Ecological replacement as a restoration tool: Disentangling the impacts and interactions of Aldabra giant tortoises (*Aldabrachelys gigantea*) using DNA metabarcoding

A thesis submitted to Cardiff University for the degree of Doctor of Philosophy

by

Rosemary Jane Moorhouse-Gann

November 2017

Declaration

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

Signed ……(candidate) Date…….

STATEMENT 1

This thesis is being submitted in partial fulfillment of the requirements for the degree of ……(insert MCh, MD, MPhil, PhD etc, as appropriate)

Signed ……(candidate) Date…….

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated, and the thesis has not been edited by a third party beyond what is permitted by Cardiff University's Policy on the Use of Third Party Editors by Research Degree Students. Other sources are acknowledged by explicit references. The views expressed are my own.

Signed ……(candidate) Date…….

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available online in the University's Open Access repository and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed ……(candidate) Date…….

STATEMENT 4: PREVIOUSLY APPROVED BAR ON ACCESS

I hereby give consent for my thesis, if accepted, to be available online in the University's Open Access repository and for inter-library loans **after expiry of a bar on access previously approved by the Academic Standards & Quality Committee.**

Signed ……(candidate) Date…….

For my granddad Michael
Who makes the impossible possible

Acknowledgements

First and foremost, I would like to thank Bill and Nik for your encouragement, support and perpetual enthusiasm for this PhD research.

Thank you to the rest of my supervisory team: Mike, Rich and Nicolas. Special thanks go to Mike and Rich for your patient feedback and 'Big Picture' thinking.

Extra special thanks go to Martine (without you this project would never have been possible) and Carl (for your support and wisdom).

Thank you to Vikash and Debby for your support. Special thanks to all MWF staff, volunteers and students, and the Durrell 'dippers' for your assistance with the project, especially those who played 'who dung it' and those who answered my never-ending plant questions. Particular thanks go to Divya, Auerlie Hector, Debs Fogell, Abdula, Teesha, Christel and Issabelle. Thank you to Nadine for so patiently organising my work and residence permits for Mauritius.

Thank you to Rouben, Dany and Stephan for the smiles, laughs, plants and skink poop.

Thank you to those I lived with on Ile aux Aigrettes – especially for the Bananagrams and delicious meals. Thank you to all those who made my time working on Round Island so magical, especially Natalie Summers, Rob Hays and Jean-Hugues for your help. Thank you to Gabby Salazar for your stellar company and beautiful photos. Special thanks to the Copsey family for having me to stay in Black River.

Thank you to Claudia Baider and Kersley Pynee (Mauritius Herbarium), Isabel Larridon (Kew Gardens), and Ashok Khadun and Jean-Claude Sevathian (MWF) for assistance with plant identification.

Thank you to team Durrell and especially to Lianne, Catherine, Ros, Spike, Finella and Gwen for the motivational pep talks. Thanks to John Ewen for the nutrition chats.

Thanks to everyone in the Cardiff molecular ecology lab (family). You have shown me such incredible encouragement and support and I will never forget the laughs and, more importantly, the creative arguments - long live the 'angry fifth floor'. Special thanks to

Isa-Rita and Dave (Dr. Stantlepants) - without your guidance this PhD would look very different (and not for the better). Thank you to Pabs and Frank (and Sula) whose doors were always open for high-fives. Thank you to Josie (a wonderful human), Hannah, Nia, Sophie and Caitlin who have been the most incredible friends and the most excellent sources of distraction and advice. Thank you to Mafalda (and Tiago), Silke (Milky-Pilky), Juan, Oliver, Holly, Sam Dunkley (Lord of Weighing), Sky Smile, Xiangjiang, Niall (special thanks for the adventure chats and ensuing psych), Pier, Mario (of the week), Stephen Short, Jenny Dunn, Ian, Alex Tuña, Daevid Mike, Alex McCubbin, Elia…

From the wider OnE community, I would like to say a special thank you to Marian Pye, Dr. Dray, Steve Thomas, James Vaf, Rob Thomas, Eleanor Kean, Liz Chadwick, Beky Weiser, and Jo Cable for all of your great help, advice and support. Extra special thanks to Ian Vaughan for being a wonderful internal assessor until the very end of the PhD and for your help with the analyses.

Thank you to Helen Hipperson, Deborah Dawson and the rest of the NBAF team at the University of Sheffield for your assistance with the Illumina sequencing and bioinformatics. Thank you to Fred Boyer for your tips on the analyses.

This thesis was written in beautiful North Wales and I would like to thank the truly wonderful group of inspirational people who supported me through it and accompanied me on adventures to keep me sane! Special thanks to Marian and Chris (let's play Catan!), to Claire, Catrin and Trystan for the fun times, to Lindsey, Pete, Kimbo (for the mountain runs) and Spike, and last but by no means least to 'Jammy' James. Marian and James, I will be forever in your debt for the support and encouragement that you have given me. You've both been nothing but amazing - so full of wisdom, so patient, and so keen for adventures (and data entry!?). Thank you to my family for their support and encouragement.

Thank you to my funders - this work was funded by the Natural Environment Research Council (NE/K00719X/1) in the form of an iCASE studentship awarded to Cardiff University, co-funded by CASE partners the Durrell Wildlife Conservation Trust with support from the Mauritian Wildlife Foundation. Additional funding was provided by NBAF. Thank you to the Mauritian National Parks and Conservation Service for their permission to carry out work in Mauritius.

Table of contents

Summary

Species extinctions on islands are commonplace throughout history. Such extinctions can lead to dysfunctional ecosystems, especially when keystone species are lost. When the target species is extinct, an analogue species can be introduced to restore ecosystem function (known as ecological replacement).

In Mauritius, exotic giant tortoises (*Aldabrachelys gigantea*) have been introduced to restore ecosystem function after the loss of their endemic counterparts, which were thought to be keystone grazers. Dietary analysis is essential to understand the impact that tortoises have on the ecological network. Metabarcoding of plant DNA from faecal samples is an invaluable tool to recover detailed dietary information. Such dietary analysis is often inhibited by the absence of comprehensive DNA barcode libraries.

The aim of this PhD research was to use DNA metabarcoding to assess the impacts and interactions of introduced Aldabra giant tortoises on Mauritian islands undergoing restoration. Here, the direct effect of tortoise dietary preferences on the plant community and the knock-on effects on two vertebrate species endemic to Mauritius, the Telfair's skink (*Leiolopisma telfairii)* and Pink Pigeon (*Nesoenas mayeri),* were investigated.

A comprehensive DNA barcode library of the plants present on Ile aux Aigrettes and Round Island was created using the second internal transcribed spacer (ITS2) in order to maximize taxonomic discrimination at the species level in the dietary analyses. Ninety-nine percent of the Islands' angiosperms were successfully sequenced. This is the first time island plant communities have been so comprehensively DNA barcoded in order to carry out dietary analyses and the library lays the foundations for the construction of more comprehensive food webs to further current understanding of ecological restoration.

Universal short-amplicon plant DNA metabarcoding primers for the ITS2 region, capable of amplifying the degraded plant DNA found in faecal samples were designed. To increase the breadth of the application of these primers, they were tested on both Mauritian and UK plant species to prove that they can be successfully applied in both tropical and temperate systems. *In silico* testing suggested that 88% of 1,111 UK and

Mauritian plants were a good match with the novel primers. In practice, 99% of 202 UK and Mauritian plants amplified successfully.

The diets of introduced Aldabra giant tortoises, Telfair's skinks and Pink Pigeons were analysed by metabarcoding the plant DNA found in faecal samples, using novel primers. Giant tortoise grazing alters the plant community structure by cropping both exotic and native vegetation. This engineering of the vegetation structure indicates that tortoises create and maintain tortoise lawns in open areas, which together with the established forested areas constitutes a vegetation mosaic that may be beneficial for biodiversity. The giant tortoises also play a role in controlling the invasive weed species on Ile aux Aigrettes, by reducing plant biomass through grazing. However, Telfair's skinks and Pink Pigeons exhibit dietary preferences for some exotic plant species. Thus, it is important to increase the availability and variation of native plant species that these endemic vertebrates prefer to consume in order to buffer the effect of reducing the availability of preferred exotic plants.

Chapter One – General introduction

"What escapes the eye…is a much more insidious kind of extinction: the extinction of ecological interactions"

Daniel H. Janzen, 1994

1.1 Background

1.1.1 Species extinctions and rewilding

Catastrophic recent declines in biodiversity and habitat modification have led scientists to debate both the concept of a sixth mass extinction and the dawn of a new geological epoch, the Anthropocene, where humans are the main driver of environmental change (Chapin et al. 2000; Crutzen 2002; Sanderson et al. 2002; Wake & Vredenburg 2008; Dirzo et al. 2014; Birnie-Gauvin et al. 2017). The loss of keystone species can have cascading effects on ecosystems, triggered by the loss of seed dispersers, apex predators, reduced nutrient cycling and a change in grazing or browsing pressure on plant communities (Paine 1969, 1980; Kaiser-Bunbury et al. 2010; Estes et al. 2011; Dirzo et al. 2014). Trophic cascades such as these can leave ecosystems devoid of their structural and functional complexity and less resilient to global change (Griffiths et al. 2010; Fernandez et al. 2017).

Ecological restoration has the potential to reverse ecosystem degradation by reestablishing both lost species composition and lost ecosystem function (Suding 2011; Wortley *et al.* 2013; Corlett 2016). Restoration has traditionally focused on rebuilding plant communities (Perring *et al.* 2015), whereas threatened species recovery programmes have focused on reinstating lost animal communities (Jones & Swinnerton 1997). Rewilding, on the other hand, has been proposed as an ambitious alternative to more traditional approaches to conservation. Rewilding has no strict definition, but encompasses a range of approaches that look to conserve or increase biodiversity and minimize or reduce anthropogenic impacts by restoring ecosystem function (Lorimer *et al.* 2015). This can be achieved by restoring species interactions, reinstating natural processes and functions, and subsequently nurturing self-sustaining ecosystems (Zimov *et al.* 1995; Zimov 2005; Lorimer *et al.* 2015; Corlett 2016; Svenning *et al.* 2016; Fernandez *et al.* 2017).

Typically the term 'rewilding' is used in the context of restoring populations of apex consumers to resurrect top-down control in ecosystems (Galetti 2004; Donlan *et al.* 2005; Estes *et al.* 2011; Seddon *et al.* 2014; Svenning *et al.* 2016). Rewilding interventions range from the very passive, where communities are allowed to reestablish themselves, to reintroductions and ecological replacement/taxon substitution, through to the very active introduction of analogues for the Pleistocene

megafauna (Fernandez *et al.* 2017). Rewilding projects and proposals vary in scale but a common theme is that they are typically ambitious. There are four projects that have been described as flagships for rewilding: the Yellowstone National Park (United States), Oostvaardersplassen (Netherlands), Pleistocene Park (Russia), and Mascarenes (Lorimer *et al.* 2015).

Grey wolves, *Canis lupus*, were reintroduced to the Yellowstone National Park, which had multiple cascading effects throughout the Yellowstone ecosystem. For example, elk (*Cervus elaphus*) grazing pressure was modified, which increased the availability of berries as food for threatened grizzly bears (*Ursus arctos horribilis)* (Ripple *et al.* 2014) and also allowed aspen (*Populus tremuloides*) to regenerate (Ripple *et al.* 2001; Ripple & Beschta 2007). Elsewhere, large-bodied herbivores have been introduced to reinstate lost grazing or browsing pressure. Konik horses (*Equus ferus caballus*) and Heck cattle (*Bos taurus*) have been introduced as analogues for extinct tarpans (*Equus ferus ferus*) and aurochs (*Bos taurus primigenius*) in the Oostvaardersplassen (Marris 2009). In northern Siberia, the Pleistocene Park is now home to reindeer (*Rangifer tarandus*), horses (*Equus caballus*), musk ox (*Ovibos moschatus*), moose (*Alces alces*) and bison (*Bison bison*) in the hope that they will re-create a steppe ecosystem that would play important carbon storage roles (Zimov *et al.* 1995; Zimov 2005). Two species of non-native giant tortoises, Aldabra giant tortoises (*Aldabrachelys gigantea*) and radiated tortoises (*Astrochelys radiata*), have been introduced to Mauritian islands as surrogates for their extinct counterparts (*Cylindraspis triserrata* and *C. inepta*) and have been shown to disperse the seeds of those native plants with large fleshy fruits and also play a role in alien weed control (Griffiths *et al.* 2010; Griffiths *et al.* 2011; Griffiths *et al.* 2012; Griffiths *et al.* 2013). In addition to these flagship sites, there are growing numbers of rewilding projects and proposals (e.g. Rewilding Europe 2015). Although rarely labeled with the term rewilding, domestic animals have often been introduced to graze systems and have reinstated lost ecosystem functions. This has been seen, for example, in the Pantanal where horses, cows (*Bos taurus*) and feral hogs (*Sus scrofa*) play important seed dispersal and weed control roles (Galetti 2004). Species reintroductions play a key role in many of these projects. However, in many instances this is not possible, due to species extinctions, and here ecological replacement is becoming increasingly important.

1.1.2 Ecological replacement

Ecological replacement is defined by the IUCN as 'the intentional movement and release of an organism outside its indigenous range to perform a specific ecological function' (IUCN/SSC 2013). This conservation intervention is increasingly being applied across the globe where a keystone species has become extinct (e.g. Zimov 2005; Griffiths *et al.* 2013). Here the species being introduced are referred to as ecological analogues, taxon substitutes or proxy species, and these terms are used interchangeably throughout this thesis.

