.312 ± 5.425	4.062 ± 2.205	7.188 ± 4.430	8..812 ± 5.718	8.688 ± 5.665	7.312 ± 4.715	12.312 ± 8.538	9.250 ± 6.319	13.750 ± 9.511
H_o	0.724 ± 0.201	0.661 ± 0.171	0.667 ± 0.190	0.592 ± 0.250	0.668 ± 0.232	0.668 ± 0.201	0.643 ± 0.205	0.679 ± 0.239	0.672 ± 0.180	0.655 ± 0.210	0.659 ± 0.188
H_e	0.689 ± 0.188	0.679 ± 0.199	0.682 ± 0.186	0.556 ± 0.234	0.668 ± 0.209	0.671 ± 0.207	0.670 ± 0.191	0.665 ± 0.220	0.682 ± 0.190	0.671 ± 0.196	0.680 ± 0.200
Fis	0.053	0.027	0.021	-0.067	0	0.003	0.041	-0.021	0.015	0.025	0.03
N_{e_rand} [95%CI]	86.5 [25.7 ; ∞]	161.7 [69.8 ; ∞]	∞ [303.2 ; ∞]	2.9 [2.4 ; 4.2]	9.4 [7.7 ; 11.3]	48.1 [35.6 ; 69.8]	117.0 [67.8 ; 325.1]	∞ [128.4 ; ∞]	991.8 [386.3 ; ∞]	246.1 [129.7 ; 1255.6]	824.8 [456.7 ; 3092.2]
N_{e_mono} [95%CI]	174.9 [52.9 ; ∞]	325.8 [141.3 ; ∞]	∞ [607.8 ; ∞]	8.8 [6.6 ; 11.4]	21 [18.0 ; 24.6]	97.7 [72.8 ; 141.1]	235.5 [137.2 ; 651.6]	∞ [259.0 ; ∞]	1984.8 [774.1 ; ∞]	493.6 [261.0 ; 2512.6]	1651.1 [915.0 ; 6186.7]

Table 9 : Pairwise Φ_{st} values calculated from the concatenation of COI, ND2 and DLP mtDNA genes (above the diagonal) and F_{st} values (and N_m, the number of effective migrants among populations) calculated from microsatellite markers (below the diagonal). Significant values after Bonferroni's correction are in bold (* = P < 0.05; ** = P < 0.01; *** = P < 0.001). For pairwise Φ_{st} values calculated from each mtDNA individually, see Appendix Table S3 and S4.

	BAH	USA	CUB	CAY	JAM	DOM	GDP	MTQ
BAH		0.036	-0.010	**0.261*****	0.011	0.033	-0.002	-0.003
USA	0.007 (34.472)		0.013	**0.166*****	0.029	**0.039*****	**0.039****	0.019
CUB	0.005 (48.770)	0.003 (99.750)		**0.197*****	0.016	**0.028****	0.012	0.001
CAY	**0.089***** **(2.575)**	**0.084***** **(2.740)**	**0.083***** **(2.751)**		**0.238*****	**0.226*****	**0.228*****	**0.235*****
JAM	**0.031***** **(7.867)**	**0.021***** **(11.827)**	**0.031***** **(11.827)**	**0.099***** **(2.283)**		**0.046****	0.024	0.006
DOM	0.007 (37.629)	**0.013**** **(18.689)**	0.006 (42.853)	**0.080***** **(2.867)**	**0.033***** **(7.442)**		**0.039***	0.030
GDP	**0.007**** **(38.212)**	**0.011***** **(23.560)**	**0.007***** **(37.629)**	**0.068***** **(3.410)**	**0.028***** **(8.808)**	**0.010***** **(25.003)**		-0.009
MTQ	**0.021*** **(11.432)**	**0.011*** **(23.560)**	0.011 (22.477)	**0.089***** **(2.550)**	**0.024***** **(10.210)**	0.015 (15.984)	0.008 (29.871)	

Table 10 : AMOVA results for populations of white-crowned pigeons based on the genetic differentiation revealed by pairwise Φst comparisons. CAY was not included in this analysis because samples were collected from a breeding program population. Significant F-statistic are in bold.

	df	**Sum of squares**	**Variance components**	**% of variation**	**Φ-statistics**	**P**
GA and LA, including DOM in GA						
Between LA and GA	1	4.064	-0.019	-0.57	Φ_{CT} = -0.006	0.619
Among populations within LA and GA	5	26.429	0.0716	2.15	Φ_{SC} = 0.021	**0.039**
Within populations	199	655.424	3.294	98.43	Φ_{ST} = 0.016	**0.032**
Total	205	685.917	3.346			
GA and LA, excluding DOM from GA						
Between LA and GA	1	5.137	0.027	0.79	Φ_{CT} = 0.008	0.196
Among populations within LA and GA	4	12.409	-0.012	-0.34	Φ_{SC} = -0.004	0.566
Within populations	164	559.202	3.41	99.56	Φ_{ST} = 0.004	0.393
Total	169	576.747	3.425			
GA vs. LA vs. DOM						
Among LA, GA and DOM	2	18.085	0.097	2.85	Φ_{CT} = 0.029	**0.029**
Among populations within LA and GA (and DOM)	4	12.409	-0.007	-0.22	Φ_{SC} = -0.002	0.562
Within populations	199	655.424	3.294	97.37	Φ_{ST} = 0.026	**0.035**
Total	205	685.917	3.383			

Microsatellite polymorphism

We genotyped a total of 237 individual using twenty different microsatellites (Table 8). Screening procedure for scoring errors or null alleles evidenced four markers (Table S1). Accordingly, departures from HWE were found only for these markers due to heterozygote deficiency. Considering all samples, we found an average of 13.750 ± 9.511 (± sd) alleles per

locus, with a mean (± sd) observed heterozygosity equal to 0.679 (± 0.239), varying from 0.592 (± 0.250; CAY) to 0.724 (± 0.201; BAH, see Table 8). Global F_{is} was estimated at 0.030, extending from -0.067 (CAY) and 0.053 (BAH; Table 8). A summary of genetic data for all populations is presented in Table 8.

According to pairwise comparison of F_{st} values, the strongest genetic differentiation was detected between the captive breeding population of CAY and all other natural island-populations (F_{st} =0.069 - 0.099, Table 9). Otherwise, pairwise comparisons of F_{st} values support the existence of three genetic populations, (i) Greater Antilles (minus JAM), (ii) Lesser Antilles and (iii) JAM, although differentiation values are relatively low. However, DOM was also significantly differentiated from GDP in the Lesser Antilles and from USA in the Greater Antilles, but not from other populations (excepted JAM and CAY). These levels of genetic differentiation were illustrated using STRUCTURE plots (Figure 26). At the optimal number of

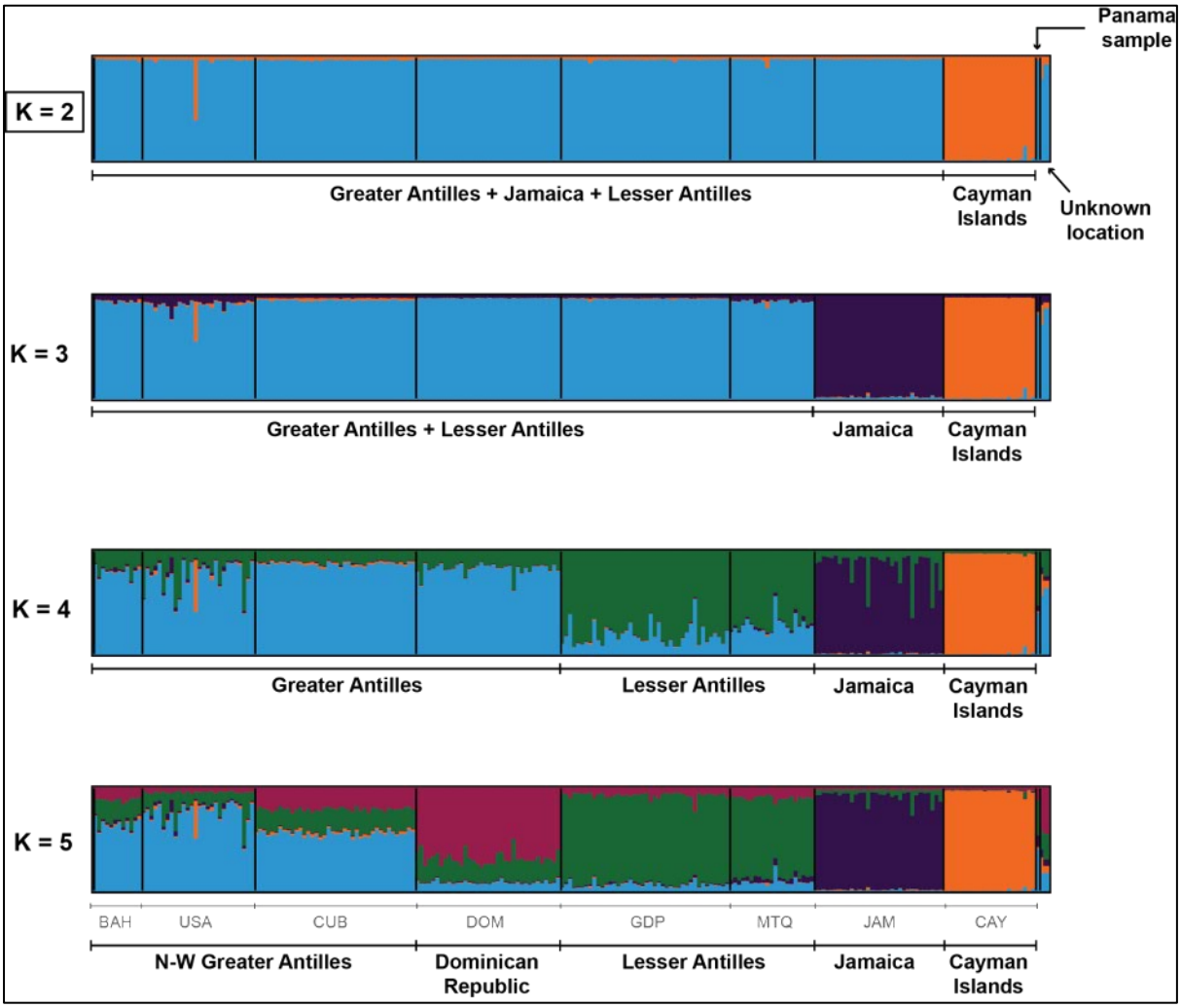


Figure 26: Structure clustering plots estimated from White-crowned Pigeon island-populations used in this study. Framed "K" where the optimal number of genotype clusters according to the method describes by Evanno et al. (2005). Evanno's plot were presented in Appendix Figure S4.

genotype clusters K = 2, as recommended by Evanno's method (Evanno et al. 2005), the breeding program population of CAY appeared as a genotype cluster, illustrating its strong genetic differentiation from other natural-islands. Considering higher Ks (3 to 5; Figure 26), other genotype clusters appeared, suggesting the differentiation of (i) JAM population (K = 3), (ii) Lesser Antilles (GDP and MTQ; K = 4), (iii) Greater Antilles (K = 4-5) and (iv) DOM population (K = 5), in accordance with pairwise comparison based on F_{st}. In contrast, when visualising genetic cluster using PCoA, only CAY appeared to be differentiated from other island-populations according to the first principal component (Figure 27).

Similar to mtDNA analyses, AMOVA results indicated that genetic variation was more important within populations (> 99.004 %) than among populations (< 1.602 %), and between GA and LA (< 1.093 %), excluding or not DOM and JAM populations from GA, or considering DOM and JAM as groups independently of GA (Table 11). This suggests a high level of connectivity among island populations of WCPi, in accordance with mtDNA data. However, although F-statistics values were very low, F_{ST} and F_{SC} estimates were significantly different to zero in all cases (Table 10), reflecting a weak, albeit significant, genetic differentiation between DOM and/or JAM and some other island populations, as suggested by pairwise F_{st} comparison and STRUCTURE analysis (Table 9 and 11).

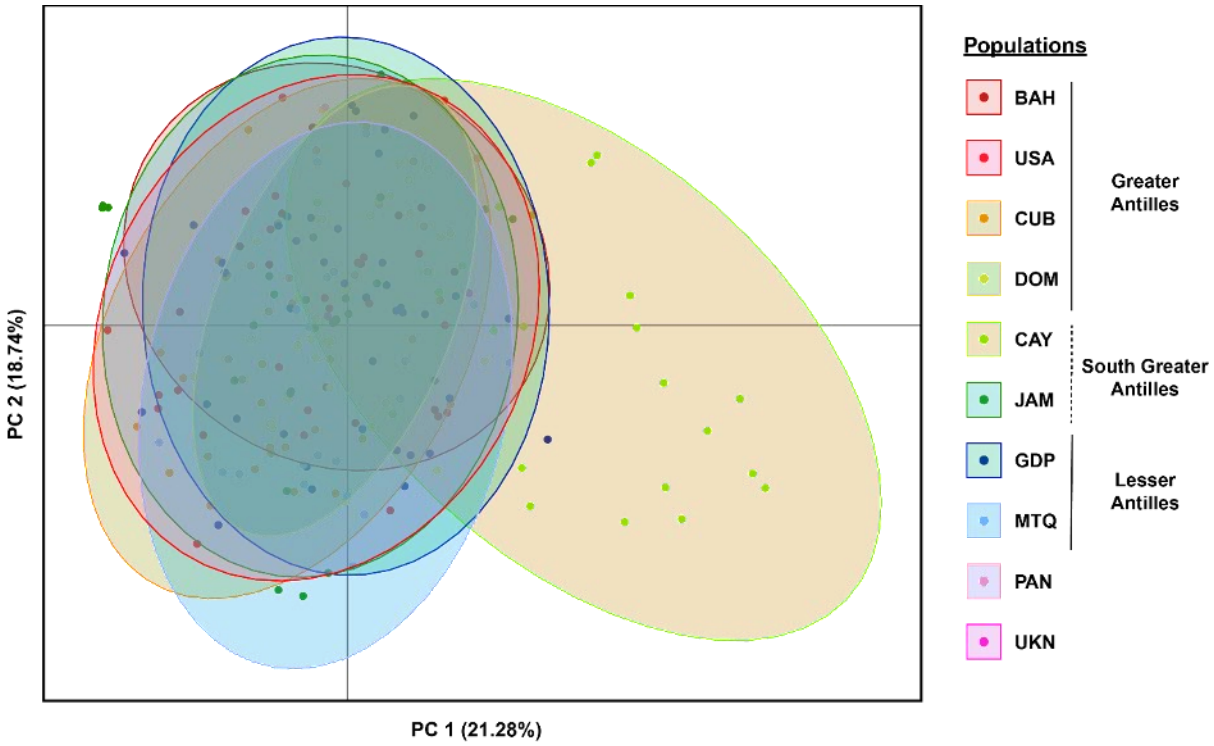


Figure 27 : Plot from the principal coordinate analysis (PCoA) relying on the genetic distance estimated among each individual and calculated using GENALEX.

Table 11 : AMOVA results for populations of white-crowned pigeons based on the 16 microsatellite markers. CAY was not included in this analysis because samples were collected from a captive population. In bold are significant F-statistic.

	Sum of squares	Variance components	% of variation	F-statistics	P
GA and LA, including DOM and JAM in GA					
Between LA and GA	10.657	-0.003	-0.059	F_{CT} = -0.001	0.478
Among populations within LA and GA	51.325	0.085	1.555	F_{SC} = 0.016	**0.000**
Within populations	2235.877	5.390	98.504	F_{ST} = 0.015	**0.000**
Total	2297.860	5.472			
GA(JAM) and LA, excluding DOM from GA					
Between LA and GA	10.889	-0.003	-0.048	F_{CT} = -0.001	0.480
Among populations within LA and GA	40.537	0.088	1.598	F_{SC} = 0.016	**0.000**
Within populations	1854.988	5.395	98.451	F_{ST} = 0.016	**0.000**
Total	1906.414	5.480			
GA(DOM) and LA, excluding JAM from GA					
Between LA and GA	10.841	0.016	0.291	F_{CT} = 0.003	0.071
Among populations within LA and GA	30.819	0.042	0.762	F_{SC} = 0.008	**0.000**
Within populations	1899.330	5.399	98.947	F_{ST} = 0.003	**0.000**
Total	1940.991	5.456			
GA and LA, excluding DOM and JAM from GA					
Between LA and GA	10.570	0.021	0.376	F_{CT} = 0.004	**0.030**
Among populations within LA and GA	21.393	0.032	0.620	F_{SC} = 0.006	**0.006**
Within populations	1518.442	5.407	99.004	F_{ST} = 0.010	**0.000**
Total	1550.405	5.462			
GA(JAM) vs. LA vs. DOM					
Among LA, GA and DOM	21.446	-0.006	-0.107	F_{CT} = -0.001	0.535
Among populations within LA and GA (and DOM)	40.537	0.088	1.602	F_{SC} = 0.016	**0.000**
Within populations	2235.877	5.390	98.505	F_{ST} = 0.015	**0.000**
Total	2297.860	5.472			
GA(DOM) vs. LA vs. JAM					
Among LA, GA and JAM	31.164	0.060	1.093	F_{CT} = 0.011	**0.000**
Among populations within LA and GA (and JAM)	30.819	0.042	0.759	F_{SC} = 0.008	**0.000**
Within populations	2235.877	5.390	98.147	F_{ST} = 0.019	**0.004**
Total	2297.860	5.492			
GA vs. LA vs. DOM vs. JAM					
Among LA, GA, DOM and JAM	40.589	0.058	1.058	F_{CT} = 0.011	**0.006**
Among populations within LA, GA, DOM and JAM	21.393	0.034	0.624	F_{SC} = 0.006	**0.008**
Within populations	2235.877	5.390	98.318	F_{ST} = 0.017	**0.000**
Total	2297.860	5.482			

In addition, when considering JAM as an independent group in AMOVA, F_{CT} were significantly different from zero, suggesting than the JAM population is actually genetically differentiated from LA, GA and/or DOM (Table 9 and 11). Moreover, when considering GA and LA without

DOM and JAM in GA in the AMOVA analysis, we found a low level of differentiation between LA and GA (Table 11), as suggested by STRUCTURE analysis (K = 4; Figure 26). Low values of F-statistics suggested however an important connectivity between island populations. Indeed, overall, 17 WCPis (~7%) were identified as F_0 migrants (Table 12). Among the total of identified F_0 migrants, 65% came from GA and 35% from LA, regardless of their final destination. However, the proportion of F_0 migrants did not differ between GA and LA (Fisher's test; GA = 11/171 LA = 6/63; P = 0.574). In addition, the proportion of F_0 migrants moving from GA to LA did not differ from that in the reverse direction (Fisher's test; $F_{0\ GA \rightarrow LA}$ = 3/11 and $F_{0\ LA \rightarrow GA}$ = 3/6; P = 0.643). Interestingly, movements of individuals did not necessarily occur between neighbour populations (Table 12). For instance, WCPis collected in MTQ and GDP were assigned to CUB and DOM island-populations, while individuals collected in CUB and DOM population-islands were assigned to GDP. In this respect, no correlation was found between genetic differentiation and geographic distance, excluding or not the CAY population (Mantel's tests, $r_{\text{with CAY}}$ = -0.157, P = 0.920; $r_{\text{without CAY}}$ = 0.025, P = 0.384). However, we did not identify F_0 migrants in CAY and JAM (Table 12), what is in accordance with the genetic structure totally or partially evidenced by analyses of pairwise F_{st} comparison (Table 9), STRUCTURE (Figure 26), PCoA (Figure 27) and AMOVA (Table 11). No bottleneck was found in our samples (Wilcoxon signed-rank tests, P > 0.05 for a deficit of heterozygosity), but an excess of heterozygosity was detected for CAY (Wilcoxon signed-rank test, P = 0.002) and JAM (Wilcoxon signed-rank test, P = 0.009).

Table 12 : Percentage of first-generation (F_0) migrants of white-crowned pigeons assessed using GENECLASS 2 to assign genotypes to island-populations sampled in this study. In parenthesis are the absolute number of white-crowned pigeons

	Assigned to								
Sampled in	BAH	USA	CUB	DOM	CAY	JAM	GDP	MTQ	Immigrants
BAH (12)	**91.7% (11)**	-	**8.3% (1)**	-	-	-	-	-	**8.3% (1)**
USA (28)	-	**85.7% (24)**	**7.1% (2)**	**3.6% (1)**	-	**3.6% (1)**	-	-	**14.3% (4)**
CUB (40)	**2.5% (1)**	-	**90.0% (36)**	**2.5% (1)**	-	-	**5.0% (2)**	-	**10.0% (4)**
DOM (36)	**2.8% (1)**	-	-	**94.4% (34)**	-	-	**2.8% (1)**	-	**5.6% (2)**
CAY (23)	-	-	-	-	**100.0% (23)**	-	-	-	-
JAM (32)	-	-	-	-	-	**100.0% (32)**	-	-	-
GDP (42)	-	-	-	**4.8% (2)**	-	-	**90.5% (38)**	**4.8% (2)**	**9.5% (4)**
MTQ (21)	-	-	**4.8% (1)**	-	-	-	**4.8% (1)**	**90.5% (19)**	**9.5% (2)**
Emigrants	**11.8% (2)**	-	**23.5% (4)**	**23.5% (4)**	-	**5.9% (1)**	**23.5% (4)**	**11.8% (2)**	

Effective population size, N_e [95%IC], was estimated to be 991.8 [386; ∞] and 1,984.8 [774.1; ∞] for GA, and 246.1 [129.7; 1255.6] and 493.6 [261.0; 2512.69] for LA (Table 8), considering random mating system or monogamy, respectively. At the regional scale, the effective population size (N_{e_rand} = 824.8 [456.7; 3092.2] and N_{e_mono} = 1651.1 [915.0; 6186.7]) was lower than GA but with a larger confidence interval suggesting possible higher values. The highest within-island estimate was USA, with N_{e_rand} = 161.7 [69.8; ∞] and N_{e_mono} = 325.8[141.3; ∞] pigeons, while the second highest estimate was for GDP with N_{e_rand} = 117.0 [386.3; ∞] and N_{e_mono} = 235.5 [137.2; 651.6]. However, CUB and MTQ exhibited infinite values with the lowest values of the %95CI higher than estimates of USA and GDP, suggesting higher N_e. As expected, the lowest N_e was estimated for the CAY captive breeding population (N_{e_rand} = 2.9 [2.4; 4.2] and N_{e_mono} = 8.8[6.6;11.4]).

Discussion

Genetic diversity

Following studies on the Zenaida Dove (Monceau et al. 2013) and the Scaly-naped Pigeon (Cambrone et al. 2021), the present study is only the third one investigating the genetic diversity and structure of a Caribbean-endemic columbid species at the regional scale, using both mtDNA genes and neutral nuclear markers. The present study allowed us to unravel high levels of genetic diversity among WCPi populations at different geographical scales, especially using mtDNA (Table 13). Indeed, genetic diversity estimated through microsatellite markers was quite similar to what has been reported for other columbid species, except for the subspecies *Columba janthina nitens*, which is considered as locally critically endangered and shows critical low genetic diversity (Table 14, Ando et al. 2014). In contrast, based on mtDNA, haplotype diversity estimated for WCPi is the highest ever reported for a columbid species (Table 13). We are confident that this high value is reliable as we sequenced and aligned the forward and reverse sequences of each sample, such that risk of error during sequence read processing was low.