When evaluating the suitability of candidate species to be used as ecological analogues, there are three main criteria to consider: (i) conservation importance; (ii) taxonomic closeness to the extinct counterpart; and (iii) ecological equivalence to it. If the ecological analogue itself requires conservation action, introduction outside its natural range as an analogue species to restore lost ecosystem function can be a mutually beneficial approach (IUCN/SSC 2013). It is important to note that taxonomic closeness does not necessarily mean ecological closeness, since many closely related taxa differ markedly in their ecology. Thus, ecological closeness may be a more important criterion than taxonomy, since the role of the analogue species is to restore ecological function (Jones 2002).

Ecological replacement remains a controversial conservation intervention. Many conservationists are wary of this approach, due to the numerous ecological, evolutionary, practical and societal issues and questions, which remain unsolved and unanswered (Caro 2007). There is a risk of introducing novel diseases to wildlife and domestic animals (Donlan *et al.* 2005); there is concern that ecological analogues could respond in unpredictable ways, thus potentially reducing biodiversity (Smith 2005); and there could be any number of unforeseen interactions with native taxa and livestock (Rubenstein *et al.* 2006; Ricciardi & Simberloff 2009a, b). It is also difficult, due to a deficit of data and empirical studies, to support or refute the hypothesis that ecological analogues will provide the same selection pressures on plant and animal communities as their extinct counterparts (Donlan *et al.* 2006; Svenning *et al.* 2016; Fernandez *et al.* 2017).

1.1.3 Ecological restoration in Mauritius

In comparison to continental systems oceanic island ecosystems are typically characterised by high levels of endemism and small population sizes. The anthropogenic colonisation of islands and subsequent hunting and habitat destruction have lead to the extinction of many native, and often endemic, species (Cheke & Hume 2008). This has been exacerbated by the introduction of exotic species that compete with and predate the native fauna, which have commonly evolved in the absence of such competitors and predators (Cheke & Hume 2008). As a consequence, species extinctions on oceanic islands are commonplace throughout history, for example in plants (Myers *et al.* 2000); mammals (Alcover *et al.* 1998); birds (Boyer & Jetz 2014), (Athens *et al.* 2002); and reptiles (Richman *et al.* 1988). In the tropics, megafaunal extinctions are mostly, but not exclusively, large herbivores (Corlett 2013). Such extinctions disrupt plant-animal mutualisms and can lead to a loss of invaluable ecosystem services such as seed dispersal and pollination (for a review see Kaiser-Bunbury *et al.* 2010). Large herbivores in particular are thought to act as keystone species, as their feeding and trampling behaviours can shape the structure of landscapes, for example by maintaining vegetation heterogeneity, and ecosystem dynamics (Owen-Smith 1987, 1988; Dirzo & Miranda 1991; Estes *et al.* 2011; Hunter *et al.* 2013; Bakker *et al.* 2016). The reduced functional redundancy in island systems may leave them particularly sensitive to megafaunal loss in comparison to continental systems. These depauperate and relatively simple systems may experience a more rapid and devastating trophic cascade of species loss (Hansen & Galetti 2009).

In Mauritius, many species have been saved from the brink of extinction because of intensive conservation efforts, such as captive breeding, (re)introductions, supplementary feeding, forest regeneration and the control of invasive predators and alien weeds (Jones & Swinnerton 1997; Cole *et al.* 2013). For example, there is now a viable population of Mauritius kestrels (*Falco punctatus*), which recovered from just two pairs in the 1970s (Cade & Jones 1993). Similarly, the wild Pink Pigeon (*Nesoenas mayeri*) population consisted of ten birds in the 1990s, whereas there are around 400 individuals today (Jones & Swinnerton 1997; Concannon 2014). The Mascarene islands (Mauritius, Réunion and Rodrigues) however, are not exempt from the threats that other oceanic islands face and numerous native species have been extirpated. Indeed one of the most famous examples of a species extinction took place in the Mascarenes: the dodo, *Raphus cucullatus,* which was once found in Mauritius. In reality a host of Mascarene species have shared the same fate as the dodo. These include many species of

bird (Hume 2011, 2014, 2015), reptiles, including giant tortoises (Cheke & Hume 2008), and the lesser Mascarene flying-fox (*Pteropus subniger*) (Mickleburgh *et al.* 2008).

The Mascarenes have suffered the loss of their large herbivore species. Namely, five endemic species of giant tortoise in the genus *Cylindraspis*, which became extinct during the Holocene (Cheke & Hume 2008; Corlett 2013). Two of these five extinct *Cylindraspis* tortoises, *C. inepta* and *C. triserrata*, inhabited Mauritius and were still extant until extirpation around 1840 (Cheke & Hume 2008). Giant tortoises were keystone herbivores on these islands: they modulated plant community structure through grazing and trampling, dispersed seeds, and played an important role in nutrient cycling. In the absence of tortoises, plant-herbivore interactions are disrupted and plant community structure is expected to have changed and seed dispersal reduced (Griffiths *et al.* 2011). Any changes in the plant community are likely to have cascading effects on native consumers. Unfortunately, the reintroduction of native tortoises to resurrect lost species interactions was impossible, but ecological replacement had conservation potential.

1.1.4 Tortoises as analogue species

Mauritius had already lost two endemic species of giant tortoise, *Cylindraspis inepta* and *C. triserrata*, before detailed ecological records were made. Thus, what is known of their ecology is assembled from brief accounts by sailors, the fossil record and also the native vegetation: many species have relict features to deter herbivory, which are thought to have evolved under high evolutionary pressure produced by high tortoise densities (Cheke & Hume 2008).

Vetevaria arguta, a tussock-forming grass endemic to the Mascarenes, has coarse unpalatable leaves, which are ignored by grazers in favour of other grasses. Consequently, it was the most common grass on Round Island, Mauritius, until invasive mammalian herbivores were eradicated and invasive grasses were permitted to flourish, outcompeting the endemic tussocks. In addition, heterophylly is common amongst native plants. Here, young and adult leaves have markedly different forms, the latter of which are found approximately 1.2 m above the ground and thus out of the reach of a giant tortoise. In feeding trials, these adult leaves are readily eaten by Aldabra giant tortoises (from the Seychelles), whereas the young leaves found close to the ground are ignored (Jones 2002; Eskildsen *et al.* 2004) adding weight to the hypothesis that heterophylly evolved in Mauritius in response to tortoise herbivory. Furthermore,

many plant species have large, fleshy and palatable fruits, which is likely to be an adaptation to aid seed dispersal (Cheke & Hume 2008).

The saddlebacked shells of *C. triserrata* (Cheke & Hume 2008) are adaptations to allow the neck to extend upwards and exploit higher vegetation by browsing (Griffiths *et al.* 2010) up to 1.2 m above the ground (Jones 2002) (Fig. 1.1). These tortoises were probably too large to manoeuver through dense inland hardwood forests, so it is likely that they utilised the more open coastal palm forests (Griffiths *et al.* 2010). *Cylindraspis inepta* were smaller, dome shelled tortoises (Cheke & Hume 2008), which grazed vegetation closer to the ground and whose smaller size allowed the exploitation of hardwood fruits in dense inland forests (Griffiths *et al.* 2010). This niche partitioning between tortoises with different morphologies has been observed on Pinta Island (Galápagos islands) where two phenotypes of *Chelonoidis nigra* were introduced as ecological replacements for the extinct *C. abingdonii.* Here, tortoises with a domed shell phenotype settled at higher and moister locations, whereas the saddle backed tortoises exploited lower and more arid zones (Hunter *et al.* 2013).

Figure 1.1 A bronze statue of *Cylindraspis triserrata* on Ile aux Aigrettes. This extinct giant tortoise was endemic to the Mascarenes. This species had saddlebacked shells, allowing them to browse vegetation up to approximately 1.2 m (Jones 2002). Professor Bill Symondson is standing beside the statue for scale.

Two candidate species for *C. inepta* and *C. triserrata* ecological analogues are the Aldabra giant tortoise and the Madagascar radiated tortoise, *Astrochelys radiata.* These tortoises meet the criterion of taxonomic closeness: both belong to the same family (Testudinidae) as the *Cylindraspis* tortoises. *Astrochelys radiata* is Critically Endangered and its extinction is forecast for within 45 years time (Leuteritz *et al.,* 2008) and *A. gigantea* is classified as Vulnerable (Tortoise and Freshwater Turtle Specialist group 1996). Thus, the introduction of these species as ecological analogues outside their natural range may be beneficial for their own conservation, whilst simultaneously restoring ecosystem function (Griffiths *et al.* 2012). *Astrochelys radiata* is known to utilise both coastal sand dune habitats and high inland plateaus (Leuteritz *et al.,* 2008). On the Aldabra Atoll, the highest densities of *A. gigantea* are found in coastal mixed scrub habitats and they feed mostly on tortoise turf (a community of cropped grasses and herbaceous plants), long grasses, and leaf litter (Gibson & Hamilton 1983). This foraging behaviour and also their physiology is thought to be similar to the extinct *Cylindraspis* tortoises (Waibel *et al.* 2013).

The introduction of *A. gigantea* and *A. radiata* to two Mauritian offshore islands began in 2000: *A. gigantea* to Ile aux Aigrettes in 2000 and both *A. gigantea* and *A. radiata* to Round Island in 2007 (Griffiths *et al.* 2010) (Fig. 1.2, Fig. 1.3). The aim was to restore lost plant-herbivore mutualisms and reverse ecosystem dysfunction in a reversible rewilding experiment. At this time, the IUCN guidelines for reintroductions precluded ecological replacement. Introductions outside a species' native range could be considered only as a last resort if it would benefit that species (benign/conservation reintroduction) (IUCN 1998). Thus, practitioners operated outside the guidelines and in 2013 new guidelines for (re)introductions were set and ecological replacement was included (IUCN/SSC 2013).

Early work has shown that introduced giant tortoises have not only increased seed dispersal, but also increased seedling success in a dispersal-limited Critically Endangered endemic ebony (*Diospyros egrettarum*) (Griffiths *et al.* 2011). In addition to seed dispersal, giant tortoises can also influence plant community composition by suppressing some species through selective grazing. Griffiths *et al* (2013) found that introduced tortoises reduced the vegetation height, biomass, and cover as well as the abundance of seedlings, flowers and seeds. It has been suggested that introduced giant tortoises avoid native plants, due to the availability of exotic plants that may not be strongly defended against herbivory. Thus these tortoises may contribute to the control

of palatable exotic plants (Griffiths *et al.* 2010; Griffiths *et al.* 2013). However, previous assessments of the diet of introduced giant tortoise have been limited by small sample sizes and inherent difficulties in delineating diet from feeding observations and the morphological identification of dietary items in faecal samples (Holechek *et al.* 1982; Pompanon *et al.* 2012; Griffiths *et al.* 2013). In addition, some of the effects of tortoise grazing on the plant community are known, but the knock-on effects of these ecological analogues on the native fauna remain unknown. Advances in molecular methods of diet assessment have the capacity to rapidly generate larger volumes of data of a greater precision (Symondson 2002; King *et al.* 2008). These molecular techniques allow for trophic interactions to be studied in great detail, including the cascading effects of introducing nonindigenous giant tortoises.

Figure 1.2. Introduced Aldabra giant tortoises, *Aldabrachelys gigantea,* on Round Island, Mauritius. An endemic Telfair's skink, *Leiolopisma telfairii,* can be seen basking on a rock in the background.

Figure 1.3. An introduced radiated tortoise, *Astrochelys radiata,* on Round Island, Mauritius. The tortoise is beside *Aloe tormentorii,* a plant endemic to Mauritius.

1.1.5 Studying trophic interactions

A sound understanding of trophic interactions is fundamental to monitoring and assessing the health of the species that have been restored, and also to inform, monitor and assess ambitious conservation strategies. According to the IUCN, it is best practice to monitor trophic interactions, such as predation and herbivory, subsequent to (re)introductions or translocations (IUCN/SSC 2013). This monitoring is necessary in both species recovery programmes and where conservation interventions such as ecological replacement have been used. Such monitoring allows us to detect dietary overlap and competition with both native (Jung *et al.* 2015) and non-native species (Brown *et al.* 2014), to preempt or monitor human-wildlife conflict (Kowalczyk *et al.* 2011); it also makes it possible to estimate the need for supplementary feed (Edmunds *et al.* 2008), and understand seed dispersal and pollination mechanisms to inform ecosystem restoration (Pernetta *et al.* 2005). An understanding of trophic links also allows species at risk due to inflexible niches to be identified, isolates particularly vulnerable interaction networks, and allows for suitable (re)introduction sites to be identified (Pernetta *et al.* 2005; Clare 2014; Soorae 2016); it also provides a better understanding of the reasons for the successes and failures of threatened species recovery programmes.

There are a variety of methods available for studying trophic interactions, and each one has its own strengths and weaknesses. Traditional methods include the morphological examination of faecal samples, gut contents and feeding observations. The morphological identification of plant remains in faecal samples requires high levels of expertise and considerable time to identify chewed and partially digested plant fragments (Holechek *et al.* 1982). Analysing stomach contents instead can provide information on dietary items that are more susceptible to digestion (Britton *et al.* 2006), but this method can only be implemented after the death of the subject or by invasively inducing regurgitation (Alonso *et al.* 2014). When directly observing feeding behaviours there are likely to be biases associated with disturbing the subject and the need to identify dietary items from a distance (Kruuk 1995) and this method also precludes working with any elusive, nocturnal or soil dwelling species (Pompanon *et al.* 2012). Molecular methods provide an alternative suite of approaches that can generate greater volumes of data more rapidly and with greater precision (Symondson 2002; King *et al.* 2008). For example, species-specific primers can be used to amplify the DNA of particular focal dietary items in gut contents or faecal samples (Pumarino *et al.* 2011; Leal *et al.* 2013; Wallinger *et al.* 2013). However, this approach is only appropriate if *a*

priori dietary information is available and if the diet range is small. It cannot unravel the effects non-focal species may be having on dietary selection by a polyphagous predator or herbivore. In order to overcome such problems, and determine whole dietary ranges, DNA barcodes coupled with next generation sequencing, known as DNA metabarcoding or simply metabarcoding, have been widely adopted. In most cases, metabarcoding provides greater sensitivity and specificity in comparison to morphological methods (Soininen *et al.* 2009; Ando *et al.* 2013; Alonso *et al.* 2014).