Table 13: Comparison of genetic diversity and differentiation estimated using mtDNA genes among columbid species. Region refers to the study region. When possible, values of genetic diversity (h, i.e., haplotype diversity) were provided as the range between the minimun and maximum values estimated among populations studied and at the global scale, i.e., considering all geographic samples as one (h_{global}). Genetic differentiation values were presented as the range between the minimun and maximum pairwise populations values of genetic differentiation (Φ_{st}) estimated among populations. This symbol "‡" means that values in the table were not presented in the original research article, but estimated here using their original data. Regarding the WCPi, the CAY population were not considered because it was a captive population. Population size estimations were extracted from the IUCN Red List. Species are sorted in descending order according to their h_{global} estimate.

Species	Region	Continent /island	IUCN	Population size	mtDNA gene	Genetic diversity	Genetic differentiation	Distance isolation	References
WCPi *P. leucocephala*	Caribbean	Islands	NT	550 k	COI + DLP + ND2	h_{COI} = 0.30 - 0.82 $h_{COI\ global}$ = 0.95 h_{DLP} = 0.55 - 0.91 $h_{DLP\ global}$ = 0.98 h_{ND2} = 0.55 - 0.89 $h_{ND2\ global}$ = 0.98 $h_{COI+\ DLP+ND2}$ = 0.58 - 0.97 h_{global} = 0.99	$\Phi_{st\ COI}$ = -0.024 - 0.181 $\Phi_{st\ DLP}$ = -0.029 - 0.070 $\Phi_{st\ ND2}$ = -0.029 - 0.070 $\Phi_{st\ COI+DLP+ND2}$ = -0.010 - 0.046	NS	This study†
Emerald Dove *Chalcophaps indica*	Vanuatu	Islands	LC	NA	DLP	h_{global} = 0.94	$\Phi_{st} \approx 0$	-	Kirchman & Franklin (2007)
European Turtle Dove *Streptopelia turtur*	Europe + Africa	Continental	VU	12,8 m	Cyt b + DLP	$h_{Cyt\ b}$ = 0.80 - 1.00 $h_{\ Cyt\ b\ global}$ = 0.91 h_{DLP} = 0.83 - 0.98 $h_{DLP\ global}$ = 0.94	$\Phi_{st\ Cyt\ b}$ = -0.035 - 0.234 $\Phi_{st\ DLP}$ = -0.030- 0.207	-	Calderon et al. (2016) Prakas et al. (2021)
Woodpigeon *Columba palumbus*	Europe + Marocco	Continental	LC	51 m	DLP	h = 0.00 - 0.95 h_{global} = 0.89	Φ_{st} = -0.008 - 0.128	-	Butkauskas et al. (2019)
Hill Pigeon *C. rupestris*	South Korea	Continental	LC	NA	DLP + Cyt b + COI	$h_{DLP+Cytb}$ = 0.75 - 0.91 h_{global} = 0.87	Φ_{st} = 0.02 - 0.21	-	Kim et al. (2022)
White-winged Dove *Z. asiatica*	Texas, U.S.A.	Continental	LC	19 m	DLP	h = 0.74 - 0.89	Φ_{st} = Ø	r = 0.200 ; P < 0.001	Pruett et al. (2011)
Eurasian Collared Dove *S. decaocto*	Europe + Caribbean (Invasive)	Both	LC	40 m	COI	h = 0.49 - 0.96 h_{global} = 0.84	Φ_{st} = 0.010	-	Bagi et al. (2018)
New Zealand Pigeon *Hemiphaga novaeseelandiae*	New Zealand	Islands	NT	NA	DLP	h = 0.56 - 0.72 h_{global} = 0.62	Φ_{st} = -0.017 - -0.007‡	-	Goldberg et al. (2011)
Eurasian Collared Dove *S. decaocto*	Japan (Invasive)	Islands	LC	40 m	COI + DLP	h_{global} = 0.62	Φ_{st} = Ø	-	Nagai et al. (2020)

Table 8 (suite)

Species	Region	Continent /island	IUCN	Population size	mtDNA gene	Genetic diversity	Genetic differentiation	Distance isolation	References
Plain Pigeon *P. inornata*	Caribbean	Islands	NT	1 k	DLP	h = 0.48 - 0.59 h_{global} = 0.56 ‡	Φ_{st} = 0.116 ‡	-	Young & Allard (1997)
Pink Pigeon *Nesoenas mayeri*	Mauritius Island	Islands	VU (EN)	250	DLP	h_{global} = 0.45	Φ_{st} = Ø	-	Swinnerton et al. (2004)
Zenaida Dove *Z. aurita*	Caribbean	Islands	LC	500 k	COI	h = 0.00 - 0.75 ‡ h_{global} = 0.40 ‡	Φ_{st} = -0.013 - 0.839	NS	Monceau et al. (2013)
Scaly-naped Pigeon *P. squamosa*	Caribbean	Islands	LC	NA	COI	h = 0.00 - 0.40 h_{global} = 0.15	Φ_{st} = -0.025 - 0.242	NS	Cambrone et al. (2021)
Japanese Wood Pigeon *Columba janthina*	Japan	Islands	NT	NA	DLP	h = 0.11 - 1.00 h_{global} = 0.12	Φ_{st} = 0.000 - 0.962	r = 0.617 ; P = 0.042	Seki et al. (2007) Ando et al. (2011, 2014)

Table 14 : Comparison of genetic diversity and differentiation estimated using microsatellite markers among columbid species. Species are sorted in descending order according to their estimated heterozygosity (H_o). Region refers to the study region. When possible, values of genetic diversity (H_o and N_a, i.e., observed heterozygosity and means of alleles per locus, respectively) were provided as the range between the minimun and maximum values estimated in populations studied. Genetic differentiation values were presented as the range between the minimun and maximum pairwise populations values of genetic differentiation (F_{st} or R_{st}) estimated among populations. Regarding the WCPi, the CAY population were not considered because it was a captive population.

Species	Region	Continent/ island	IUCN	Population size	Number of microsatellite markers	Genetic diversity	Genetic differentiation	Distance isolation	References
Feral Pigeon *Columba livia domestica*	Europe (Invasive)	Continental	-	NA	7	N_a = 5.92 - 9.87 H_o = 0.62 - 0.87	R_{st} = 0.00 - 0.33	P = 0.001	Jacob et al. (2014)
Zenaida Dove *Z. aurita*	Caribbean	Islands	LC	500 k	13	N_a = 6.23 - 10.08 H_o = 0.66 - 0.79	F_{st} = 0.020 - 0.097	NS	Monceau et al. (2013)
Scaly-naped Pigeon *P. squamosa*	Caribbean	Islands	LC	NA	7	N_a = 7.14 - 8.29 H_o = 0.68 - 0.77	F_{st} = 0.008 - 0.059	NS	Cambrone et al. (2021)
WCPi *P. leucocephala*	Caribbean	Islands	NT	555 k	16	N_a = 4.06 - 8.81 H_o = 0.59 - 0.72	F_{st} = 0.003 - 0.033	NS	This study
White-winged Dove *Z. asiatica*	Texas, U.S.A.	Continental	LC	19 m	10	N_a = 6.40 - 7.00 H_o = 0.58 - 0.62	F_{st} = 0.020	r = 0.083, P = 0.039	Pruett et al. (2011)
Feral Pigeon *C. livia domestica*	Egypt (Invasive)	Continental	-	NA	11	N_a = 1.76 - 4.56 H_o = 0.31 - 0.76	F_{st} = 0.114 - 0.318	-	Ramadan et al. (2018)
Japanese Wood Pigeon *Columba janthina (nitens)*	Japan	Islands	NT	NA	11	N_a = 1.00 - 3.13 H_o = 0.00 - 0.30	F_{st} = 0.063 - 0.806	-	Seki et al. (2007) Ando et al. (2011, 2014)
Galapagos Dove *Z. galapagoensis*	Galapagos archipelago	Islands	NT	NA	9	N_a = 4.60 - 5.80 H_o = Ø	F_{st} = -0.004 - 0.037	P > 0.25	Santiago-Alarcon et al. (2006)

Among the species with the highest haplotype diversity values ($h > 0.80$, Table 13), only WCPi shows a distribution area mainly consisting of islands, although the study on the predominantly continental Emerald Dove concerned only the Vanuatu island populations (Kirchman & Franklin 2007). In contrast, all other species with high haplotype diversity are mainly continental with wide distribution areas and large population sizes. These features are known to be related to higher genetic diversity (Frankham 1996, 1997), whereas islands species are expected to have lower genetic diversity (Frankham 1997). Still, WCPi is characterized by very high haplotype diversity, with no marked phylogeographic structure. These features are evidence that the species has experienced recent demographic and spatial expansion (Avise 2000), what was also confirmed by neutrality tests (especially Fu's Fs tests) and tests on the mismatch distribution of haplotypes (SSD and Harpending's raggedness index) performed in the present study (Ray et al. 2003, Sæther et al. 2005). Moreover, the absence of obvious geographic structure associated with haplotype networks and their star-shape are also a signal of recent demographic and spatial expansion (Slatkin & Hudson 1991, Avise 2000). Interestingly, extensive gene flow and recent demographic expansion were also evidenced in the Vanuatu populations of the Emerald dove (Kirchman & Franklin 2007), which also shows very high haplotype diversity (Table 13). Such demographic and spatial expansion within an archipelago may take place sequentially, island by island. Due to genetic drift or founder events, newly settled populations may experience a decrease in genetic diversity during a certain time (Frankham 1996, 1997, Bollmer & Nims 2018). Then, isolation and local selective pressures may contribute to genetic diversification from other island populations (Brown et al. 2013, Bollmer & Nims 2018). The presence of second star-shape structures found in haplotype networks displayed by the WCPi illustrates this recurrent process of colonisation-partial isolation-dispersion-colonisation. Given the ability of WCPi to easily disperse over water (Bancroft et al. 2020)), the strong connectivity among island populations may mitigate the effect of both isolation by distance among islands and local adaptation (Frankham 1996, 1997, Bollmer & Nims 2018), thereby maintaining high genetic diversity at the local and regional scales.

Genetic structure

At first sight, results obtained with STRUCTURE (Evanno's optimal K) and the PCoA analysis support the existence of one panmictic population at the regional scale, as only the breeding program population of Cayman Islands was strongly differentiated from other natural-populations (Φst > 0.261; Fst > 0.099). This suggests that island-populations of WCPi are interconnected by gene flow. This is in accordance with the high-flying ability of the species, its seasonal presence in the Lesser Antilles, and year-round residency in the Greater Antilles (Delcroix et al. 2016, Bancroft et al. 2020, Cambrone et al. 2021). However, when considering other STRUCTURE plots (K = 3 to 5), Φ_{st} and F_{st} pairwise values, and AMOVA analysis three, possibly four, genetic populations appear at the regional scale, although differentiation rates are relatively low. This would suggest a more complex genetic structure than expected among island-populations of WCPi, with the existence of relatively high connectivity. Indeed, two large populations seem to be differentiated, one corresponding to GA and the other to LA. Then, JAM population appear to be differentiated from all other island populations. Finally, the DOM populations deserve particular attention as the latter is not totally differentiated from all other populations, suggesting it might be regarded as an intermediate population, or an intermediate gene pool, located at the crossroad of gene flow between the two large populations. Accordingly, DOM on Hispaniola is at the intersection between several possible flyways, with connexion to The Bahamas in the north through the Turk Caicos islands (not sampled here), with the Florida Keys (U.S.A.) on the west through Cuba, and with the Lesser Antilles on the south east through Puerto-Rico. Unfortunately, we were unable to collect DNA samples from Puerto-Rico, the Virgin Islands (U.K. and U.S.A.) and islands in the north of the archipelago of Guadeloupe, such as Antigua. The possibility therefore remains that the DOM population, together with populations from neighbour islands constitute a third major population, intermediate between the GA and LA populations.

When comparing pairwise Φ_{st} and F_{st} values obtained for WCPi with other columbid species (Table 13), values are among the lowest for the WCPi, just after the New Zealand Pigeon (Φ_{st} = -0.017 – 0.007; calculated Goldberg et al. 2011) and similar to the Galapagos Dove (F_{st} = -0.004 - 0.037; Santiago-Alarcon et al. 2006). For both species, extensive gene flow was related to their strong flying ability allowing frequent dispersal events. In this respect, the WCPi is able to perform long-distance flight, up to 150 km (Bancroft et al. 2020).

Moreover, we identified first-generation migrants in all natural island-populations of WCPi that we investigated, excepted in Jamaica, with some individuals collected at one extremity of the distribution area of the species being assigned to an island-populations located at the opposite extremity. The number of estimated effective migrants within the two major populations was relatively high, both in GA (~34-100 pigeons/generation) and LA populations (~30 pigeons/generation), and above that between GA and LA (11-38 pigeons/generation). This is in accordance with an analysis of banding recoveries suggesting inter-island movements (Bancroft et al. 2020). The dynamics of movements among island populations remains however unclear and deserves further investigations. It may however correspond to erratic or vagrant movements of individuals in response to environmental features (Gibbs et al. 2001, Bancroft et al. 2020), such as spatial and temporal variation of fruit abundance (Strong & Johnson 2001, Meyer & Zimmerman 2006), or following severe climatic events, such as hurricanes, affecting resource availability (e.g., Wiley & Wunderle 1993, Imbert 2018, Rivera-Milàn et al. 2022). Indeed, both WCPis and Scaly-naped pigeons were observed in unusual higher numbers in Guadeloupe following the passage of hurricane Maria in 2017 on Dominica (C. Cambrone, pers. obs.), an island located about 40km South of Guadeloupe, suggesting that Guadeloupe, that was not much affected by the hurricane that year, received pigeons from Dominica. Both phenomena probably contribute to explain the observed low values of differentiation and important gene flow among island populations of WCPi, particularly as the breeding season (April/May to late September, Gibbs et al. 2001, Bancroft et al. 2020) partially overlap the hurricane season (June to November) in the Caribbean.

Effective population size

The estimated effective population size, N_e, was much lower than expected, based on estimations by Bancroft et al. (2020) of about 7,500 pairs in the Florida Keys, 30,000 pairs in BAH, and between 5,000 and 10,000 in DOM. On average, N_e is one-tenth of N_c, ranging for birds between 0.05 and 0.74 (Frankham 1995). Based on estimates provided by Bancroft et al. (2020) and N_e considering random mating, N_e/N_c values for WCPi's populations of USA, BAH and DOM can be estimated at 0.011, 0.0014, and between 0.0048 and 0.0024, respectively. At the regional scale, population size was estimated at 550,000 adults (BirdLife International 2020), corresponding according to our estimation to a N_e/N_c ratio [95%CI] of

0.0015 [0.00099; 0.0056]. Except for USA population, with a N_e/N_c almost similar to what is ordinarily found in avian species, other estimates are considerably smaller, even considering higher values calculated with assumptions considering monogamy as mating system. Although N_e estimates must be interpreted cautiously due to potential biasing factors (Nunney & Elam 1994, Charlesworth 2009, Heller et al. 2013, Wang et al. 2016), the pattern of estimates is similar to the extinct Passenger Pigeon, *Ectopiste migratorius* (Hung et al. 2014), a sister taxon of *Patagioenas* species (Johnson et al. 2010, Fulton et al. 2012). Because N_e is sensitive to demographic fluctuations (Nei & Chakraborty 1975, Motro & Thomson 1982), Hung et al. (2014) suggested that the extremely low value of N_e/N_c was probably due to historical demographic fluctuations, as also suggested by previous studies (Motro & Thomson 1982, Frankham 1997, Vucetich et al. 1997). In the present case, the low value of N_e/N_c could result from several phenomena of "expansion-contraction" in terms of both population size and spatial distribution due to past climatic, food-resource or other ecological variations, as suggested to explain low N_e/N_c values in the European Turtle Dove (Calderon et al. 2016).

Implication for conservation and conclusion

The high genetic diversity in the WCPi reported here is suggestive of a low extinction risk (Reed and Frankham 2003, Spielman et al. 2004, Bollmer and Nims 2018). However, it does not mean that the species is not vulnerable, based on evidence from the critically endangered Chatham Island Taiko, *Pterodroma magentae* (Lawrence et al. 2007) or the extinct Passenger Pigeon (Hedrick 2018), for which a large population size was not a reliable indicator of extinction risk (Stanton 2014). In particular, the WCPi remains vulnerable to anthropogenic threats, such as massive habitat destruction and unregulated hunting. In that respect, we recommend to perform regular census at local and regional scales, using adequate methods such as the call-broadcast technique (Cambrone et al. 2021) to better document fluctuations in local population sizes. In addition, genetic samples from Puerto-Rico and the North of the Lesser Antilles, as well as from offshore islands of Central America, would be helpful to obtain a more complete phylogeographic structure of the species. It is also important to better document movement pattern of WCPis in its distribution area, possibly through long-term

tracking of individuals from different island-population using of increasingly accessible modern technologies such as GPS tag devices.

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Appendices

Figures

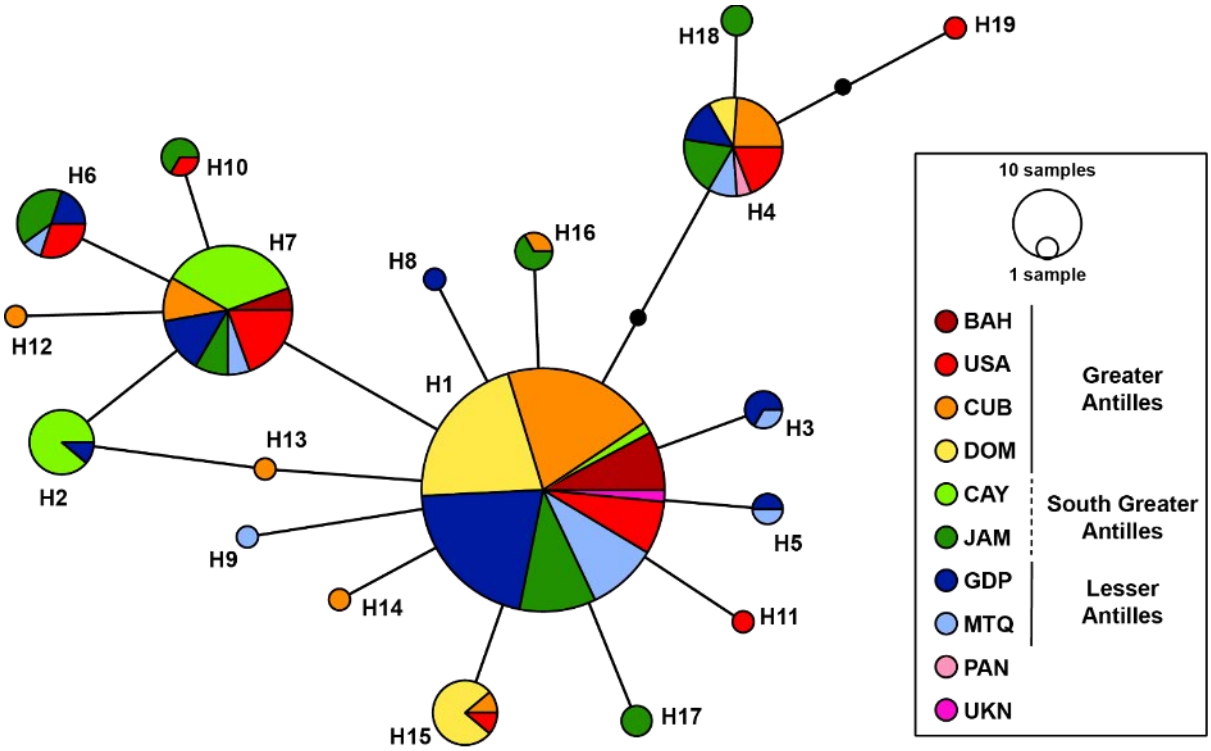


Figure S1: Median joining haplotypes network based on COI sequences obtained from *Patagieonas leucocephala*. Each circle represents a haplotype and its size is proportional to the number of individuals sharing this haplotype. Colors of circles indicate the geographic origin of individuals and the area within each circle is proportional to the number of individuals originating from the same geographic location. One continuous segment indicates one nucleotide difference.

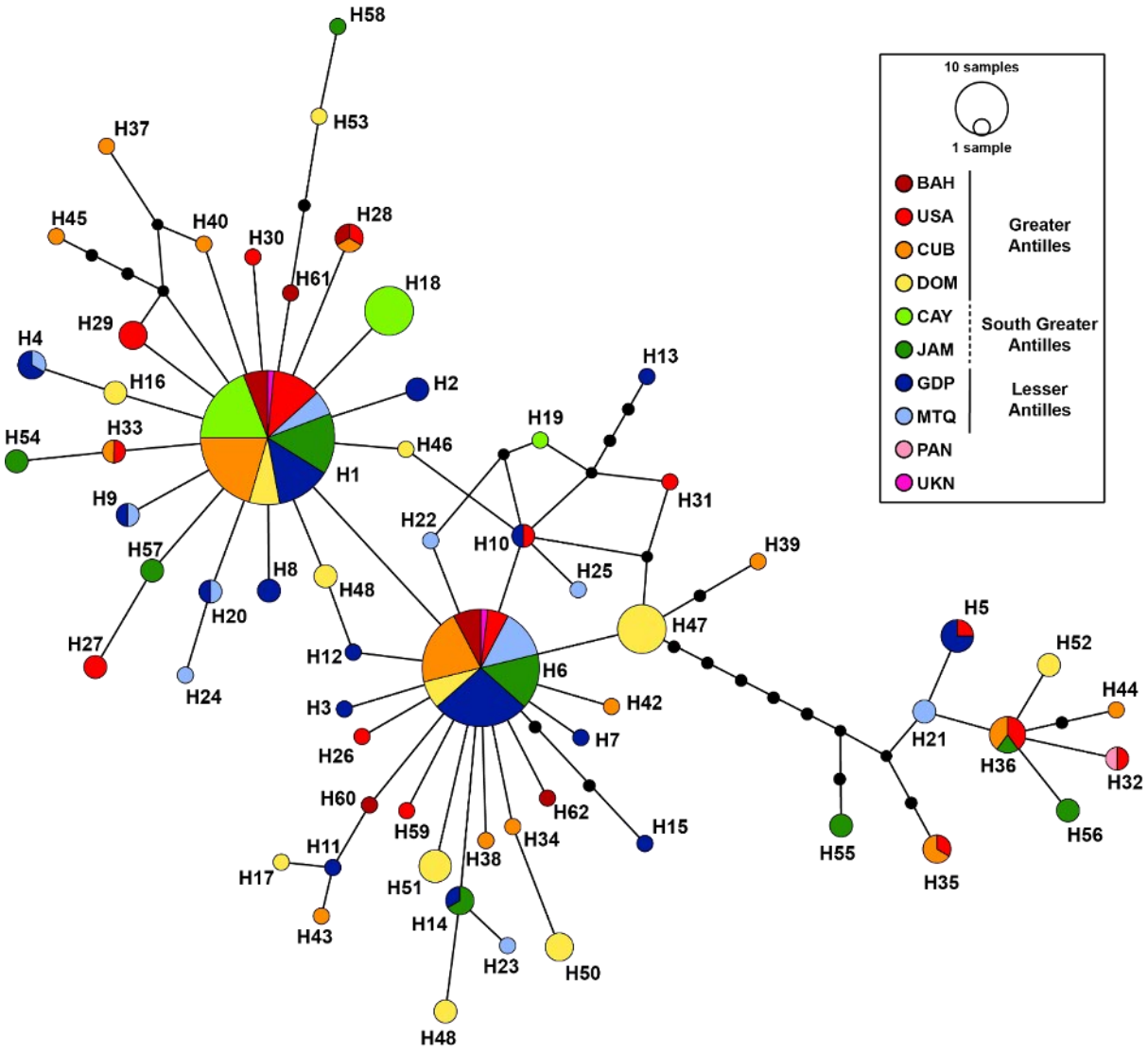


Figure S2: Median joining haplotypes network based on DLP sequences obtained from *Patagioenas leucocephala*. Each circle represents a haplotype and its size is proportional to the number of individuals sharing this haplotype. Colors of circles indicate the geographic origin of individuals and the area within each circle is proportional to the number of individuals originating from the same geographic location. One continuous segment indicates one nucleotide difference.