However, DNA metabarcoding is itself not without its limits. Metabarcoding cannot differentiate between different tissue types originating from the same species, it cannot reliably detect cannibalism or secondary consumption; presence or absence can be reliably determined, but not the biomass consumed (Deagle *et al.* 2009; Coissac *et al.* 2012; Hibert *et al.* 2013; Elbrecht & Leese 2015; Ford *et al.* 2016). There can be problems with taxonomic resolution, especially if comprehensive DNA reference libraries are unavailable (Taberlet *et al.* 2007; Valentini *et al.* 2009; Garcia-Robledo *et al.* 2013a; Garcia-Robledo *et al.* 2013b) and this technique provides no information on nutrition. In truth, no single technique can comprehensively provide all of the information that might be need about a trophic link. However, some of the limitations of metabarcoding can be partially overcome by careful study design. Taxonomic resolution can be maximized by carefully choosing the DNA barcoding region(s) that will maximize taxonomic differentiation in a particular study system (Taberlet *et al.* 2007; Hollingsworth *et al.* 2009; Hollingsworth 2011; Hollingsworth *et al.* 2011; Pompanon *et al.* 2012; Hollingsworth *et al.* 2016). A comprehensive DNA barcode library is necessary to identify dietary items to the level of species (de Vere *et al.* 2012; Garcia-Robledo *et al.* 2013a; Garcia-Robledo *et al.* 2013b). The issues surrounding quantification can be partially overcome by using large sample sizes. When sample sizes are large, those dietary items that occur more frequently across the dataset can more reliably be considered as important species in the diet (frequency of occurrence). By combining methodologies, it is possible to determine which plant tissue types were consumed. For example, metabarcoding can provide a list of taxa that are consumed alongside their frequency of occurrence in the samples. This can be used as a guide for subsequent morphological examinations of faecal samples where specific seeds or leaf morphologies are targeted. By simultaneously assessing the nutritional value of those species in the diet, the importance of those species in terms of the fitness of the consumer can be determined. Despite these limitations, DNA metabarcoding is largely accepted to be the

most accurate and sensitive method for dietary analysis (Soininen *et al.* 2009; Pompanon *et al.* 2012; Ando *et al.* 2013; Alonso *et al.* 2014).

1.2 Project aims

1.2.1 Aims of this PhD project

The overarching aim of this PhD project was to use DNA metabarcoding to determine the impacts of introduced Aldabra giant tortoises on the plant community and the knock-on effects on two endemic species: the Pink Pigeon (*Nesoenas mayeri*) and the Telfair's skink (*Leiolopisma telfairii*). The primary focus of the PhD was on Ile aux Aigrettes, although work was also carried out on Round Island. The majority of the work on Round Island is not mentioned in the thesis but will be published elsewhere.

Specific aims were to (i) lay the foundations for the use of metabarcoding to study trophic interactions on Mauritian islands by building a comprehensive DNA barcode library of the plant communities of Round Island and Ile aux Aigrettes; (ii) design DNA metabarcoding PCR primers that were suitable for us not only in the Mauritian system but also elsewhere to study trophic interactions; (iii) analyse the trophic links between Aldabra giant tortoises, Pink Pigeons and Telfair's skinks via the plant community, by analysing herbivory by these three consumers and assessing their dietary preferences. This was achieved by metabarcoding the plant DNA found in faecal samples and comparing the food eaten to its availability. The final aim was to (iv) determine the direct affect of tortoise grazing and grazing preferences on the plant community by conducting a tortoise exclusion experiment.

1.2.2 Chapter structure

Chapter two is the first data chapter of the thesis. Here a comprehensive DNA barcode library covering the Ile aux Aigrettes and Round Island plant communities is presented. The library was scrutinised to determine the taxonomic resolution that could be obtained using the ITS2 DNA barcode.

Chapter three details novel PCR primers that are suitable for DNA metabarcoding studies in both the Mauritian system and in the UK. The results of testing the primers in respect of species amplification biases and species-level taxonomic discrimination capacity are presented. Analyses to determine which parameters should be used in the downstream bioinformatics analysis of metabarcoding data were also determined.

Chapter four introduces the dietary data from Pink Pigeons and Telfair's skinks. In addition to presenting the plants consumed, the proportion of native and introduced plants in the diet were compared and deviations from random herbivory were tested for to determine dietary preferences. For Telfair's skinks only, diet as determined by DNA metabarcoding was compared to that determined by the morphological identification of plant remains in faecal samples. Finally, variation in supplementary feed use by Pink Pigeons between sexes and seasons was tested for.

Chapter five is the final data chapter of the PhD thesis. Here the diet of Aldabra giant tortoises on Ile aux Aigrettes was investigated by DNA metabarcoding. Food available was compared to what was consumed to test for dietary preferences. These data were combined with the Pink Pigeon and Telfair's skink dietary data from Chapter four in order to begin to understand the knock-on effects that the tortoises may have on the endemic fauna. To disentangle this further and also to assess the direct affects of tortoise grazing on the plant community, the results of a tortoise exclusion experiment are presented.

Finally, in Chapter six the findings from the PhD project were summarised and the extent to which research aims were met was discussed. Based on the findings, suggestions for conservation management in Mauritius were made and avenues for future research discussed.

Chapter Two – DNA barcoding of island plant communities: towards understanding the role of trophic dynamics in ecological restoration

2.1 Abstract

Dietary analysis of animals is essential for describing food webs, determining trophic dynamics and improving understanding of ecosystem functioning. The inclusion of trophic dynamics in ecological restoration has considerable potential for management of rare species and whole communities. High throughput sequencing technologies can provide detailed information through metabarcoding dietary remains in the faecal samples or regurgitates of herbivores and predators. However, to reliably identify dietary taxa, a comprehensive DNA barcode library of all available food is essential. In this study the plants on two Mauritian islands, one coralline the other volcanic, which are the focus of long-term restoration projects were DNA barcoded. Here, a comprehensive ITS2 DNA barcode library containing 99% of angiosperms, plus a fern and a moss (165 species and 469 sequences) is presented. Species assignment tests using the BLASTn algorithm indicated that 98.6% of taxa could be assigned to species. This is the first time that island plant communities have been comprehensively barcoded for dietary analysis (in this case herbivory) with precision, paving the way for a comprehensive understanding of the trophic dynamics within island ecosystems that support rare species and are undergoing restoration.

2.2 Introduction

While the concept of a sixth mass extinction remains debatable, catastrophic recent declines in island biodiversity are now a matter of record (Chapin *et al.* 2000; Wake & Vredenburg 2008; Dirzo *et al.* 2014; Spatz *et al.* 2017). Island ecosystems feature high levels of endemism and small populations in comparison to continental systems. Species extinctions on oceanic islands are especially commonplace and include plants (Myers *et al.* 2000), mammals (Alcover *et al.* 1998), birds (Athens *et al.* 2002; Boyer & Jetz 2014) and reptiles (Richman *et al.* 1988; Cole *et al.* 2005). Anthropogenic colonisation of islands and subsequent habitat destruction, coupled with the intentional and accidental introduction of invasive non-native species, has extirpated many plant and animal species, which have evolved in the absence of such competitors and predators (Cole *et al.* 2005; Cheke & Hume 2008; Maggs *et al.* 2015). For the effective restoration and conservation of such ecosystems, an understanding of species interactions is required. However, incorporating trophic dynamics is yet to be fully realised in the field of restoration ecology (Perring *et al.* 2015). The first step towards understanding trophic

dynamics is to analyse the diet of the animals within a system to determine food web structure.

DNA metabarcoding is known to have the capacity to identify dietary items from faecal samples or stomach contents to a greater taxonomic resolution than is possible using more traditional methods (Soininen *et al.* 2009; Pompanon *et al.* 2012; Ando *et al.* 2013; Alonso *et al.* 2014). To implement such an approach, a database containing reference sequences from known species, a DNA barcode library, is required to identify taxa. Publicly available databases include GenBank (Benson *et al.* 2014) and the Barcode of Life Database (Ratnasingham & Hebert 2007). However, these databases rarely hold DNA barcodes from all of the species in the focal study system and the discriminatory power of a DNA barcode can be greatly increased by excluding species that are not present (Hofreiter *et al.* 2003; Garcia-Robledo *et al.* 2013b). Thus, a bespoke DNA barcode library for the study system should be a prerequisite (Taberlet *et al.* 2007; Valentini *et al.* 2009; Garcia-Robledo *et al.* 2013a). DNA barcode libraries have already been assembled for some systems, for example progress towards DNA barcoding all of Alaska's non-marine arthropods (Sikes *et al.* 2017), Canada's hemiptera (Gwiazdowski *et al.* 2015), Germany's fauna and flora (Geiger *et al.* 2016), and an ambitious project to DNA barcode all eukaryotes on and around the island of Moorea (French Polynesia) is underway (Check 2006). For plants, comprehensive DNA barcode libraries have been assembled for the native Welsh flora (de Vere *et al.* 2012) and the flora of the Canadian Arctic archipelago (Saarela *et al.* 2013). Plant barcode libraries can be used beyond dietary studies; for example for species identification during early life stages (Gonzalez *et al.* 2009) or tissue types (Burgess *et al.* 2011) that are difficult to identify by morphology alone; identifying cryptic species (Lahaye *et al.* 2008); better understanding of plant community ecology (Kress *et al.* 2009); determining historic species composition from ancient samples to reconstruct past ecosystems (Sonstebo *et al.* 2010), monitoring future climate induced changes in plant distribution (Saarela *et al.* 2013); and pollinator studies (Hawkins *et al.* 2015).

Here, a comprehensive plant DNA barcode library for two contrasting Mauritian islands that have been the focus of restoration efforts, including the use of ecological surrogates, to restore ecosystem functioning (Griffiths *et al.* 2010; Griffiths *et al.* 2011; Griffiths *et al.* 2013) is presented. It is believed that this is the first time that plant communities on islands have been so comprehensively DNA barcoded, and the first time that such large-scale barcoding has been completed for dietary analysis, to determine trophic

interactions in systems that are the direct focus of conservation initiatives.

2.3 Methods

2.3.1 Study sites

Ile aux Aigrettes is a 26 ha low coralline island located in the Indian Ocean approximately 600 m off the southeast coast of the main island of Mauritius (Fig. 2.1). The island harbours one of the last remnants of dry Ebony-rich forest that was once widespread on the coastal region of mainland Mauritius. Invasion by non-native plants, mammals, birds, and reptiles, in addition to partial clear-felling, had a severe impact on the native plant and animal communities on the island, resulting in the extirpation of numerous species. The island was declared a nature reserve in 1965, but tree felling and coppicing continued until 1985. The restoration of the island's floral and faunal communities began in 1986 with the removal of invasive plants, the elimination of exotic vertebrate species, the re-establishment of native plant species, and the (re)introduction of native animals and ecological surrogates (Aldabra giant tortoises, *Aldabrachelys gigantea*) (Jones & Hartley 1995; Cheke & Hume 2008; Griffiths *et al.* 2011). Today, despite eradication attempts one exotic mammal remains, the Asian musk shrew (*Suncus murinus*), (Seymour *et al.* 2005), along with several exotic reptiles including the wolf snake (*Lycodon capucinus*), three species of gecko, one species of agamid lizard (*Calotes versicolor*) (Cheke & Hume 2008), five bird species, plus 59 exotic plant species (Table 2.1).

Round Island (Fig. 2.1) is a 219 ha basaltic volcanic cone, which rises to 280 m at its highest point. The island was declared a nature reserve in 1957 and is now closed to the public. Introduced goats and rabbits were eradicated in 1979 and 1986, respectively, and the island has never featured introduced rodents, or other predatory vertebrates (Cheke & Hume 2008). The last remnant of the Mascarene lowland palm rich plant community is found on Round Island, in addition to the last wild individual of the endemic and critically endangered hurricane palm, *Dictyosperma album var. conjugatum* (Page 1988). Round Island is significant for its breeding populations of reptiles and seabirds. Until recently, three endemic reptile species were restricted to Round Island and found nowhere else in Mauritius before they were (re)introduced to other Mauritian islands in order to reduce extinction risks and rebuild lost reptile communities (Cole et al. 2014). Ecosystem restoration efforts on Round Island include the removal of exotic plants, the planting of native plant species and taxon substitution

(Aldabra tortoises alongside radiated tortoises, *Astrochelys radiata*, introduced to replace the native, but extinct, Mauritian giant tortoises as top grazer) (Griffiths *et al.* 2010).

Figure 2.1 Map of the Mauritius mainland and the surrounding islands. Both Round Island and Ile aux Aigrettes are indicated in dark red. Shapefiles supplied by Nik Cole and the Mauritian Wildlife Foundation and the map was constructed using QGIS (QGIS Development Team 2014).