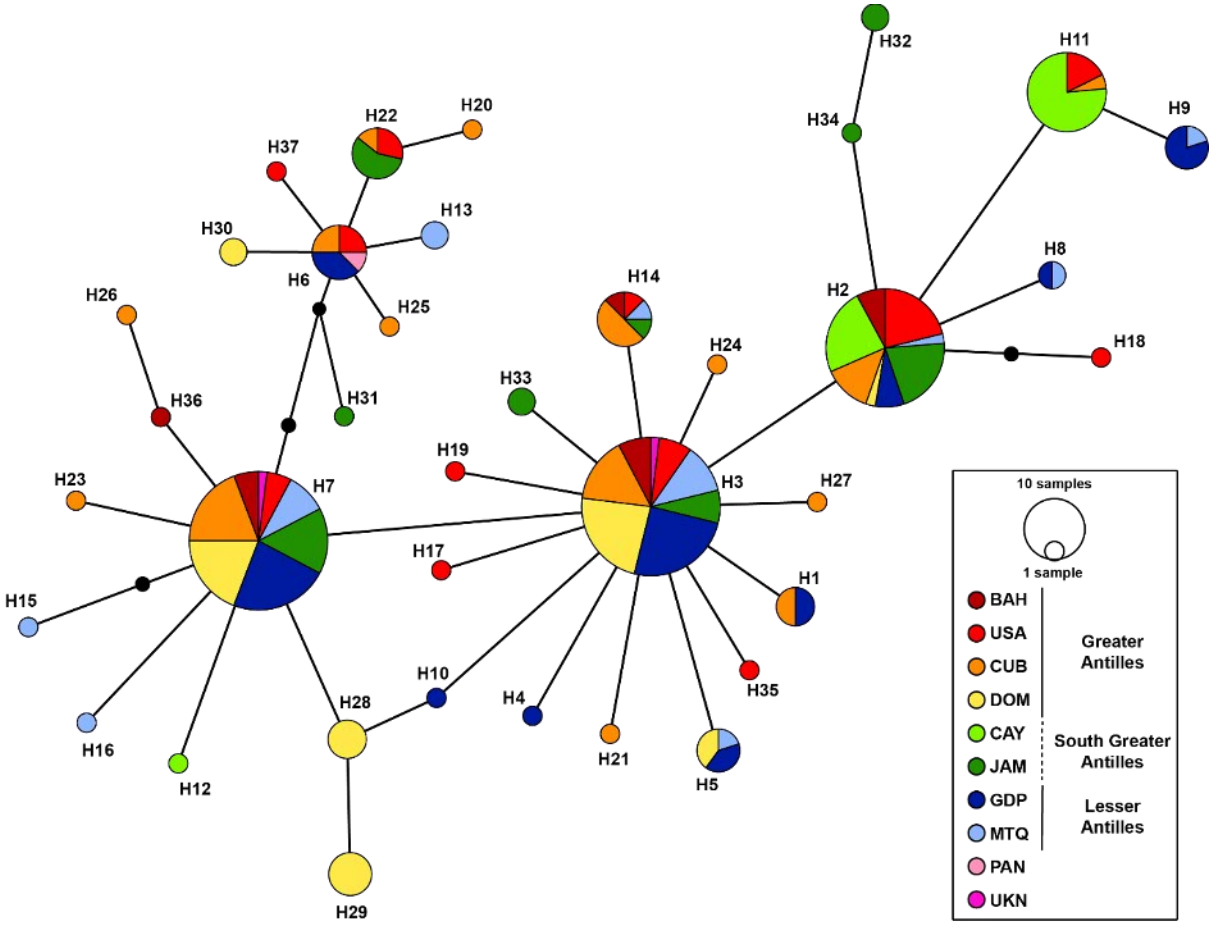


Figure S3: Median joining haplotypes network based on ND2 sequences obtained from *Patagieonas leucocephala*. Each circle represents a haplotype and its size is proportional to the number of individuals sharing this haplotype. Colors of circles indicate the geographic origin of individuals and the area within each circle is proportional to the number of individuals originating from the same geographic location. One continuous segment indicates one nucleotide difference.

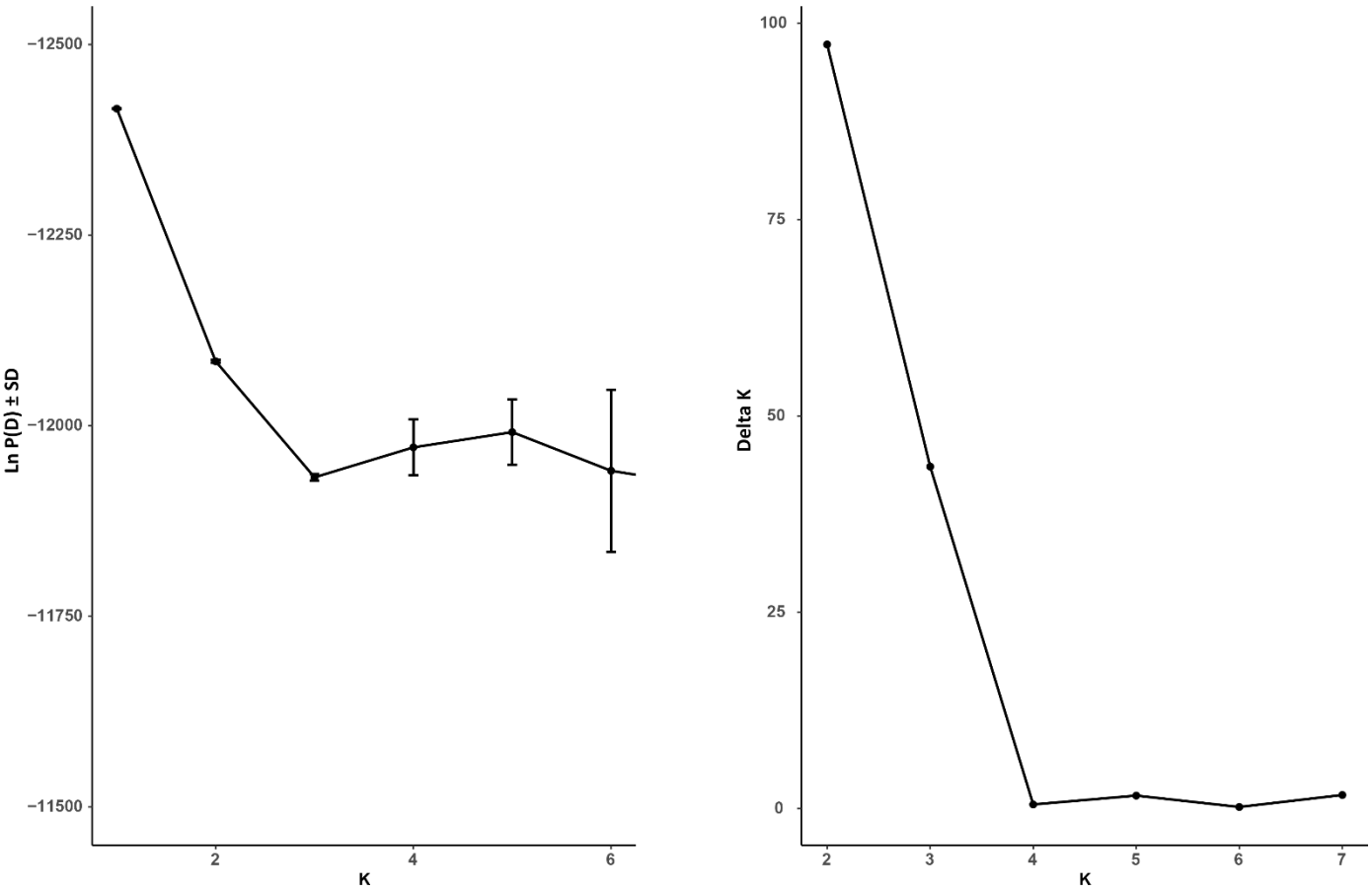


Figure S4: Plots to determine the optimal number of genotype cluster (K) according to Evanno et al. 2005 (Mol. Ecol. 14:2611-2620).

Tables

Table S1 : Microsatellite primers and motifs used to genotype white-crowned pigeons. In bold are markers having evidenced scoring errors or null alleles in our study according to Microchecker analysis.

Primer name	Repeats	Primer Sequence (5'-3')
N1010040-F	(TGGC)6	GGTGGATGGATGGATAGGTG
N1010040-R		GGAGGAAGAAGTGGTGTCCA
N1016506-F	(CCT)6	TTGCTTGCTATGGCTGAGAA
N1016506-R		CCAGAGCTCCTCCTCAGAGT
N1020077-F	(AAT)5	ACCTCATGTGGGGAAAACAG
N1020077-R		AAACCACAAAGGTGTCTCGG
N1022518-F	(TTG)12	GTTACCTTATGCCCCAGCAA
N1022518-R		GCCCTTGGTATATAGCAGCTTG
N1023651-F	(ATT)15	AATTGCTGCCCAAACTCAAG
N1023651-R		CCCTAAAACCATGCTCCTCC
N1026645-F	(TCT)8	TGGAAGGAACAGTGTTGGTG
N1026645-R		AACACAGCACTGCCACAAAG
N1035855-F	(AAAC)7	CAGGGCAGTTATGGAGCAAT
N1035855-R		CATCCTGCAGATGTTGTTTCA
N1044493-F	(ATT)10	GAATCACAGCCAGAGAAGCC
N1044493-R		AGCATGGCTGCCTTTATGAG
N1046621-F	(AAAT)5	TGGAGTCACACAAGTGGCTT
N1046621-R		TTTCCCCAAATTACTCACTGC
N1047610-F	(TAGA)8	TGTTTGCATCTTTTGCCAGA
N1047610-R		GGTTGACAGCTCCTGAGAGG
N1047812-F	(TAA)7	AGAGGGCACGTTCACCATAG
N1047812-R		TTCTTCATACAGGCATCCTCC
N1053119-F	(TAT)6	TCCCATTGTGTCTTGCAGAA
N1053119-R		AAATGTCCCTGCTGCTGAAT
N1053277-F	(CATC)18	CATCCATGTGTGCATCCTTC
N1053277-R		ACAGATGGTGGGAGAGTTGG
N1054896-F	(CCAT)7	AAACGAGGTCTCGTCTGTGG
N1054896-R		GGCAGCCACCACTTGTTTAT
N1082841-F	(ATA)12	CAGGACAGCAAAGGAAGAGAG
N1082841-R		AATACTGCTGGTCGGGAATG
N1083822-F	(AAC)5	AGTGTCCAGGGACCAGAGTG
N1083822-R		CTTAAGCTGCCAGCAGTGTG
N1065936-F	(TTTC)5	AATTGTCAACCAGCGAGACC
N1065936-R		GCTGACAGCAAGACCAAACA
N1075248-F	(TGTA)5	AACACCAGTAGACATGGGGC
N1075248-R		GCTGTCCTGGCTGAGAGAAC
N105361-F	(TGTT)8	GAAAAGTGGTGGGTTTGGTC
N105361-R		TGCTTAACAAAGACCTCCCAA
N1054205-F	(TATC)16	TCTTCCAACCAAAACAAAATGA
N1054205-R		CATCTGTCTGATGCCACAAA

Table S2 : Genetic characteristic of island-populations of white-crowned pigeons sampled for the study, based on each mtDNA genes and the result of their concatenation. Neutrality statistic were also presented. For island acronyms, see Table 1. GA and LA means Greater Antilles and Lesser Antilles, respectively. GA gathers samples collected in island-populations of BAH, USA, CUB, DOM and JAM, and LA gathers samples of GDP and MTQ populations. CAY was not included in GA because samples were collected from a captive population. Variable are: Sample size (N), Number of polymorphic sites (S), Haplotypic diversity (h), Average number of nucleotide differences between haplotypes (π), Nucleotide diversity (average over loci, k); s.d. = standard deviation. Nominal significance level was at 5%.(* = P < 0.05; ** = P < 0.01; *** = P < 0.001)

	BAH	USA	CUB	CAY	JAM	DOM	GDP	MTQ	GA	LA	All locations
COI (652 bp)											
N	12	28	40	23	32	36	42	20	116	62	233
N_h	2	8	8	3	8	3	8	7	21	15	47
S	1	9	8	2	8	3	8	7	13	9	19
h ± s.d.	0.303 ± 0.148	0.823 ± 0.044	0.563 ± 0.087	0.577 ± 0.069	0.804 ± 0.057	0.408 ± 0.086	0.575 ± 0.085	0.642 ± 0.118	0.876 ± 0.017	0.771 ± 0.046	0.946 ± 0.006
π ± s.d.	0.303 ± 0.337	1.738 ± 1.042	0.923 ± 0.651	0.640 ± 0.517	1.756 ± 1.046	0.538 ± 0.457	0.916 ± 0.647	1.047 ± 0.726	1.001 ± 0.679	0.946 ± 0.657	1.177 ± 0.760
k ± s.d. ($x10^{-3}$)	0.465 ± 0.582	2.666 ± 1.779	1.416 ± 1.109	0.982 ± 0.885	2.693 ± 1.785	0.825 ± 0.779	1.405 ± 1.102	1.606 ± 1.243	1.535 ± 1.154	1.450 ± 1.117	1.806 ± 1.289
SSD	**0.00191***	0.00140 (N.S.)	0.00320 (N.S.)	**0.0390***	0.00385 (N.S.)	2.274 e-5 (N.S.)	0.00241 (N.S.)	0.00343 (N.S.)	0.002 (N.S.)	0.002 (N.S.)	0.002 (N.S.)
Harpending's Raggedness	0.247 (N.S.)	0.0478 (N.S.)	0.0613 (N.S.)	0.215 (N.S.)	0.0405 (N.S.)	0.136 (N.S.)	0.0538 (N.S.)	0.0408 (N.S.)	0.047 (N.S.)	0.049 (N.S.)	0.039 (N.S.)
Tajima's D	-0.195 (N.S.)	-0.785 (N.S.)	-1.452 (N.S.)	0.405 (N.S.)	-0.347 (N.S.)	-0.574 (N.S.)	-1.432 (N.S.)	**-1.543***	**-1.560***	-1.365 (N.S.)	**-1.638***
Fu's Fs	0.297 (N.S.)	-1.917 (N.S.)	**-3.735***	0.373 (N.S.)	-1.619 (N.S.)	0.328 (N.S.)	**-3.676** *	**-3.373****	**20.833*****	**-13.242*****	**-11.852*****
DLP											
N	12	28	40	23	30	36	42	20	116	62	231
N_h	6	15	16	3	9	12	16	10	49	26	87
S	5	21	26	5	20	22	26	18	40	28	55
h ± s.d.	0.818 ± 0.084	0.905 ± 0.042	0.823 ± 0.046	0.549 ± 0.060	0.821 ± 0.048	0.897 ± 0.029	0.846 ± 0.042	0.853 ± 0.063	0.962 ± 0.008	0.915 ± 0.022	0.977 ± 0.003
π ± s.d.	1.212 ± 0.829	4.587 ± 2.322	3.685 ± 1.903	0.846 ± 0.623	4.209 ± 2.150	3.233 ± 1.707	3.005 ± 1.600	3.258 ± 1.751	3.574 ± 1.829	3.051 ± 1.610	3.341 ± 1.721
k ± s.d. ($x10^{-3}$)	3.100 ± 2.387	11.732 ± 6.612	9.424 ± 5.406	2.163 ± 1.776	10.765 ± 6.118	8.269 ± 4.853	7.685 ± 4.545	8.332 ± 5.002	9.141 ± 5.179	7.802 ± 4.566	8.544 ± 4.869
SSD	0.0275 (N.S.)	**0.0384***	0.0251 (N.S.)	**0.0342** **	0.0255 (N.S.)	0.0153 (N.S.)	**0.0119** *	0.0166 (N.S.)	0.030 (N.S.)	0.010 (N.S.)	0.026 (N.S.)
Harpending's Raggedness	0.186 (N.S.)	0.0510 (N.S.)	0.0557 (N.S.)	0.218 (N.S.)	0.0419 (N.S.)	0.0566 (N.S.)	0.0455 (N.S.)	0.0366 (N.S.)	0.035 (N.S.)	0.035 (N.S.)	0.040 (N.S.)
Tajima's D	-0.988 (N.S.)	-0.532 (N.S.)	-1.350 (N.S.)	-1.102 (N.S.)	-0.579 (N.S.)	-1.329 (N.S.)	**-1.695***	1.346 (N.S.)	**-1.579***	**-1.566***	**-1.877****
Fu's Fs	**-2.741****	**-4.265***	**-4.687***	0.968 (N.S.)	0.515 (N.S.)	-2.226 (N.S.)	**-5.932****	-2.417 (N.S.)	**-25.916*****	**-17.059*****	**-25.983*****
ND2											
N	12	28	40	23	31	36	42	20	116	62	232
N_h	5	12	15	3	9	7	10	10	39	20	71
S	4	14	17	4	11	9	12	14	27	17	38
h ± s.d.	0.818 ± 0.070	0.889 ± 0.040	0.883 ± 0.030	0.549 ± 0.060	0.850 ± 0.036	0.795 ± 0.039	0.817 ± 0.038	0.863 ± 0.055	0.959 ± 0.007	0.903 ± 0.021	0.976 ± 0.003
π ± s.d.	1.227 ± 0.836	2.680 ± 1.470	2.265 ± 1.272	0.775 ± 0.587	2.559 ± 1.412	1.727 ± 1.030	1.876 ± 1.094	2.347 ± 1.336	2.188 ± 1.220	2.010 ± 1.148	2.234 ± 1.236
k ± s.d. ($x10^{-3}$)	1.224 ± 0.939	2.672 ± 1.632	2.259 ± 1.408	0.772 ± 0.652	2.551 ± 1.566	1.722 ± 1.141	1.870 ± 1.212	2.340 ± 1.488	2.181 ± 1.347	2.004 ± 1.269	2.227 ± 1.363
SSD	0.0273 (N.S.)	0.0146 (N.S.)	**0.0157****	**0.0327****	0.0191 (N.S.)	0.0257 (N.S.)	0.0066 (N.S.)	0.0046 (N.S.)	**0.009***	0.005 (N.S.)	0.004 (N.S.)
Harpending's Raggedness	0.171 (N.S.)	0.0525 (N.S.)	**0.0810****	0.218 (N.S.)	0.0776 (N.S.)	0.0509 (N.S.)	0.0502 (N.S.)	0.0384 (N.S.)	**0.049*****	0.043 (N.S.)	**0.041***
Tajima's D	-0.258 (N.S.)	-0.864 (N.S.)	-1.405 (N.S.)	-0.790 (N.S.)	-0.226 (N.S.)	-0.610 (N.S.)	-0.999 (N.S.)	-1.483 (N.S.)	**-1.733***	-1.343 (N.S.)	**-1.85****
Fu's Fs	-1.388 (N.S.)	-4.084 (N.S.)	**-7.203****	0.774 (N.S.)	-1.176 (N.S.)	-0.625 (N.S.)	-2.591 (N.S.)	**-3.826***	**-27.035*****	**-13.149*****	**-27.035*****

Table S3 : Pairwise Φst values based on COI and ND2 mtDNA genes below and above the diagonal, respectively. Significant values considering Bonferroni's correction method are in bold (* = P < 0.05; ** = P < 0.01; *** = P < 0.001)

	BAH	USA	CUB	CAY	JAM	DOM	GDP	MTQ
BAH		-0.005	-0.025	**0.260***	-0.011	0.008	-0.012	-0.025
USA	0.146		0.013	**0.127***	0.001	**0.080***	0.053	0.042
CUB	0.002	0.077		**0.222*****	0.005	0.024	0.002	-0.008
CAY	**0.444*****	**0.154****	**0.358*****		**0.215*****	**0.310*****	**0.284*****	**0.284*****
JAM	0.105	-0.004	0.038	**0.236*****		0.064	0.041	0.030
DOM	0.041	**0.181*****	0.030	**0.484*****	**0.122***		0.009	-0.003
GDP	-0.005	0.070	-0.013	**0.337*****	0.034	0.043		-0.019
MTQ	0.009	0.037	-0.024	**0.317*****	0.004	0.043	-0.032	

Table S4 : Pairwise Φst values based on DLP. Significant values considering Bonferroni's correction method are in bold (* = P < 0.05; ** = P < 0.01; *** = P < 0.001)

	BAH	USA	CUB	CAY	JAM	DOM	GDP	MTQ
BAH								
USA	0.003							
CUB	-0.029	0.003						
CAY	0.173	**0.129***	**0.135***					
JAM	-0.024	0.013	-0.008	**0.151***				
DOM	0.061	0.050	**0.069***	**0.204*****	**0.070***			
GDP	-0.018	0.028	0.009	**0.194*****	0.006	**0.067***		
MTQ	-0.024	0.029	0.006	**0.213*****	0.004	0.062	-0.016	

END OF ARTICLE 6

DISCUSSION, PERSPECTIVES & CONCLUSION

Implications for the conservation

Caribbean columbid species

Results from Article 1 (Chapter 1) have revealed an important disparity in scientific attention towards certain columbid species, with the most threatened ones being the least studied. This unequal acquisition of knowledge, both quantitatively and qualitatively, creates gaps that deserve to be filled in order to have a good appreciation of species extinction risk and associated threats. To sum up, according to findings presented in Article 1, a strong improvement in the knowledge on columbid species has to be achieved by paying particular attentions to habitat loss, unregulated hunting, invasive mammals, insularity, and socioeconomic features of countries and territories in which columbid species live, which are all strongly associated with extinction risk among *Columbidae* species.

Caribbean columbid species have particular ecological and biological features that have been shown to be strongly associated with high extinction risk in *Columbidae* (Chapter 1, Article 1). Indeed, most of them are insular species, what makes them quite vulnerable to disturbances as their biogeographical history in the region has been marked by a particular combination of evolutionary process (genetic drift or founder effects) and relaxed selective pressures (predation and parasitism). In addition, because most of the Caribbean columbid species are forest-dependent, they are exposed to a high rate of habitat loss within the region as it remains only about 3% of primary forests at the regional scale (Hedges et al. 2018; FAO 2021). Besides, they are exposed to invasive predators such as mongooses, rats or feral cats which can induce direct mortality on Caribbean columbid populations and modify ecosystems (e.g., le Corre et al. 2015; Miller-ter Kuile et al. 2021). Unfortunately, so far, this impact has not been assessed quantitatively. Finally, several Caribbean columbid species are much appreciated by local hunters for their meat, but also for the shooting challenge they represent thanks to their flying ability.

Equally important are the socioeconomic features of Caribbean countries, which may affect extinction risk of Caribbean columbid species. Indeed, the Caribbean region consists of 26 different sovereign countries and 19 overseas territories associated to France, the Netherlands, the United Kingdom or the United States of America, each one having its own environmental policy, because of differences in terms of economic vulnerability and biodiversity use. Consequently, this results in heterogeneous investment by countries in the preservation of the regional biodiversity. For instance, the hunting regulation of columbid

species, which are species often inhabiting several islands, is not homogeneous across the Caribbean region, with countries applying regulations (French West indies, Puerto-Rico, Jamaica) or even prohibiting hunting activities (Antigua), whereas others do not have any regulation plan or are poorly efficient at enforcing hunting bans (Haiti).

Table 15: Extinction risk (i.e., Probability to be threatened) of Caribbean columbid species estimated from the training random forest model developed in the Article 1 (Chap. 1). IAS means invasive or alien species. In bold are the two species concerned by this PhD study.