2.3.2 DNA barcode library preparation

Plant tissue samples were collected from at least three individuals, where possible, belonging to every known species of plant found on Ile aux Aigrettes and Round Island (n = 171 species) and dried over silica gel. Samples were collected in triplicate to detect intraspecific genetic variation if present or cryptic species, and to better identify any errors. Samples from the islands were supplemented by material from the mainland or other nearby islands when fewer than three individuals were available on the study islands (Table 2.1). Images of each species were captured and compiled into a photo library (Goder *et al.* 2017). DNA extractions were carried out using the method described in Randall *et al.* (2015), after samples were ground under liquid nitrogen, or using the Qiagen DNeasy plant kit (Qiagen, Manchester, UK). The complete second internal transcribed spacer of nuclear ribosomal DNA (ITS2) and partial 5.8S and 26S sequences were amplified using primer pair S2F and S3R (Chen *et al.* 2010). Where amplification with this primer pair failed, a second primer pair was used, ITS-p3 and ITS-p4 (Cheng *et al.* 2016). Both primer pairs are located in the 5.8S and 26S regions, which flank ITS2. Polymerase chain reactions (PCRs) were carried out in 10 µL reaction volumes containing 2 µL DNA template, 1 X PCR buffer, 2.0 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP and 1 U *Taq* DNA polymerase. For problematic samples (amplification or sequencing failure), a multiplex PCR mix from Qiagen (Manchester, UK) was used, with primers and DNA at the same concentration and volume described above. PCR cycling conditions were as follows, initial denaturation at 95°C for 10 minutes; 40 cycles of 95°C for 30 seconds, 56°C for 30 seconds, 72°C for 1 minute; and a final extension of 72°C for 10 minutes. PCR products were sequenced in both directions by Eurofins Genomics (Wolverhampton, UK). Contigs were constructed and a consensus sequences created in Sequencher (Broveak 1996) after manually checking all sequences.

2.3.3 Species assignment

All sequences were trimmed to ITS2 only, following Chen *et al.* (2010), using ITSx (Bengtsson-Palme *et al.* 2013). These trimmed sequences were then compared against the comprehensive reference database for the two islands using a BLASTn search to determine whether species assignments were correct (Altschul *et al.* 1990). A correct species assignment was defined as a query sequence matching to the correct species with the highest BIT-Score, where that BIT-Score was never equal to other incorrect candidate species (Kress *et al.* 2009; de Vere *et al.* 2012; Hawkins *et al.* 2015; de Vere *et al.* 2017). Matches to the query sequence itself were ignored and species where only one

individual was sequenced were excluded from the main analysis, but included in the reference library. This exclusion is to avoid a circular test where a query sequence is only confirmed as a correct species assignment because it is a match to itself. Those species represented by a single sequence were compared, also using a BLASTn search, against the bespoke database as a precautionary measure to ensure that they were not a close match to any other species present on the islands. A BLASTn approach to assess taxonomic resolution was chosen since this is the most common method for assigning sequences to taxa in DNA metabarcoding studies (e.g. Ando *et al.* 2013; Garcia-Robledo *et al.* 2013a; Hawkins *et al.* 2015).

2.4 Results

2.4.1 DNA barcode library preparation

A total of 684 plant tissue samples were collected from 171 species. For ten putative taxa, it was not possible to collect a sample from three individuals (Table 2.1) as some species occur at very low abundance on the islands, and thus were more difficult to find, and examples were not found on the mainland or other islands. At least one sample from every species of plant known to be present on Ile aux Aigrettes and/or Round Island was collected.

Sequencing of at least one sample per species was successful for 99% of angiosperms (n=164), 20% of ferns (n=5) and one moss from an unknown number of mosses present on the islands. No DNA sequences were successfully obtained from the single species belonging to the Lycopodiophyta division (Table 2.1). The single angiosperm from which no DNA sequence was obtained was the critically endangered hurricane palm, *Dictyosperma album* var. *conjugatum* (Page 1988). The DNA barcode library contains 469 sequences overall, which have been uploaded to GenBank (Table 2.1).

2.4.2 Species assignment

ITSx (Bengtsson-Palme *et al.* 2013) confirmed that all 469 sequences were authentic, and complete ITS2 sequences were detected for 454 sequences (97%). Only single sequences were obtained for 18 species and were removed from the BLASTn assignment test. Therefore, 436 complete and trimmed ITS2 sequences, representing 145 species (85% of those species present on the islands) were used for the BLASTn species assignment test for taxonomic discrimination. The assignment tests revealed

that 143 species were correctly assigned (98.6%). The two species that could not be differentiated are both in the genus *Fimbristylis* and whether they belong in the same species is currently in question. None of the 18 species that were represented by a single sequence were a close match to any other taxa in the database, eliminating the possibility of incorrect assignments to other taxa on the islands.

Table 2.1. Species and sample list for Ile aux Aigrettes and Round Island.

Division	Order	Family	*Binomial name*	Located on	Sample no.	Sample origin	Endemic/ native/ Introduced	Sequenced	Local name	GenBank Accession number
Angiosperm	Apiales	Araliaceae	*Polyscias maraisiana*	IAA, RI	3	IAA	Endemic	TRUE	Bois de Boeuf, Bois d'éponge	KY700450 - KY700452
Angiosperm	Arecales	Arecaceae	*Dictyosperma album var. conjigatum*	IAA, RI	3	IAA	Endemic	FALSE	Hurricane palm	-
Angiosperm	Arecales	Arecaceae	*Hyophorbe lagenicaulis*	IAA, RI	3	IAA	Endemic	TRUE	Palmiste Bouteille, Palmiste gargoulett	KY700379 - KY700381
Angiosperm	Arecales	Arecaceae	*Latania loddigesii*	IAA, RI	3	IAA	Endemic	TRUE	Latanier bleu, Latanier de Maurice, Latanier de l'Ile Ronde	KY700544, KY700571
Angiosperm	Asparagales	Amaryllidaceae	*Zephyranthes rosea*	IAA	3	IAA	Introduced	TRUE	Sourire	KY700535 - KY700537
Angiosperm	Asparagales	Asparagaceae	*Asparagus setaceus*	IAA	6	IAA	Introduced	TRUE	Liane asperge	KY700230 - KY700232
Angiosperm	Asparagales	Asparagaceae	*Asparagus umbellatus*	IAA, RI	6	IAA	Native	TRUE	Asperge sauvage	KY700233 - KY700235
Angiosperm	Asparagales	Asparagaceae	*Dracaena concinna*	IAA, RI	3	IAA	Endemic	TRUE	Bois de chandelle	KY700317, KY700568, KY700569
Angiosperm	Asparagales	Xanthorrhoeaceae	*Aloe tormentorii*	IAA, RI	3	IAA	Endemic	TRUE	Mazambron	KX689270 - KX689272
Angiosperm	Asparagales	Orchidaceae	*Angraecum eburneum*	IAA	3	IAA	Native	TRUE	-	KY700223, KY700224
Angiosperm	Asparagales	Orchidaceae	*Disperis tripetaloides*	IAA	3	IAA	Endemic	TRUE	-	KY700567
Angiosperm	Asparagales	Orchidaceae	*Oeoniella polystachys*	IAA	3	IAA	Native	TRUE	-	KY700424
Angiosperm	Asterales	Asteraceae	*Ageratum conyzoides*	IAA, RI	4	IAA (3); RI (1)	Introduced	TRUE	Herbe de bouc	KY700212 - KY700214
Angiosperm	Asterales	Asteraceae	*Bidens pilosa*	IAA, RI	6	IAA (3); RI (3)	Introduced	TRUE	Herbe Villebague	KY700236 - KY700238
Angiosperm	Asterales	Asteraceae	*Chromolaena odorata*	IAA, RI	4	IAA (3); RI (1)	Introduced	TRUE	-	KY700271 - KY700273
Angiosperm	Asterales	Asteraceae	*Conyza canadensis*	RI	5	RI	Introduced	TRUE	-	KY700284, KY700285
Angiosperm	Asterales	Asteraceae	*Psiadia arguta*	IAA, RI	4	IAA	Endemic	TRUE	Baume de l'Ile Plate	KY700461 - KY700463
Angiosperm	Asterales	Asteraceae	*Sonchus asper*	IAA, RI	8	IAA (3); RI (5)	Introduced	TRUE	Lastron piquant (Mauritian)	KY700486 - KY700488, KY700492 - KY700494
Angiosperm	Asterales	Asteraceae	*Tridax procumbens*	IAA, RI	7	IAA (3); RI (4)	Introduced	TRUE	Herbe Caille	KY700519 - KY700522
Angiosperm	Asterales	Campanulaceae	*Lobelia cliffortiana*	IAA	3	IAA	Introduced	TRUE	Brède mamzelle	KY700400 - KY700402
Angiosperm	Asterales	Goodeniaceae	*Scaevola taccada*	IAA, RI	3	IAA	Native	TRUE	Veloutier vert	KY700472 - KY700474
Angiosperm	Boraginales	Boraginaceae	*Cordia curassavica*	IAA	7	IAA	Introduced	TRUE	Herbe Condé	KY700286, KY700287
Angiosperm	Boraginales	Boraginaceae	*Hilsenbergia petiolaris*	IAA, RI	3	IAA	Native	TRUE	Bois de pipe	KY700373 - KY700375

Table 2.1. Species and sample list for Ile aux Aigrettes and Round Island.

Division	Order	Family	*Binomial name*	Located on	Sample no.	Sample origin	Endemic/ native/ Introduced	Sequenced	Local name	GenBank Accession number
Angiosperm	Boraginales	Boraginaceae	*Tournefortia argentea*	IAA, RI	3	IAA	Native	TRUE	Veloutier blanc	KY700514 - KY700516
Angiosperm	Brassicales	Caricaceae	*Carica papaya*	IAA	3	IAA	Introduced	TRUE	Papayer, Papaye, Pawpaw	KY700258 - KY700260
Angiosperm	Caryophyllales	Aizoaceae	*Sesuvium ayresii*	RI	3	RI	Native	TRUE	Pourpier marin	KX689335, KX689336
Angiosperm	Caryophyllales	Amaranthaceae	*Achyranthes aspera*	RI	5	RI	Introduced	TRUE	-	KY700202 - KY700204, KY700208
Angiosperm	Caryophyllales	Amaranthaceae	*Aerva congesta*	IAA, RI	3	IAA	Endemic	TRUE	-	KY700209 - KY700211
Angiosperm	Caryophyllales	Amaranthaceae	*Amaranthus dubius*	IAA, RI	4	IAA (2); mainland (2); RI (1)	Introduced	TRUE	Brède malabar	KY700217 - KY700219, KY700229
Angiosperm	Caryophyllales	Amaranthaceae	*Amaranthus viridis*	IAA, RI	3	RI (3)	Introduced	TRUE	-	KY700220 - KY700222
Angiosperm	Caryophyllales	Nyctaginaceae	*Boerhavia coccinea*	RI	9	RI	Native	TRUE	Herbe pintade	KY700239 - KY700244, KY700563, KY700564
Angiosperm	Caryophyllales	*Petiveriaceae*	*Rivina humilis*	IAA	10	IAA	Introduced	TRUE	Petite groseille	KY700467 - KY700469
Angiosperm	Caryophyllales	Portulacaceae	*Portulaca oleracea*	IAA, RI	11	IAA (4), Ile aux Fouquets (3); RI (3); mainland (1)	Introduced	TRUE	Pourpier rouge, Pourpier	KY700453 - KY700456
Angiosperm	Celastrales	Celastraceae	*Cassine orientalis*	IAA, RI	3	IAA	Endemic	TRUE	Bois d'olive	KY700255 - KY700257
Angiosperm	Celastrales	Celastraceae	*Maytenus pyria*	IAA, RI	3	IAA	Endemic	TRUE	Bois à poudre	KY700412 - KY700414
Angiosperm	Commelinales	Commelinaceae	*Commelina benghalensis*	RI	5	RI	Introduced	TRUE	Herbe cochon	KY700280, KY700281
Angiosperm	Ericales	Ebenaceae	*Diospyros egrettarum*	IAA, RI	6	IAA	Endemic	TRUE	Bois d'ébène lie aux Aigrettes	KY700301 - KY700303
Angiosperm	Ericales	Ebenaceae	*Diospyros tesselaria*	IAA, RI	4	IAA (1); mainland (2); RI (1)	Endemic	TRUE	Bois d'ébène noir, ébenier	KY700307 - KY700310
Angiosperm	Ericales	Lecythidaceae	*Foetidia mauritiana*	IAA, RI	3	IAA	Endemic	TRUE	Bois puant	KY700361, KY700362

Table 2.1. Species and sample list for Ile aux Aigrettes and Round Island.

Division	Order	Family	*Binomial name*	Located on	Sample no.	Sample origin	Endemic/ native/ Introduced	Sequenced	Local name	GenBank Accession number
Angiosperm	Ericales	Sapotaceae	*Sideroxylon boutonianum*	IAA	3	IAA	Endemic	TRUE	Bois de fer	KX689341 - KX689343
Angiosperm	Fabales	Fabaceae	*Albizia lebbeck*	IAA	3	IAA	Introduced	TRUE	Bois noir	KY700215, KY700216
Angiosperm	Fabales	Fabaceae	*Caesalpinia bonduc*	IAA	6	IAA	Native	TRUE	Cadoque, Cadoc, Bonduc	KY700251 - KY700254
Angiosperm	Fabales	Fabaceae	*Dendrolobium umbellatum*	IAA	3	IAA	Native	TRUE	Bois malgache	KY700565, KY700566, KX689290
Angiosperm	Fabales	Fabaceae	*Desmanthus virgatus*	IAA, RI	3	IAA	Introduced	TRUE	Petit acacia	KY700299, KY700300
Angiosperm	Fabales	Fabaceae	*Desmodium incanum*	IAA, RI	7	Mainland (1); RI (6)	Introduced	TRUE	Herbe gallon	KY700295 - KY700298
Angiosperm	Fabales	Fabaceae	*Erythrina variegata*	IAA	1	IAA	Cryptogenic	TRUE	Mourouque	KY700321, KY700322
Angiosperm	Fabales	Fabaceae	*Gagnebina pterocarpa*	IAA, RI	4	IAA	Native	TRUE	Acacia indigene	KY700363 - KY700365
Angiosperm	Fabales	Fabaceae	*Leucaena leucocephala*	IAA, RI	3	IAA	Introduced	TRUE	Acacia	KY700392 - KY700394
Angiosperm	Fabales	Fabaceae	*Millettia pinnata*	IAA	4	IAA (3); mainland (1)	Introduced	TRUE	Pongame, Coqueluche	KY700415 - KY700417
Angiosperm	Fabales	Fabaceae	*Pithecellobium dulce*	IAA	6	IAA	Introduced	TRUE	Cassie de Manille	KY700366 - KY700369, KY700448, KY700449
Angiosperm	Fabales	Fabaceae	*Rhynchosia viscosa*	IAA	3	IAA	Introduced	TRUE	Liane lastic	KX689329 - KX689331
Angiosperm	Fabales	Fabaceae	*Senna occidentalis*	IAA	3	IAA	Introduced	TRUE	Casse puante	KY700480 - KY700482
Angiosperm	Fabales	Fabaceae	*Sophora tomentosa*	IAA	3	IAA	Native	TRUE	Bois chapelet	KY700495 - KY700497
Angiosperm	Gentianales	Apocynaceae	*Catharanthus roseus*	IAA	6	IAA	Introduced	TRUE	Saponaire, Pervenche de Madagascar	KY700261 - KY700263
Angiosperm	Gentianales	Apocynaceae	*Cynanchum staubii*	IAA	3	IAA	Endemic	TRUE	Liane calle	KX689283 - KX689285
Angiosperm	Gentianales	Apocynaceae	*Ochrosia borbonica*	IAA	3	IAA	Endemic	TRUE	Bois jaune, Quinquina du pay	KX689310 - KX689312
Angiosperm	Gentianales	Apocynaceae	*Secamone dilapidens*	IAA	4	IAA	Endemic	TRUE	Liane á cornes	KX689337 - KX689340
Angiosperm	Gentianales	Apocynaceae	*Secamone volubilis*	IAA	2	IAA	Endemic	TRUE	Liane bois d'olive, liane a ouate	KY700483, KY700558
Angiosperm	Gentianales	Apocynaceae	*Tylophora coriacea*	IAA, RI	6	IAA	Native	TRUE	Ipéca du Pays	KY700526, KY700527
Angiosperm	Gentianales	Rubiaceae	*Coffea myrtifolia*	IAA	3	IAA	Endemic	TRUE	-	KY700288 - KY700290
Angiosperm	Gentianales	Rubiaceae	*Coptosperma borbonica*	IAA, RI	3	IAA	Endemic	TRUE	Bois de rat	KY700282, KY700283
Angiosperm	Gentianales	Rubiaceae	*Fernelia buxifolia*	IAA, RI	6	IAA	Endemic	TRUE	Bois buis, Bois bouteille, Bois chauve-souris	KY700341, KY700342
Angiosperm	Gentianales	Rubiaceae	*Morinda citrifolia*	IAA	4	IAA	Introduced	TRUE	Bois tortue	KY700418, KY700419

Table 2.1. Species and sample list for Ile aux Aigrettes and Round Island.

Division	Order	Family	*Binomial name*	Located on	Sample no.	Sample origin	Endemic/ native/ Introduced	Sequenced	Local name	GenBank Accession number
Angiosperm	Gentianales	Rubiaceae	*Oldenlandia sieberi*	IAA	3	IAA	Native	TRUE	-	KX689313 - KX689315
Angiosperm	Lamiales	Acanthaceae	*Asystasia gangetica*	IAA	3	IAA	Introduced	TRUE	Herbe á pistache	KY700228, KY700560
Angiosperm	Lamiales	Acanthaceae	*Barleria observatrix*	IAA, RI	6	IAA	Endemic	TRUE	-	KY700561, KY700562, KX689273
Angiosperm	Lamiales	Bignoniaceae	*Tabebuia pallida*	IAA	3	IAA	Introduced	TRUE	Técoma	KX689347 - KX689349
Angiosperm	Lamiales	Lamiaceae	*Premna serratifolia*	IAA, RI	4	IAA	Native	TRUE	Bois sureau	KY700459, KY700460
Angiosperm	Lamiales	Lauraceae	*Clerodendrum heterophyllum*	IAA, RI	6	IAA	Endemic	TRUE	Bois cabris	KY700274 - KY700276
Angiosperm	Lamiales	Oleaceae	*Chionanthus ayresii*	IAA	3	IAA	Endemic	TRUE	Bois blanc	KX689274, KX689275
Angiosperm	Lamiales	Oleaceae	*Olea europaea var. africana*	IAA	4	IAA	Native	TRUE	Olivier de bourbon	KY700425 - KY700427
Angiosperm	Lamiales	Scrophulariaceae	*Myoporum mauritianum*	RI	4	RI	Endemic	TRUE	-	KY700421 - KY700423
Angiosperm	Lamiales	Verbenaceae	*Lantana camara*	IAA	3	IAA	Introduced	TRUE	Vieille fille	KY700389 - KY700391
Angiosperm	Lamiales	Verbenaceae	*Stachytarpheta jamaicensis*	IAA	3	IAA	Introduced	TRUE	-	KY700547
Angiosperm	Laurales	Lauraceae	*Cassytha filiformis*	IAA	3	IAA	Native	TRUE	-	KY700543
Angiosperm	Laurales	Lauraceae	*Litsea glutinosa*	IAA	3	IAA	Introduced	TRUE	Bois d'oiseaux	KY700399
Angiosperm	Malpighiales	Erythroxylaceae	*Erythroxylum sideroxyloides*	IAA, RI	4	IAA	Endemic	TRUE	Bois de ronde	KY700318 - KY700320
Angiosperm	Malpighiales	Euphorbiaceae	*Acalypha indica*	IAA	3	IAA	Introduced	TRUE	Herbe chatte	KY700205 - KY700207
Angiosperm	Malpighiales	Euphorbiaceae	*Euphorbia heterophylla*	IAA	3	IAA	Introduced	TRUE	-	KY700323 - KY700325
Angiosperm	Malpighiales	Euphorbiaceae	*Euphorbia hirta*	IAA	3	IAA	Introduced	TRUE	Jean Robert	KY700326, KY700327, KY700329
Angiosperm	Malpighiales	Euphorbiaceae	*Euphorbia hypericifolia*	IAA	3	IAA	Introduced	TRUE	Herbe malélevé, Herbe colique	KY700328, KY700330, KY700331
Angiosperm	Malpighiales	Euphorbiaceae	*Euphorbia prostrata*	RI	3	IAA	Introduced	TRUE	Rougette	KY700340, KY700550, KY700551
Angiosperm	Malpighiales	Euphorbiaceae	*Euphorbia thymifolia*	RI, IAA	6	RI	Cryptogenic	TRUE	Petite rougette	KY700335 - KY700339, KY700552
Angiosperm	Malpighiales	Euphorbiaceae	*Ricinus communis*	IAA	2	IAA	Introduced	TRUE	Ricin	KY700464, KY700465
Angiosperm	Malpighiales	Euphorbiaceae	*Stillingia lineata*	IAA, RI	9	IAA (2); RI (7)	Endemic	TRUE	Fangame	KY700505 - KY700507
Angiosperm	Malpighiales	Passifloraceae	*Passiflora suberosa*	IAA, RI	4	IAA	Introduced	TRUE	Liane poc poc	KY700430 - KY700432
Angiosperm	Malpighiales	Passifloraceae	*Turnera angustifolia*	IAA	3	IAA	Introduced	TRUE	-	KX689353 - KX689355
Angiosperm	Malpighiales	Phyllanthaceae	*Phyllanthus amarus*	IAA, RI	6	RI	Introduced	TRUE	Petit tamarin blanc	KY700439 - KY700441,

Table 2.1. Species and sample list for Ile aux Aigrettes and Round Island.

Division	Order	Family	*Binomial name*	Located on	Sample no.	Sample origin	Endemic/ native/ Introduced	Sequenced	Local name	GenBank Accession number
										KY700445, KY700555
Angiosperm	Malpighiales	Phyllanthaceae	*Phyllanthus casticum*	IAA	3	IAA	Native	TRUE	Bois castique, Castique, Bois de demoiselle	KY700442 - KY700444
Angiosperm	Malpighiales	Phyllanthaceae	*Phyllanthus mauritianus*	IAA, RI	3	IAA	Native	TRUE	-	KX689319 - KX689321
Angiosperm	Malpighiales	Phyllanthaceae	*Phyllanthus niruroides*	IAA	3	IAA	Introduced	TRUE	Petite castique, Curanellie blanche	KX689322, KX689323, KX689328
Angiosperm	Malpighiales	Phyllanthaceae	*Phyllanthus revaughanii*	IAA, RI	3	IAA	Endemic	TRUE	-	KX689324 - KX689326
Angiosperm	Malpighiales	Phyllanthaceae	*Phyllanthus tenellus*	IAA	4	IAA	Introduced	TRUE	-	KY700446, KY700447, KY700557
Angiosperm	Malpighiales	Phyllanthaceae	*Margaritaria anomala*	IAA, RI	3	IAA	Endemic	TRUE	Bois chenille	KY700409 - KY700411
Angiosperm	Malpighiales	Salicaceae	*Flacourtia indica*	IAA	3	IAA	Introduced	TRUE	Prune malgache	KY700356 - KY700360
Angiosperm	Malpighiales	Salicaceae	*Ludia mauritiana*	IAA	3	IAA	Endemic	TRUE	Bois mozambique	KY700403 - KY700405
Angiosperm	Malpighiales	Salicaceae	*Scolopia heterophyla*	RI	6	RI	Endemic	TRUE	Bois goyave	KX689332 - KX689334
Angiosperm	Malvales	Malvaceae	*Abutilon indicum*	RI	4	RI	Introduced	TRUE	Mauve du pays	KY700199 - KY700201
Angiosperm	Malvales	Malvaceae	*Dombeya mauritiana*	IAA	3	IAA	Endemic	TRUE	-	KY700311 - KY700313
Angiosperm	Malvales	Malvaceae	*Hibiscus fragilis*	RI	3	RI	Endemic	TRUE	Mandrinette, Augerine (fide Bojer)	KX689303 - KX689305
Angiosperm	Malvales	Malvaceae	*Hibiscus tiliaceus*	IAA, RI	6	IAA	Native	TRUE	Var, Vaur	KY700376 - KY700378, KY700511
Angiosperm	Malvales	Malvaceae	*Sida pussila*	RI	6	RI	Native	TRUE	-	KX689344 - KX689346
Angiosperm	Malvales	Malvaceae	*Thespesia populnea*	IAA, RI	7	IAA	Native	TRUE	Mahoe, Ste Marie, Porcher	KY700512, KY700513
Angiosperm	Malvales	Malvaceae	*Trochetia boutoniana*	IAA	3	IAA	Endemic	TRUE	-	KY700517, KY700518
Angiosperm	Malvales	Malvaceae	*Urena lobata var.sinuata*	IAA	3	IAA	Endemic	TRUE	Herbe panier	KY700528 - KY700530
Angiosperm	Malvales	Thymelaeaceae	*Wikstroemia indica*	IAA	3	IAA	Introduced	TRUE	Herbe tourterelle	KY700531 - KY700533
Angiosperm	Myrtales	Combretaceae	*Terminalia bentzoe*	IAA, RI	3	IAA	Endemic	TRUE	Bois benjoin	KX689350 - KX689352
Angiosperm	Myrtales	Lythraceae	*Pemphis acidula*	IAA	3	IAA	Native	TRUE	Bois matelot	KY700436 - KY700438
Angiosperm	Myrtales	Myrtaceae	*Eugenia lucida*	IAA, RI	3	IAA	Endemic	TRUE	Bois clou, Bois de clous	KY700332 - KY700334
Angiosperm	Oxalidales	Oxalidaceae	*Oxalis corniculata*	IAA, RI	3	IAA	Introduced	TRUE	Petite oseille, Petit trèfle, Alleluia a fleurs jaunes	KY700428, KY700429
Angiosperm	Pandanales	Pandanaceae	*Pandanus vandermeeschii*	IAA, RI	3	IAA	Endemic	TRUE	Vacoas	KY700545
Angiosperm	Poales	Cyperaceae	*Cyperus dubius*	IAA, RI	10	IAA (7), RI (3)	Native	TRUE	-	KY700386 - KY700388, KY700466
Angiosperm	Poales	Cyperaceae	*Cyperus exilis*	RI	6	RI	Native	TRUE	-	KX689279 - KX689282

Table 2.1. Species and sample list for Ile aux Aigrettes and Round Island.