#Rank	Species	Extinction risk	IUCN consideration	Endemicity status	Natural distribution
1	*Leptotila wellsi*	0.696	Threatened (CR)	Island	Grenada
2	*Geotrygon leucometopia*	0.558	Threatened (EN)	Island	Hispaniola
3	*Patagioenas caribaea*	0.515	Threatened (VU)	Island	Jamaica
4	*Starnoenas cyanocephala*	0.508	Threatened (EN)	Island	Cuba
5	*Geotrygon caniceps*	0.482	Threatened (VU)	Island	Cuba
6	*Geotrygon versicolor*	0.470	Not threatened (NT)	Island	Jamaica
7	*Geotrygon mystacea*	0.462	Not threatened (LC)	Regional	Lesser Antilles + Puerto Rico
8	***Patagioenas leucocephala***	**0.295**	**Not threatened (NT)**	Regional	Caribbean Basin
9	*Columba livia*	0.266	Not threatened (LC)	IAS	Globally widespread
10	*Patagioenas inornata*	0.247	Not threatened (NT)	Regional	Greater Antilles
11	***Patagioenas squamosa***	**0.210**	**Not threatened (LC)**	Regional	Caribbean islands
12	*Geotrygon chrysia*	0.168	Not threatened (LC)	Regional	Greater Antilles
13	*Patagioenas corensis*	0.138	Not threatened (LC)	Non endemic	South America coasts
14	*Streptopelia decaocto*	0.108	Not threatened (LC)	IAS	Globally widespread
15	*Zenaida aurita*	0.107	Not threatened (LC)	Regional	Caribbean Basin
16	*Columbina passerina*	0.086	Not threatened (LC)	Non endemic	Central + North + South America
17	*Ducula bicolor*	0.065	Not threatened (LC)	IAS	New Providence, The Bahamas (South-eastern Asia and Oceania origin)
18	*Leptotila jamaicensis*	0.060	Not threatened (LC)	Regional	Jamaica + Central America
19	*Geopelia cuneata*	0.056	Not threatened (LC)	IAS	Puerto Rico, local escape (Australia origin)
20	*Zenaida macroura*	0.045	Not threatened (LC)	Non endemic	North + Central America
21	*Geotrygon montana*	0.043	Not threatened (LC)	Non endemic	Central + South America
22	*Zenaida auriculata*	0.026	Not threatened (LC)	Non endemic	Lesser Antilles + South America
23	*Zenaida asiatica*	0.026	Not threatened (LC)	Non endemic	Central + North America
24	*Spilopelia chinensis*	0.021	Not threatened (LC)	IAS	U.S. Virgin Islands (Asia origin)
25	*Streptopelia roseogrisea*	0.006	Not threatened (LC)	IAS	Puerto Rico, Cayman Islands (Africa origin)
26	*Turtur tympanistria*	0.004	Not threatened (LC)	IAS	Puerto Rico, local escape (Africa origin)

As a final endeavour, we predicted the extinction risk of pigeon and dove species occurring in the insular Caribbean region, considering their biological and ecological features, as well as environmental and socio-political features of the different Caribbean islands (Table 15). To that end, the same random forest model than in Article 1 (Chap. 1) was run, but Caribbean species were excluded from the training model in order to make predictions about them. Overall, results from model predictions were consistent with current conservation status. Species considered as the least vulnerable obtained extinction risk values lower than species with higher conservation considerations. Interestingly, species endemic to a single island were associated with the highest extinction risks, while non-endemic and alien columbid species were associated with lowest extinction risks (Table 15). Both WCPi and SNPi were credited with intermediate levels of extinction risk (Table 15).

The White-crowned Pigeon and the Scaly-naped Pigeon – Results summary and conservation implications

Here, we first showed that the call-broadcast method is a more efficient detection method than the passive one for both WCPi and SNPi, at least in areas where the two species occur at low density (Article 2). Indeed, we showed that the method resulted in a better estimation of both the occurrence and abundance of these two pigeon species. An unbiased spatial and temporal estimation of both occurrence and abundance is essential to assess population dynamics through time, and thus, to inform conservation and management plans, especially for game species (Morris 2003; Dourado et al. 2014). Conversely, the use of an unsuitable or poorly reliable method could induce underestimation and misinterpretation of crucial parameters used to determine extinction risk and sustainable harvest pressure (Leopold 1987; Caro et al. 2014). Furthermore, tropical insular species are known to be highly vulnerable to natural habitat loss and predation by invasive mammals (Courchamp et al. 2003; Doherty et al. 2016; Melo et al. 2018), that are major threats in the insular Caribbean. Therefore, a reliable detection method is important to quantitatively assess the spatial and temporal variation of abundance and occurrence of key species, at different levels of exposition to disturbances and/or degradations (St-Laurent et al. 2009). For instance, the abundance of the European turtle doves (*Streptopelia turtur*) is affected by landscape changes and agricultural practices (Carboneras et al. 2022; Sauser et al. 2022). In addition, the

abundance and occupancy of the New Zealand Pigeon (*Hemiphaga novaeseelandiae*) is limited by both forest loss and the predation by alien mammals (Carpenter et al. 2021). We therefore recommend the systematic use of the playback method to estimate abundance and occurrence of the two species in areas where they are at low density. Such surveys should be carried out each year, especially where the SNPi and/or the WCPi are exposed to hunting. This approach will allow researchers to advise authorities in charge of hunting regulations, such that management decisions could be based upon quantitative data collected using a scientifically proven method.

We also showed that both SNPi and WCPi populations presented relatively high genetic diversity, especially the WCPi for which genetic diversity values estimated from mitochondrial genes were the highest ever reported in a *Columbidae* species (Article 5 & 6). Such information is essential for the management and conservation of these two game species. Indeed, genetic diversity is positively correlated with species adaptedness, and long-term persistence of populations (Humphries 1995; Lande & Shannon 1996; Reed & Frankham 2003; Frankham 2010a, 2010b; Vitorino et al. 2019; Schreiber 2020). Our results thus suggest that both WCPi and the SNPi have a certain potential to adapt in response to environmental change (Westermeier et al. 1998, Frankham et al. 2002, Frankham 2005, Evans and Sheldon 2008), and a relatively long-term low risk of extinction (McNeely et al. 1990; Spielman et al. 2004; Evans & Sheldon 2008). However, the high genetic diversity revealed in WCPi does not mean that the species is not threatened, as evidenced in the critically endangered Chatham Island Taiko, *Pterodroma magentae* (Lawrence et al. 2008), or the extinct Passenger Pigeon (*Ectopistes migratorius*; Hedrick 2018). Moreover, genetic studies performed on the WCPi and SNPi only considered a small portion of their genome, that might not be representative of their entire adaptive potential (Reed & Frankham 2003). Besides, we should carefully consider genetic evidence of demographic fluctuations of WCPi's populations, as this species could be particularly sensitive to human-related threats, similar to the related Passenger Pigeon (Hung et al. 2014). Consequently, genetic results obtained here are rather encouraging for the future of the two pigeon species, but this does not mean that species are not of conservation concern. Conservation and managing efforts should therefore be taken to at least maintain this high genetic diversity, especially by maintaining large enough populations to avoid genetic drift or inbreeding depression (Frankham 1998; Hedrick & Garcia-Dorado 2016).

Genetic analyses have also highlighted the high gene flow existing among island populations suggesting significant inter-island movements for both SNPi and WCPi. This information is crucial as it provides some information about the spatial scale at which the species should be managed, particularly in relation to current threats such as hunting. Indeed, the definition of genetic management units is crucial for conservation since they allow the prioritisation of conservation efforts when differences in conservation and management needs exist between populations (Phillimore & Owens 2006; Haig & D'Elia 2010). Here, no genetic structure was found among investigated natural island populations of SNPi, suggesting there exists one single, large population, at least from Puerto-Rico to Martinique. Regarding the WCPi, only a weak genetic structure was found, pointing to the existence of four genetic populations: Greater Antilles, Dominican Republic, Lesser Antilles and Jamaica. However, genetic differentiation was relatively low, suggesting regular movements of individuals between Caribbean islands. For both species, it would be ideal to have a unique management plan at the scale of the Caribbean region given the weak genetic structure evidenced in our studies. However, as mentioned in the introduction and at the beginning of this last chapter, the geopolitical context of the Caribbean region is complex and makes the initiation of such a management plan particularly difficult. Constituting a regional consortium of columbid specialists could however be a first step towards developing such management plans and coordination of hunting regulations.

Finally, we revealed a high prevalence of haemosporidian parasites within populations of WCPi (Article 4). Our results further suggest that screening other Caribbean columbid species for blood parasites may allow the detection of new lineages and complete existing phylogenies of this widely studied group (Perkins & Schall 2002; Santiago-Alarcon et al. 2010; Galen et al. 2018). In addition, the difference in infection prevalence among screened island populations of WCPi is interesting as it suggests the existence of ecological or environmental underlying factors that would deserve to be investigated. The same approach could be performed with the SNPi or other Caribbean columbid species. Interestingly, the high observed prevalence of blood parasites in WCPi populations suggests that the species might be tolerant to costs induced by haemosporidians infections. However, studies investigating the relationship between fitness cost and malarial infections produced contradictory results (e.g., Lachish et al. 2011; Zylberberg et al. 2015; Podmokła et al. 2017; Romano et al. 2019),

as fitness costs can vary according to species, populations or even the sex of individuals (e.g., Fletcher et al. 2019). However, similar studies are lacking for Caribbean columbid species and further investigations are necessary to assess to what extent infections by haemosporidians, using both intensity and prevalence, might influence the body and physiological condition of WCPis and SNPis (e.g., Motta et al. 2013; Schoenle et al. 2017). In addition, it would be also relevant to assess whether co-infection with several haemosporidian species has a significant impact on pigeons' fitness (Pigeault et al. 2018), as we suspected simultaneous infection by several malarial lineages or species in several screened pigeons. The same is true for other parasites and pathogens (Genovart et al. 2010; Rouffaer et al. 2018).

Future research and perspectives

In order to provide reliable, consensual, and reproducible estimates of the abundance and occurrence of game species, we recommend to standardize survey methods and to make them accessible to the largest possible number of people. For instance, the call-broadcast method is an easy-to-use method, which could be used by anyone wishing to collect data to inform conservation of a given columbid species, although prior training would be recommended to recognize species' vocalizations. In addition, the use of camera traps (Jean-Pierre et al. 2022) and/or automated acoustic recorders (Pérez-Granados & Traba 2021) would simultaneously allow the collection of important amounts of data, which can be stored and made accessible to inform management decisions in full transparency. Coordinated data collection programs could involve both hunters and amateur conservationists alongside scientists, and thus help re-establishing mutual trust among stakeholders and with local authorities in charge of hunting regulation.

Besides, game species are not only threatened by unsustainable hunting. Indeed, they are also exposed to habitat loss and the unregulated presence of alien predators. The contribution of these threats to populations declines is no less or more important than that associated with the impact of hunting. Therefore, it seems important to see to what extent alien predators such as rats, feral cats or mongooses, as well as habitat degradation, contribute to the decline of WCPi and SNPi populations, and more generally Caribbean avian game species. Regarding the impact of alien predators, it would be possible to identify ingested preys by collecting their faeces or stomach contents, using DNA meta-barcoding

(e.g., Subrata et al. 2021). This method consists in using universal primers for a given taxon and sequencing all sequences targeted by these primers. Thus, by comparing produced sequences with a reference database, it becomes possible to identify prey species. Another way to quantify the impact of alien predators would be to monitor game species nests with camera traps or to rely on artificial nests so as to identify nest visitors and estimate visit rate by various exotic predators (Matysek et al. 2019; Holopainen et al. 2021; de Aguiar et al. 2022). Regarding habitat loss and urbanization, identifying the particular features of urban columbid species may help determining the ability of species to adapt to urbanization and global change. For instance, in Barbados, where it has been accidently introduced, SNPi flourish in urban areas. Comparing its foraging, social or breeding behaviour, or even morphological features with natural populations of SNPi could be of interest as this may contribute to refine the estimation of pigeon species extinction risk.