Division	Order	Family	*Binomial name*	Located on	Sample no.	Sample origin	Endemic/ native/ Introduced	Sequenced	Local name	GenBank Accession number
Angiosperm	Poales	Cyperaceae	*Fimbristylis cymosa* **	IAA, RI	6	IAA (3); RI (3)	Native	TRUE	Hurricane grass	KY700346 - KY700350
Angiosperm	Poales	Cyperaceae	*Fimbristylis littoralis* **	IAA	3	IAA	Native	TRUE	Lesser Fimbristylis	KY700351 - KY700353
Angiosperm	Poales	Poaceae	*Bothriochloa pertusa*	IAA	1	IAA	Introduced	TRUE	-	KY700268
Angiosperm	Poales	Poaceae	*Cenchrus echinatus*	RI	5	RI	Introduced	TRUE	Herbe á cateaux	KY700264, KY700265
Angiosperm	Poales	Poaceae	*Chloris barbata*	IAA, RI	6	IAA (3); RI (3)	Introduced	TRUE	-	KY700266, KY700269, KY700270
Angiosperm	Poales	Poaceae	*Chloris filiformis*	RI	6	RI	Endemic	TRUE	-	KX689276 - KX689278, KX689300 - KX689302
Angiosperm	Poales	Poaceae	*Cymbopogon excavatus*	RI	5	RI	Native	TRUE	Citronelle sauvage	KY700291, KY700292
Angiosperm	Poales	Poaceae	*Dactyloctenium ctenoides*	RI	3	RI	Native	TRUE	-	KX689286 - KX689289
Angiosperm	Poales	Poaceae	*Dichanthium annulatum**	IAA	1		Introduced	TRUE	-	KY700267
Angiosperm	Poales	Poaceae	*Digitaria horizontalis*	RI	5	RI	Introduced	TRUE	Gros Meinki	KX689291 - KX689293
Angiosperm	Poales	Poaceae	*Eleusine indica*	IAA	4	IAA	Introduced	TRUE	-	KY700395, KY700396, KY700548, KY700549
Angiosperm	Poales	Poaceae	*Eragrostis amabilis*	IAA	6	IAA	Native	TRUE	-	KY700433 - KY700435, KY700499 - KY700501
Angiosperm	Poales	Poaceae	*Heteropogon contortus*	IAA, RI	8	IAA (4); RI (4)	Introduced	TRUE	Herbe polisson	KY700371, KY700372
Angiosperm	Poales	Poaceae	*Lepturus repens*	IAA, RI	7	IAA (3); RI (4)	Native	TRUE	-	KY700397, KY700398
Angiosperm	Poales	Poaceae	*Saccharum sp.**	IAA	1	IAA	Introduced	TRUE	-	KY700370
Angiosperm	Poales	Poaceae	*Sporobolus africanus var. capensis*	RI	3	RI	Introduced	TRUE	-	KY700498, KY700573
Angiosperm	Poales	Poaceae	*Sporobolus virginicus*	RI	2	RI	Native	TRUE	-	KY700540, KY700542
Angiosperm	Poales	Poaceae	*Stenotaphrum dimidiatum*	IAA	6	IAA	Native	TRUE	Herbe bourique	KY700502 - KY700504
Angiosperm	Poales	Poaceae	*Stenotaphrum micranthum*	IAA, RI	6	IAA	Native	TRUE	-	KY700508 - KY700510
Angiosperm	Poales	Poaceae	*Vetiveria arguta*	IAA, RI	3	IAA	Native	TRUE	-	KX689356 - KX689358
Angiosperm	Poales	Poaceae	*Zoysia tenuifolia*	RI	4	RI	native	TRUE	Herbe pique fesses	KY700539, KY700541
Angiosperm	Ranunculales	Papaveraceae	*Argemone mexicana*	IAA	6	IAA	Introduced	TRUE	Chardon	KY700225 - KY700227
Angiosperm	Rosales	Moraceae	*Ficus benghalensis*	IAA	3	IAA	Introduced	TRUE	Banian, Multipliant, Lafouche	KY700343 - KY700345

Table 2.1. Species and sample list for Ile aux Aigrettes and Round Island.

Division	Order	Family	*Binomial name*	Located on	Sample no.	Sample origin	Endemic/ native/ Introduced	Sequenced	Local name	GenBank Accession number
Angiosperm	Rosales	Moraceae	*Ficus reflexa*	IAA, RI	4	IAA	Native	TRUE	La fouche bâtard, Affouche á petites feuilles	KY700354, KY700355
Angiosperm	Rosales	Moraceae	*Ficus rubra*	IAA, RI	4	IAA	Native	TRUE	Affouche rouge, La fouche, Affouche á grandes feuilles	KX689294 - KX689296
Angiosperm	Rosales	Rhamnaceae	*Colubrina asiatica*	IAA	3	IAA	Introduced	TRUE	-	KY700277 - KY700279
Angiosperm	Rosales	Rhamnaceae	*Gouania tiliifolia*	IAA	3	IAA	Native	TRUE	Liane charretier	KX689297 - KX689299
Angiosperm	Rosales	Rhamnaceae	*Scutia myrtina*	IAA	3	IAA	Native	TRUE	Liane bambara, Bambara	KY700477 - KY700479
Angiosperm	Rosales	Rhamnaceae	*Ziziphus mauritiana*	IAA	1	IAA	Introduced	TRUE	-	KY700538
Angiosperm	Rosales	Urticaceae	*Pilea microphylla*	IAA	2	IAA	Introduced	TRUE	Barbe de St. Antoine	KY700554, KY700559
Angiosperm	Santalales	Santalaceae	*Santalum album*	IAA	3	IAA	Introduced	TRUE	Bois de santal	KY700470, KY700471
Angiosperm	Sapindales	Anacardiaceae	*Poupartia borbonica*	IAA	6	IAA	Endemic	TRUE	Bois Poupart	KX689316 - KX689318
Angiosperm	Sapindales	Anacardiaceae	*Schinus terebinthifolius*	IAA	3	IAA	Introduced	TRUE	Poivrier marron	KY700475, KY700476
Angiosperm	Sapindales	Burseraceae	*Protium obtusifolium*	IAA, RI	3	IAA	Endemic	TRUE	Colophane bâtard	KY700457, KY700458
Angiosperm	Sapindales	Meliaceae	*Turraea thouarsiana*	IAA	4	IAA	Endemic	TRUE	Bois quivi	KY700524, KY700525
Angiosperm	Sapindales	Rutaceae	*Triphasia trifolia*	IAA	1	IAA	Introduced	TRUE	Orangine	KY700523
Angiosperm	Sapindales	Rutaceae	*Zanthoxylum heterophyllum*	IAA, RI	3	IAA	Endemic	TRUE	Bois de catafaille noir	KX689359, KX689360
Angiosperm	Sapindales	Sapindaceae	*Dodonaea viscosa*	IAA, RI	3	IAA	Native	TRUE	Bois de reinette	KY700314 - KY700316
Angiosperm	Sapindales	Sapindaceae	*Hornea mauritiana*	RI	2	RI	Endemic	TRUE	Arbre á l'huile	KX689361, KX689362
Angiosperm	Sapindales	Sapindaceae	*Stadmania oppositifolia*	RI	1	RI	Native	TRUE	Bois de fer	KX689363
Angiosperm	Saxifragales	Crassulaceae	*Bryophyllum pinnatum*	IAA	8	IAA	Introduced	TRUE	Soudefafe	KY700245 - KY700250
Angiosperm	Solanales	Convolvulaceae	*Dichondra repens*	RI	3	RI	Native	TRUE	-	KY700304 - KY700306
Angiosperm	Solanales	Convolvulaceae	*Ipomoea violacea*	IAA	7	IAA	Native	TRUE	-	KX689306 - KX689309
Angiosperm	Solanales	Convolvulaceae	*Ipomoea pes-caprae*	RI	7	Ile aux Fouquets (2); mainland (1); RI (4)	Native	TRUE	Batate, Patate á Durand, Batatran	KY700382, KY700383
Angiosperm	Solanales	Convolvulaceae	*Ipomoea obscura*	IAA	4	IAA	Introduced	TRUE	Amourette	KY700384, KY700385
Angiosperm	Solanales	Solanaceae	*Nicotiana tabacum*	RI	1	RI	Introduced	TRUE	Tabak	KY700575
Angiosperm	Solanales	Solanaceae	*Physalis peruviana*	RI	1	RI	Introduced	TRUE	Pocke-pocke, Cape gooseberry	KY700546

Table 2.1. Species and sample list for Ile aux Aigrettes and Round Island.

Division	Order	Family	*Binomial name*	Located on	Sample no.	Sample origin	Endemic/ native/ Introduced	Sequenced	Local name	GenBank Accession number
Angiosperm	Solanales	Solanaceae	*Solanum americanum*	RI	8	RI (7), IAA (1)	Introduced	TRUE	Brède martin	KY700484, KY700485
Angiosperm	Solanales	Solanaceae	*Solanum lycopersicum*	RI	3	RI	Introduced	TRUE	Pomme d'amour	KY700406 - KY700408, KY700574 , KY700489, KY700490, KY700491
Angiosperm	Solanales	Solanaceae	*Withania somnifera*	RI	3	RI	Introduced	TRUE	-	KY700534, KY700576
Angiosperm	Vitales	Vitaceae	*Cyphostemma mappia*	IAA	3	IAA	Endemic	TRUE	Mapou	KY700293, KY700294
Bryophyta	Pottiales	Pottiaceae	*Hydrogonium sp.*	RI	1	RI		TRUE	-	KY700420
Lycopodiophyta	Selaginellales	Selaginellaceae	*Selaginella barklyi*	RI	5	RI	Native	FALSE	-	-
Pteridophyta	Polypodiales	Polypodiaceae	*Phymatodes scolopendria*	IAA, RI	4	IAA	Native	TRUE	Fougère polypode	KY700572, KX689327
Pteridophyta	Polypodiales	Pteridaceae	*Adiantum rhizophorum*	RI	4		Native	FALSE	-	-
Pteridophyta	Polypodiales	Pteridaceae	*Pteris vittata*	IAA, RI	5	IAA (3); RI (2)	Native	FALSE	Ptéris rubané (Reunion Island)	-
Pteridophyta	Polypodiales	Thelypteridaceae	*Christella dentata*	RI	3	Mainland	Native	FALSE	-	-
Pteridophyta	Psilotales	Psilotaceae	*Psilotum nudum*	IAA, RI	2	IAA	Native	FALSE	-	-

Grasses annotated with * were not previously recorded on the island and only one specimen of each was found
Sedges annotated with ** are awaiting identification by a Cyperaceae family expert (Dr Isabel Larridon, Kew)

2.5 Discussion

2.5.1 Comprehensive DNA barcode libraries and trophic interactions

This study presents a comprehensive DNA barcode library for two Mauritian islands, which comprises 99% of the angiosperms (n=164) and 25% of the ferns (n=4) present. Previous studies have created DNA barcode libraries for their study areas to enable studies of herbivory (e.g. Valentini *et al.* 2009; Gebremedhin *et al.* 2016), but no islands or other entire study areas have been comprehensively barcoded with dietary analysis in mind. Moreover, working on well-studied island systems means that, with the assistance of local expertise and knowledge, comprehensive species lists are available and comprehensive sampling of floral diversity is possible. For species-level identification in DNA metabarcoding studies, a comprehensive DNA barcode library (where possible) is acknowledged to improve resolution considerably (Hofreiter *et al.* 2003; Taberlet *et al.* 2007; Garcia-Robledo *et al.* 2013a).

This study will facilitate the examination of plant-plant and plant-animal interactions on two islands undergoing ecological restoration. For example, the library can facilitate a better understanding of seed dispersal, pollination, grazing ecology, and the consequences of these processes on species of conservation importance. Exploring trophic dynamics is an area yet to be fully recognized in ecological restoration (Perring *et al.* 2015). Previously, on Ile aux Aigrettes, the trophic interactions of Telfair's skinks (*Leiolopisma telfairii*), Asian musk shrews (*Suncus murinus*), and the invertebrate fauna were investigated using DNA metabarcoding (Brown *et al.* 2014). However, the absence of a DNA barcode reference library for the invertebrates on the island has meant that no species level identification of dietary items was possible. This library now allows investigation of species level trophic interactions between the plant community and a host of both native and exotic herbivorous or omnivorous species.

2.5.2 DNA metabarcoding and ecological restoration

Previously, DNA metabarcoding was used to assess and monitor restoration projects and to inform management practices. For example, DNA metabarcoding was used to assess the diet of a restored population of European bison (*Bison bonasus*) to better understand their impact on woody species for forestry management (Kowalczyk *et al.* 2011). Ji *et al.* (2013) showed that DNA metabarcoding is a reliable method for obtaining biodiversity information for policymaking, including for restoration projects.

The primary reason for creating the library was to facilitate the use of DNA metabarcoding to better understand the impacts of introduced Aldabra giant tortoises on plant communities and its consequences across the food web. The tortoises have been introduced to both Ile aux Aigrettes and Round Island to replace their extinct counterparts in an experiment to restore ecosystem functioning (Griffiths *et al.* 2010; Griffiths *et al.* 2011). However, the DNA barcode library will also facilitate the understanding of herbivorous diets of any of the islands' native and introduced animal communities. In addition it will help to refine analysis of pollination networks and is already assisting with plant taxonomy to better inform ecological restoration.

2.5.3 Taxonomic discrimination of the ITS2 region

Using a BLASTn search on a database of species from these islands, 98.6% of taxa could be correctly assigned to species-level. The high taxonomic discrimination of ITS2 at the species level is increasingly well recognised (Chen *et al.* 2010; Pang *et al.* 2010; Hollingsworth *et al.* 2011; Li *et al.* 2011). For example, *rbcL* and *matK,* the formally recognized plant DNA barcoding regions for land plants, can discriminate 72% of 550 species (Hollingsworth *et al.* 2009). Despite its superior taxonomic resolution, there has been hesitation over the use of ITS as a DNA barcode due to the risk of fungal contamination, the presence of paralogous gene copies and amplification difficulties (Hollingsworth *et al.* 2011). However, research has shown that the former two concerns are minor (Hollingsworth 2011; Li *et al.* 2011) in comparison to the benefit gained by increased taxonomic resolution. The problem of amplification can be partially overcome by using ITS2 only as opposed to the longer full ITS region (Li *et al.* 2011) as done in this study. However, the presence of fungal contamination, for example a fungal endophyte, or the presence of paralogous ITS2 copies could have prevented us from successfully sequencing *D. album* var. *conjugatum.* Furthermore, it was difficult to amplify and sequence ferns (one of five fern species successfully sequenced), but this is a known drawback of the primer pairs selected for barcoding (Chen *et al.* 2010; Cheng *et al.* 2016).

Geographically restricted studies tend to have increased taxonomic discrimination (Kress *et al.* 2009; Burgess *et al.* 2011), perhaps emphasised in this study by high floral diversity (171 species from 147 genera from 66 families) with few very closely related species (only 15 genera with multiple species, and 14 genera if both *Fimbristylis sp.* are indeed the same species). However, DNA barcoding of plants in systems that cover a

larger geographic area is achievable (e.g. de Vere *et al.* 2012; Saarela *et al.* 2013) and if a single barcode does not provide sufficient taxonomic discrimination then multiple plant DNA barcodes may be required, as recommended by the Plant Working Group of the Consortium for the Barcoding of Life (Hollingsworth *et al.* 2009).

2.6 Acknowledgements

Thank you to Daevid Mike who carried out the molecular work on a subset of the Round Island plant tissue samples.

Chapter Three - New universal ITS2 primers for high-resolution herbivory analyses using DNA metabarcoding in both tropical and temperate zones

"…a life of incomprehensible loneliness awaits a world where the wild things were, but are never to be again"

William Stolzenburg, 2009

New universal ITS2 primers for high-resolution herbivory analyses using DNA metabarcoding in both tropical and temperate zones

Rosemary J. Moorhouse-Gann[1*], Jenny C. Dunn[1,2,3*], Natasha de Vere[4,5], Martine Goder[6], Nik Cole[6,7], Helen Hipperson[8], William O. C. Symondson[1]

Submitted to: This chapter is adapted from a manuscript due for submission to Scientific Reports in November 2017

[1] Cardiff School of Biosciences, The Sir Martin Evans Building, Museum Avenue, Cardiff, CF10 3AX, UK
[2] RSPB Centre for Conservation Science, Royal Society for the Protection of Birds, The Lodge, Potton Road, Sandy, Bedfordshire, SG19 2DL, UK
[3] School of Life Sciences, University of Lincoln, Joseph Banks Laboratories, Lincoln, LN6 7TS, UK
[4] National Botanic Garden of Wales, Llanarthne, Carmarthenshire, SA32 8HG UK
[5] Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth University, Penglais, Aberystwyth, Ceredigion, SY23 3DA, UK
[6] Mauritian Wildlife Foundation, Grannum Road, Vacoas, Mauritius
[7] Durrell Wildlife Conservation Trust, Les Les Augrès Manor, La Profonde Rue, Trinity, Jersey, Channel Islands, JE3 5BP
[8] NERC Biomolecular Analysis Facility, Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK

*RJMG and JCD are joint first authors on this manuscript.

Contribution by RJMG: wrote the main manuscript text and made modifications based on co-author comments alongside JCD. Primers were designed by RJMG and JCD. RJMG collected a subset of Mauritian samples and conducted all laboratory analysis on those samples. RJMG conducted all *in silico* PCR analysis on all databases and contributed to the design and implementation of the remaining analyses.

3.1 Abstract

DNA metabarcoding is a rapidly growing technique for obtaining detailed dietary information. Current metabarcoding methods for herbivory, using a single locus, typically lack taxonomic resolution. We present novel universal primers for the second internal transcribed spacer of nuclear ribosomal DNA (ITS2), which has potential to give unrivalled taxonomic resolution from a short-amplicon barcode. Primer development and *in silico* testing utilised three databases of plant ITS2 sequences from UK and Mauritian floras (native and introduced) jointly consisting of 6561 sequences from 1790 species from 174 families. *In silico* analyses found that: (i) the primers amplified 88% of species (n=1111 species and 148 families where forward and reverse priming sites were analysed simultaneously); and (ii) taxonomic discrimination was 86.1%, 99.4% and 99.9% at the species, genus and family levels respectively (n=1577species). In practice, PCR amplified 99% of Mauritian (n=169) and 100% of UK (n=33) species. We advocate taxonomic assignment based on best match as opposed to a clustering approach for this region. With a short amplicon of 187–387 bp, these primers are suitable for metabarcoding studies utilising degraded DNA, across a broad geographic range, whilst delivering unparalleled taxonomic discrimination.

3.2 Introduction

Understanding trophic interactions facilitates our understanding of community ecology and ecosystem functioning. Analysing such complex and dynamic processes has clear conservation benefits by allowing management strategies to be better informed (Kowalczyk *et al.* 2011). For example, understanding the feeding preferences of species allows us to assess the costs (Brown *et al.* 2014) and potential benefits (Ando *et al.* 2013) of alien species. Large herbivores in particular are recognised as keystone grazers (Griffiths *et al.* 2010; Kowalczyk *et al.* 2011) and determining their dietary preferences is critical to understanding their impact on plant communities and the wider food web. This is particularly relevant in the light of recent rewilding efforts, including the introduction of non-native species as ecological replacements (analogues) for extinct taxa to restore ecosystem function, or the (re)introduction of native species (Griffiths *et al.* 2010; Kowalczyk *et al.* 2011; Fernandez *et al.* 2017).

Traditional methods of dietary analysis include the morphological examination of faecal samples, gut contents and feeding observations. These techniques are fraught with methodological problems. For instance, morphological identification of plant remains is time consuming and requires high levels of expertise to identify masticated and partially digested plant fragments (Holechek *et al.* 1982). Examining stomach contents can provide information on dietary items that are more susceptible to digestion (Britton *et al.* 2006), but this invasive method can only be implemented after the death of the subject or by inducing regurgitation (Alonso *et al.* 2014). Direct observation is inherently difficult due to the need to identify dietary items from a distance, avoiding disturbance to natural feeding behaviour (Kruuk 1995) and also precludes working with any elusive, nocturnal or soil dwelling species (Pompanon *et al.* 2012). Molecular methods provide an alternative suite of approaches that can generate greater volumes of data more rapidly and with greater precision (Symondson 2002; King *et al.* 2008). For example, species-specific primers can be used to amplify the DNA of particular focal dietary items in gut contents or faecal samples (Pumarino *et al.* 2011; Leal *et al.* 2013; Wallinger *et al.* 2013). However, this approach is only appropriate if *a priori* dietary information is available and if the diet range is small. It cannot unravel the effects non-focal species may be having on dietary selection by a polyphagous predator or herbivore. In order to overcome such problems, and determine whole dietary ranges, DNA barcodes coupled with next generation sequencing

(NGS) (often referred to as DNA metabarcoding), have been widely adopted. Comparisons between morphological and molecular methods have been made, and in most cases metabarcoding has provided more dietary information (Soininen *et al.* 2009; Ando *et al.* 2013; Alonso *et al.* 2014).

Identification of animal dietary items primarily uses the mitochondrial cytochrome *c* oxidase gene, which has been shown to effectively discriminate between species (Hebert *et al.* 2003; Hebert *et al.* 2004; Hebert & Gregory 2005). However, in plants the mitochondrial genome evolves too slowly for these genes to provide sufficient variation to be useful barcodes (Taberlet *et al.* 2007). In 2009, the Consortium for the Barcode of Life approved plastid *matK* and *rbcL* as the barcode regions for use in land plants (Hollingsworth *et al.* 2009). However, these barcodes are suboptimal for dietary studies because their large fragment size (*rbcL* = 654 bp; *matK* = 889 bp) (Hollingsworth *et al.* 2011) means that they are unlikely to be recovered in degraded faecal samples. Minibarcodes have been designed within *rbcL*, but those suitable for application in dietary studies have low discriminatory power at the species level (Little 2014). An alternative approach is to design several primer pairs for overlapping sections within a DNA barcode, amplify and sequence all fragments, and subsequently piece the fragments together in to a single longer DNA barcode amplicon. Such a scaffolding approach may deliver improved taxonomic resolution but may be too complicated for many users, especially those with large sample numbers. The most commonly used single DNA barcode in herbivory studies is the P6 loop of the plastid *trn*L (UAA) gene (Taberlet *et al.* 2007; Jurado-Rivera *et al.* 2009; Soininen *et al.* 2009; Valentini *et al.* 2009; Kowalczyk *et al.* 2011; Raye *et al.* 2011; Baamrane *et al.* 2012; Ando *et al.* 2013; Coghlan *et al.* 2013; Hibert *et al.* 2013), but the taxonomic resolution of this barcoding region is low (Pompanon *et al.* 2012). The second internal transcribed spacer (ITS2) of nuclear ribosomal DNA has been suggested as a 'gold standard' barcode for identifying plants (Chen *et al.* 2010) and there is growing evidence to support this (Hollingsworth 2011; Li *et al.* 2011; Han *et al.* 2013). In a study examining 4800 species of medicinal plant, testing of ITS2 as a barcoding region revealed correct taxonomic identification at the species and genus levels was approximately 91.5% and 99.8% (Chen *et al.* 2010). Such high taxonomic resolution in a small 160–320 bp region makes ITS2 a promising DNA barcoding region for use in dietary studies.

Existing general primers for ITS2 have been designed for priming sites within the more conserved flanking regions of 5.8S and 26S (Chen *et al.* 2010; Cheng *et al.* 2016). This presents a problem for dietary studies since the resultant amplicon length (approximately 387–547 bp using S2F and S3R (Chen *et al.* 2010)) is potentially too great to be reliably detected in semi-digested samples. Designing general shorter amplicon primers closer to ITS2 within the flanking regions, or within ITS2 itself, is a challenge due to high interspecific variation. Here, we meet this challenge by designing a suite of primer pairs for a short ITS2 amplicon that are suitable for use in herbivory studies and test these against three ITS2 sequence databases: 1) a comprehensive database of plants from two Mauritian islands (Mauritian database); 2) a database consisting of UK plant sequences downloaded from GenBank (UK database). This database contains at least one representative species from each genus of plant present in the UK; 3) all species known to feature in the diet of an obligate granivore (European turtle dove *Streptopelia turtur*) (Turtle dove database). The inclusion of the turtle dove database was to ensure that novel metabarcoding primers could be used to study herbivory by this species, which has suffered rapid and sustained population declines, in order to inform on going species conservation.

We had three objectives:

To establish what proportion of species are detected, using our new primers, against all three databases *in silico* and against all available Mauritian species and a subset of UK species *in vitro*.

To determine the discrimination capacity of our primers using all three databases combined for the ITS2 region.

For the two databases with multiple sequences per species (Mauritian and a subset of the UK database), identify clustering thresholds to use in the bioinformatics pipeline for analysis of NGS data, to maximise species discrimination and minimise assignment of multiple haplotypes of the same species to different taxonomic units.

3.3 Materials and Methods

3.3.1 Databases

Mauritian database: Plant tissue samples were collected from two Mauritian islands: Ile aux Aigrettes and Round island (Chapter 2). For *in vitro* primer testing, the database consisted of 169 species from 65 families. For *in silico* analyses, available sequences were supplemented

with eight sequences downloaded from GenBank and consisted of a maximum of 464 sequences, 167 species and 63 families. Eighty-four sequences were used for primer design (Appendix 1.1).

UK database: 6054 ITS2 sequences from 1651 UK plant species from 151 families were downloaded from GenBank. Where possible, if sequences did not span both priming sites we obtained untrimmed sequences from the authors. Where available, this included at least one representative from each genus of plants listed on the Ecological Database of the British Isles (Fitter & Peat 1994) (a comprehensive list of both native and introduced plant species found in the UK). Synonyms were checked with The Plant List (2013) .

Turtle dove database: Thirty six UK plant species were collected and barcoded as part of a separate study examining the diet of European Turtle Doves, (Dunn *et al.* in prep), with an additional 14 species represented in the database by sequences downloaded from GenBank. This included 31 species previously identified in the diet of Turtle doves using microscopy, seven species known to be present within commercial seed mixes and 12 additional species commonly found in arable farmland (Appendix 1.1).

3.3.2 Processing of field-collected samples: Mauritian and turtle dove databases

DNA extractions were carried out following Randall *et al.* (2015), after samples were ground under liquid nitrogen, or using the Qiagen DNeasy plant kit (Qiagen, Manchester, UK). The complete second internal transcribed spacer of nuclear ribosomal DNA (ITS2) and partial 5.8S and 26S sequences were amplified using primer pair S2F and S3R (Chen *et al.* 2010). Where amplification with this primer pair failed, a second ITS2 primer pair was tried, ITS-p3 and ITS-p4 (Cheng *et al.* 2016). PCRs were carried out in 10 µL reaction volumes containing 2 µL DNA template, 1 X PCR buffer, 2.0 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP and 1 U *Taq* DNA polymerase. For problematic samples, a multiplex PCR mix (Qiagen, Manchester, UK) was used, with primers and DNA at the same concentration and volume described above. Reaction conditions were an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 1 min, and a final extension of 72°C for 10 min. PCR products were sequenced in both directions by Eurofins Genomics (Wolverhampton, UK). Contigs were constructed and consensus sequences created in Sequencher version 5.4.6 (Gene

Codes Corporation) or MEGA5 (Tamura *et al.* 2011) after manually editing sequences. Consensus sequences were aligned using automated ClustalW alignment in BioEdit (Beheregaray *et al.* 2003) or ClustalX (Larkin *et al.* 2007).