An important aspect of the WCPi and SNPi ecology initiated here is their interaction with parasites and pathogens. Although it would be very helpful to estimate the health of both bird populations and ecosystems, and to predict potential cases of zoonoses (e.g., West Nile virus, Avian influenza, Newcastle disease virus; Murray et al. 2010; Geffroy et al. 2021; Gainor & Ghosh 2022), such studies are lacking in the insular Caribbean region (Gainor & Ghosh 2022). Yet, post-mortem diagnosis is relatively easy with game species as they are annually harvested by hunters, what provides, in a "One Health" context (Willingham et al. 2016), an important and valuable source of tissues to monitor bird-related pathogens and diseases, instead of capturing them *in natura*. Such monitoring of pigeons' populations health is all the more important as strongly invasive columbid species, such as the Eurasian Collared-Dove (*Streptopelia decaoco*) are potential hosts for exotic pathogens. For instance, the latter is suspected of transmitting *Trichomonas* spp. to the native avifauna of Saint Kitts and Nevis Caribbean islands (Stimmelmayr et al. 2012).

Finally, the relevance of creating breeding programs for SNPi and WCPi has to be considered. Indeed, one way to compensate mortality caused by hunting, or other human-related cause such as unregulated invasive predators, would be to reinforce natural populations with pigeons bred in captivity. The case of the WCPi's breeding program in the Cayman Islands, hosted by the Cayman Turtle Centre and created in response to population depletion after hurricanes, is a great example. Indeed, each year, WCPi are released by people

from the local civil society that became involved in the conservation the species (Photo 6). However, the low genetic diversity and effective population size that we found in this population suggests that actions should be undertaken to reduce inbreeding depression risk (Boakes et al. 2007). Such conservation programs, however, are often popular and thus attract significant funding. Still, they require a solid scientific monitoring in order to ensure an efficient management of individuals' pedigrees (Boakes et al. 2007; Galla et al. 2020). For instance, an iconic and successful breeding program of a pigeon species endemic to Mauritius Island, the Pink Pigeon (*Nesoenas mayeri*), has allowed a rapid recovery of the population, raising from 10 wild pigeons in 1990 to currently about 480 (Swinnerton et al. 2004; Jackson et al. 2022). Although, where practical, care was taken to decrease inbreeding risk in reproduction and release programs, the current population of pink pigeons exhibits a low genetic diversity due to inbreeding depression and experienced bottleneck effect (Swinnerton et al. 2004). Thus, despite rapid rebound the population, the outcome of conservation efforts is still uncertain, with possible extinction within the next 100 years if no action is conducted to reverse this trend (Jackson et al. 2022)



Photo 6 : Primary students from Cayman Islands releasing 24 white-crowned pigeons in 2020. Pigeons were bred in the breeding program hosted in the Cayman Turtle Centre. Photo was found in the website of Cayman Turtle Centre.

Conclusion

The present PhD study brought some new fundamental knowledge about the ecology of both WCPi and SNPi and resulted in recommendations, in the light of obtained results, for management plans. Although these pigeon species are not the most endangered ones in the Caribbean region, we recommend developing shortly a regional management plan for both of them. Such an action would be less costly than waiting for the situation to possibly deteriorate in the future up to a point when conservation actions will be more difficult and more costly to implement (Walls 2018; Eberhard et al. 2022). Our study also highlighted the overall lack of knowledge about Caribbean bird species, including basic information such as, for instance, reproductive phenology. Therefore, although this work on the WCPi and the SNPi provided important results, important gaps in knowledge still have to be filled to improve the conservation and the management of the WCPi and SNPi, as is true of many other Caribbean bird species of patrimonial interest.

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Abstract

Due to its biogeographical history, the Caribbean region is one of the most important hotspots of biodiversity, characterized by a large diversity of plant and animal species, many of which are endemic to the region. However, this exceptional biodiversity is threatened by habitat loss, overexploitation, invasive species, pollution and global change. Although local authorities and the civil-society have, whenever possible, taken some actions to prevent biodiversity erosion or protect species at high risk of extinction, many species are still declining. For many of them, scientific data are too scarce to have a good appreciation of their ecology and biology in relation to threats and extinction risks. This is particularly the case of pigeon and dove species, which are constitute of one of the least studied avian families in the world and, particularly, in the insular Caribbean region. To remedy to this, we aimed to provide some guidelines to help prioritizing research on columbid species according to extinction risk and their ecology, biology and environment. In this respect, the present PhD study started with a review that relied on random forest models to quantitatively assess to what extent various biological, ecological, environmental and socio-political drivers influence extinction risk and population trends of columbid species throughout the world. We also investigated interspecific variation in levels of scientific attention. The PhD then focuses on two Caribbean endemic pigeon species, the Scaly-naped Pigeon (SNPi - *Patagioenas squamosa*) and the White-crowned Pigeon (WCPi - *Patagioenas leucocephala*), which respectively are classified as least concern and near-threatened in the IUCN Red List, but have been recently considered as endangered in the French West-indies. Like other columbid species and most Caribbean birds, the WCPi and SNPi are exposed to habitat loss, unregulated or poorly regulated hunting and invasive predators. The present PhD study aimed at bringing some new fundamental knowledge about the ecology of these two pigeon species in order to able, in the light of obtained results, to provide recommendations for management plans. In addition, we carried out methodological tests to improve on both the collection of DNA samples and the estimation of population trends in the two species. We thus compared two DNA tissue samples as source of DNA for Sanger sequencing analysis and showed that the less invasive method, which consisted in collecting buccal cells with swabs, was as efficient as blood samples, which is more invasive and stressing for pigeons. We also compared two census methods to track population fluctuations through time and concluded that the call

broadcast methods allowed a better estimation of species occurrence and abundance than traditional auditory point counts. Our results on population genetics in WCPi and SNPi allowed us to assess genetic diversity and the existing connectivity among island populations. Genetic analyses provided evidence for high gene flow among island populations, suggesting significant inter-island movements for both SNPi and WCPi. In addition, the two pigeon species presented relatively high genetic diversity, especially the WCPi for which genetic diversity values estimated using mitochondrial genes were the highest ever reported in a *Columbidae* species. In relation to the high connectivity found through genetic analyses, we initiated a satellite tracking study to investigate both regional and local movements of the WCPi. However, some technical and environmental issues prematurely stopped the acquisition of location data. Finally, we documented a high prevalence and diversity of haemosporidian, malarial blood parasite, within five island populations of WCPi, including the discovery of six new lineages of *Haemoproteus*, *Parahaemoproteus* and *Plasmodium*. Our findings are encouraging, in revealing high genetic diversity, meaning that the two species are not the most endangered columbid species in the Caribbean region. However, given their known exposure to several significant threats, the development of a regional management plan for both of them is highly recommended. Besides, important gaps in knowledge have still to be filled to improve the conservation and the management of the WCPi and SNPi, as is true of many other Caribbean bird species of patrimonial interest.

Résumé

En raison de son histoire biogéographique, la région des Caraïbes figure parmi les foyers de biodiversité les plus importants au monde, caractérisé par un grand nombre d'espèces animales et végétales, dont beaucoup sont endémiques à la région. Toutefois, cette biodiversité exceptionnelle est exposée à la perte d'habitats, la surexploitation, les espèces envahissantes, la pollution et le changement climatique. Bien que les autorités locales et la population civile aient pris, dans la mesure du possible, certaines précautions pour prévenir l'érosion de la biodiversité ou pour améliorer le statut de conservation d'espèces menacées d'extinction, de nombreuses espèces sont encore en déclin et pour beaucoup d'entre elles, les données scientifiques manquent pour bien comprendre leur écologie et leur biologie par rapport aux menaces et aux risques d'extinction qui leur sont associés. Ceci est particulièrement vrai pour les espèces de pigeons et de tourterelles, qui font partie de l'une des familles d'oiseaux les moins étudiées dans le monde et dans la Caraïbe insulaire. Pour remédier à cela, nous avons réalisé une synthèse quantitative afin de fournir des lignes directrices qui aideraient à prioriser la recherche scientifique sur les espèces de columbidés en fonction de leur risque d'extinction, de leur écologie, de leur biologie et de leur environnement. A cette fin, nous avons utilisé des modèles de forêts aléatoires pour évaluer quantitativement dans quelle mesure divers facteurs écologiques, biologiques, environnementaux et sociopolitiques pouvaient influencer le risque d'extinction et les tendances démographiques des espèces de columbidés dans le monde. De la même manière, nous avons étudié la variation interspécifique des niveaux d'attention scientifique, via une étude bibliométrique. Les autres recherches développées dans le cadre de cette thèse de doctorat se sont concentrées sur deux espèces de pigeons endémiques des Caraïbes, le Pigeon à cou rouge (PCR - *Patagioenas squamosa*) et le Pigeon à couronne blanche (PCB - *Patagioenas leucocephala*), qui sont respectivement classées comme de préoccupation mineure et presque menacées dans la liste rouge de l'UICN. Cependant, ces mêmes espèces ont récemment été considérées comme menacées dans les Antilles françaises. De manière générale, comme les autres espèces de columbidés et la plupart des oiseaux des Caraïbes, les PCB et PCR sont exposées à la perte d'habitat, à une pression de chasse peu ou mal maîtrisée et aux mammifères prédateurs exotiques. En conséquence, la présente étude visait à apporter de nouvelles connaissances fondamentales sur l'écologie de ces deux espèces de

pigeons afin de pouvoir, à la lumière des résultats obtenus, fournir des recommandations pour de futurs plans de gestion. Dans un premier temps, des études méthodologiques ont été réalisées afin de proposer des méthodes appropriées pour prélever des échantillons d'ADN et suivre les fluctuations démographiques des deux espèces. A cette fin, nous avons comparé l'efficacité de l'extraction d'ADN et du séquençage à partir de deux types de tissu biologique et démontré que la méthode la moins invasive, qui consistait à prélever des cellules buccales avec des écouvillons, était aussi efficace que celle consistant à récolter des échantillons de sang, méthode plus invasive et plus stressante pour les pigeons. Par ailleurs, nous avons comparé deux méthodes de détection sur le terrain afin de proposer une méthode fiable pour suivre les fluctuations des populations au cours du temps. Nous avons ainsi montré que l'utilisation de la repasse permettait d'obtenir une meilleure estimation sur la présence et l'abondance des espèces. Notre étude sur la génétique des populations de PCB et PCR a permis de documenter leur diversité génétique et la connectivité existante entre plusieurs populations insulaires. Ces analyses ont mis en évidence un flux de gènes élevé entre populations insulaires, suggérant d'importants mouvements inter-îles pour les deux espèces. En outre, les deux espèces de pigeons présentaient une diversité génétique relativement élevée, en particulier la PCB pour lequel les valeurs de diversité génétique estimées à l'aide de gènes mitochondriaux étaient les plus fortes jamais signalées chez une espèce de *Columbidae*. En ce qui concerne la connectivité élevée entre les populations constatée via les analyses génétiques, nous avons initié une étude de suivi par satellite pour étudier les mouvements régionaux et locaux du PCB. Cependant, certains problèmes techniques et environnementaux ont prématurément mis fin à l'acquisition de données de localisation GPS. Enfin, nous avons montré une forte prévalence et une diversité élevée d'hémosporidies dans cinq populations insulaires de PCB. Nous avons notamment découvert six nouvelles lignées appartenant à la famille des *Haemoproteus*, des *Parahaemoproteus* et des *Plasmodium*. La mise en évidence d'une forte diversité génétique chez les deux espèces est encourageante, et indique que le PCB et le PCR ne sont pas parmi les espèces de columbidés les plus en danger dans la Caraïbe insulaire. Cependant, étant donné leur exposition connue à plusieurs menaces importantes, la mise en place d'un plan de gestion à l'échelle régionale pour ces deux espèces est fortement recommandée. En outre, d'importantes lacunes dans les connaissances doivent encore être comblées pour améliorer la conservation et la gestion du PCB et du PCR, comme

c’est le cas pour de nombreuses autres espèces d’oiseaux d’intérêt patrimonial de la Caraïbe insulaire.