3.3.3 Short amplicon primer design and in vitro testing

A subset of aligned ITS2 and partial 26S and 5.8S sequences (Appendix 1.1) were used to design primers for a short ITS2 amplicon to maximise amplification from the degraded DNA found in faecal samples. Aligned sequences were examined by eye in MEGA5 (Tamura *et al.* 2011) in order to detect suitably conserved sites. Five forward and seven reverse primers were designed and tested *in vitro.* All *in vitro* testing involved amplification in 10 µL PCR reaction volumes with reagents and template DNA in the same concentrations as described above. Reaction conditions were also the same as above, after initially testing annealing temperatures from 46°C–56°C by gradient PCR. Successful amplification was determined by visualisation on a 2% agarose gel stained with SYBR®Safe (ThermoFisher Scientific, Paisley, UK). Primers that failed initial tests (amplification failure, faint bands, multiple banding) on a small number of plant DNA samples were rejected with no further testing (Appendix 1.2)**.** These initial *in vitro* tests revealed that one primer pair, UniPlantF and UniPlantR, had the highest amplification success so these were subjected to further *in vitro* testing against all available Mauritian plant species and the field-collected UK species.

3.3.4 In silico testing

In silico PCR was carried out on all three databases using ecoPCR within OBITools (Boyer *et al.* 2016). The primer fit criteria allowed for a maximum of three base mismatches per UniPlant primer ensuring the last two bases at the 3' end were an exact match (Bellemain *et al.* 2010), specifying a minimum amplicon length of 100 bp and a maximum of 500 bp. Where DNA sequences did not encompass both forward and reverse priming sites, primers were tested independently and reported in the supporting information (Appendix 1.3). All DNA sequences used in this study are available on NCBI GenBank (Benson *et al.* 2014) and all accession numbers are listed in Appendix 1.5.

3.3.5 Testing taxonomic discrimination

To test the taxonomic discrimination of the ITS2 region within the UniPlant amplicon (Fig. 3.1), we combined all three databases and removed identical sequences derived from the same species and those sequences of poor quality (resulting in n=3550 total sequences, representing 1659 species, 828 genera and 155 families). We used ITSx (Bengtsson-Palme *et al.* 2013) to extract the ITS2 region from our amplicons to form our ITS2 database (ITS2 successfully extracted from 2216 sequences, representing 1577 species, 821 genera and 143 families). We used the "derep_prefix" command in usearch (Edgar 2010) to identify identical sequences within each database; we then calculated the number of taxa within which multiple species had identical ITS2 sequences. We calculated the relative taxonomic discrimination of our primers at the species, genus and family levels.

3.3.6 Testing clustering thresholds

To test whether sequences resulting from NGS analysis of faecal samples using our primers should be clustered into Molecular Operational Taxonomic Units (MOTUs) within a bioinformatics pipeline, and if so at what threshold, we used reference sequences from both the Mauritian (n=167 species and 464 sequences) and UK databases (n=1116 species and 2619 sequences). We ran the sequence files through the USEARCH (v7.0)(Edgar 2010) command cluster_fast with an identity threshold of 95%. We then used the % similarity values between clustered sequences from the cluster format output file to identify, for various threshold cut-offs, what number of different species and haplotypes would be clustered together. Resolution at a range of clustering thresholds is displayed as heatmaps, at the order level. Heatmaps were created using the 'heatmap.2' function in the *gplots* package (Warnes *et al.* 2016) in R (R Core Team 2016).

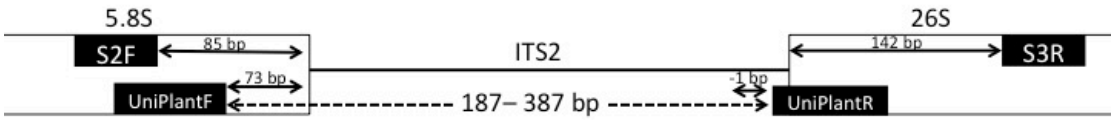


Figure 3.1. Schematic diagram of priming sites within the second internal transcribed spacer (ITS2) and flanking regions (5.8S and 26S). The location of S2F and S3R priming sites (Chen *et al*. 2010) are shown alongside UniPlantF and UniplantR from this study. The distances of the priming sites from the ITS2 region are shown (bp). Distances are based on a representative *Asparagus setaceus* sequence (NCBI Accession number KY700230). S2F and UniPlantF overlap by 7 bp. UniPlantR begins on the last 1 bp of ITS2 and continues into 26S. The amplicon size range, across all sequences assessed in this study, of the UniPlant primers is shown. Schematic not to scale.

3.4 Results

3.4.1 In vitro testing

We established that the UniPlantF (5'-TGTGAATTGCARRATYCMG-3') and UniplantR (5'-CCCGHYTGAYYTGRGGTCDC-3') primers had the greatest amplification success on a subset of plant species (Appendix 1.2), so only these primers were selected for further *in vitro* and *in silico* testing. This primer pair successfully amplified 99% of the 169 Mauritian species (Table 3.1), and 100% of 33 UK species tested (Table A1.1.2, Appendix 1.1).

3.4.2 In silico testing

Across all three databases, amplicon lengths, minus priming sites, ranged from 187–387 bp. Where coverage of both forward and reverse primer binding regions was available, 88% (n=131 species, 114 genera, 57 families) of Mauritian (Table 3.1) and 89% of UK plants (n=986 species, 561 genera and 121 families; Table 3.2) fulfilled the primer fit criteria. Poor primer matches (where <50% species fulfil match criteria) were found in only 3 families within the UK (Hydrocharitaceae = 50%, n=6; Cyperaceae = 0%, n=44, Thymelaeaceae = 50%, n=2) where multiple species were tested (Table 3.2). In the Mauritian database, *in silico* primer fit was particularly poor for Cyperaceae (0%, n=4) and Moraceae (50%, n=2). Analyses of matches for forward and reverse primers independently, due to short sequence lengths, are provided in Appendix 1.3.

3.4.3 Taxonomic discrimination

Once we had removed duplicate sequences from the same species within our combined database, our analysis showed that the taxonomic discrimination of the ITS2 region was 86.1%, 99.4% and 99.9% at the species, genus and family levels, respectively (n=1577 species). Two taxa could not be differentiated at the family level; both were ferns. All Mauritian taxa could be differentiated at the genus and family levels and just two taxa could not be differentiated at the species-level, both in the Cyperaceae family. Of the UK taxa, two, ten and 217 species could not be differentiated at the family, genus and species levels respectively.

3.4.2 Threshold analysis

At a 100% clustering threshold, the majority of species tested (n=1116 in the UK and n=165 in Mauritius, Fig 3.2) could be identified at the species level, although multiple haplotypes were present for many species. As the threshold dropped, the number of species for which discrimination was possible started to decrease, however multiple haplotypes for some species remained. This means that as the clustering threshold was reduced, a molecular operational taxonomic unit (MOTU) did not reflect species but a combination of haplotypes for a species and mixtures of haplotypes from different species. The effect of reducing the clustering threshold differed between families, particularly reducing discriminatory power in Caryophyllaceae, Myrtales, Poales and Rosales, even at high clustering thresholds (Fig. 3.2, Appendix 1)

Table 3.1. Results of in silico and in vitro analysis of primer fit for UniPlantF and UniPlantR for Mauritian plants at the species level, summarised by family. For in silico results, matches are where primers fit with a maximum of 3 bp mismatches and no mismatches in the last two bp at the 3 prime end. Data presented here are from sequences where both primer binding sites were available for analysis; details of species tested for either forward or reverse primer matches are given in Appendix 1.3.

Order	Family	Tested *in silico*	*In silico* matches	% matches	Tested *in vitro*	Amplified *in vitro*	% Amplified
Apiales	Araliaceae	1	1	100	1	1	100
Arecales	Arecaceae	-	-	-	3	3	100
Asparagales	Amaryllidaceae	1	1	100	1	1	100
Asparagales	Asparagaceae	3	3	100	3	3	100
Asparagales	Orchidaceae	1	0	0	3	3	100
Asparagales	Xanthorrhoeaceae	-	-	-	1	1	100
Asterales	Asteraceae	7	7	100	8	8	100
Asterales	Campanulaceae	1	1	100	1	1	100
Asterales	Goodeniaceae	1	1	100	1	1	100
Boraginales	Boraginaceae	1	1	100	3	3	100
Brassicales	Caricaceae	1	1	100	1	1	100
Caryophyllales	Aizoaceae	-	-	-	1	1	100
Caryophyllales	Amaranthaceae	4	4	100	4	4	100
Caryophyllales	Nyctaginaceae	1	1	100	1	1	100
Caryophyllales	Petiveriaceae	1	1	100	1	1	100
Caryophyllales	Portulacaceae	1	1	100	1	1	100
Celastrales	Celastraceae	2	2	100	2	2	100
Commelinales	Commelinaceae	1	1	100	1	1	100
Ericales	Ebenaceae	1	1	100	3	3	100
Ericales	Lecythidaceae	-	-	-	1	1	100
Ericales	Sapotaceae	1	1	100	1	1	100
Fabales	Fabaceae	13	11	85	13	13	100
Gentianales	Apocynaceae	4	4	100	6	6	100
Gentianales	Rubiaceae	5	5	100	5	5	100
Lamiales	Acanthaceae	1	1	100	2	2	100
Lamiales	Bignoniaceae	1	1	100	1	1	100
Lamiales	Lamiaceae	1	1	100	1	1	100
Lamiales	Oleaceae	1	1	100	2	2	100
Lamiales	Scrophulariaceae	1	1	100	1	1	100
Lamiales	Verbenaceae	1	1	100	2	2	100
Laurales	Lauraceae	1	1	100	3	3	100
Malpighiales	Erythroxylaceae	1	1	100	1	1	100
Malpighiales	Euphorbiaceae	8	8	100	8	8	100

Table 3.1. Results of in silico and in vitro analysis of primer fit for UniPlantF and UniPlantR for Mauritian plants at the species level, summarised by family. For in silico results, matches are where primers fit with a maximum of 3 bp mismatches and no mismatches in the last two bp at the 3 prime end. Data presented here are from sequences where both primer binding sites were available for analysis; details of species tested for either forward or reverse primer matches are given in Appendix 1.3.

Order	Family	Tested *in silico*	*In silico* matches	% matches	Tested *in vitro*	Amplified *in vitro*	% Amplified
Malpighiales	Passifloraceae	2	2	100	2	2	100
Malpighiales	Phyllanthaceae	4	4	100	7	7	100
Malpighiales	Salicaceae	2	2	100	3	3	100
Malvales	Malvaceae	7	7	100	8	8	100
Malvales	Thymelaeaceae	1	1	100	1	1	100
Myrtales	Combretaceae	1	1	100	1	1	100
Myrtales	Lythraceae	1	1	100	1	1	100
Myrtales	Myrtaceae	1	1	100	1	1	100
Oxalidales	Oxalidaceae	1	1	100	1	1	100
Pandanales	Pandanaceae	1	1	100	1	1	100
Poales	Cyperaceae	4	0	0	4	4	100
Poales	Poaceae	12	11	92	16	16	100
Polypodiales	Lomariopsidaceae				1	0	0
Polypodiales	Polypodiaceae	1	0	0	1	1	100
Polypodiales	Pteridaceae	1	0	0	2	2	100
Polypodiales	Thelypteridaceae				1	0	0
Pottiales	Pottiaceae	1	0	0	1	1	100
Psilotales	Psilotaceae	1	0	0	1	1	100
Ranunculales	Papaveraceae	1	0	0	1	1	100
Rosales	Moraceae	2	1	50	3	3	100
Rosales	Rhamnaceae	3	3	100	4	4	100
Santalales	Santalaceae	1	1	100	1	1	100
Sapindales	Anacardiaceae	2	2	100	2	2	100
Sapindales	Burseraceae				1	1	100
Sapindales	Meliaceae	1	1	100	1	1	100
Sapindales	Rutaceae	1	1	100	2	2	100
Sapindales	Sapindaceae	2	2	100	3	3	100
Saxifragales	Crassulaceae	1	1	100	1	1	100
Selaginellales	Selaginellaceae				1	1	100
Solanales	Convolvulaceae	3	3	100	4	4	100
Solanales	Solanaceae	5	3	60	4	4	100
Vitales	Vitaceae	1	1	100	1	1	100
	Total	**131**	**115**	**88**	**169**	**167**	**99**

Table 3.2. Results of in silico analysis of primer matching for UniPlantF and UniPlantR for plant families within the two UK databases, at the species level. Primer matches are where primers fit with a maximum of 3bp mismatches and no mismatches in the last two bp at the 3 prime end. Data presented here are from sequences where both primer binding sites were available for analysis; details of species tested for forward and reverse primer matches separately are given in Appendix 1.3.

		UK database		Turtle Dove database		Overall		
Order	Family	No. tested	No. matches	No. tested	No. matches	No. tested	No. matches	% match
Acorales	Acoraceae	1	1	-	-	1	1	100
Alismatales	Alismataceae	6	6	-	-	6	6	100
Alismatales	Aponogetonaceae	1	1	-	-	1	1	100
Alismatales	Araceae	4	4	-	-	4	4	100
Alismatales	Butomaceae	1	1	-	-	1	1	100
Alismatales	Cymodoceaceae	1	0	-	-	1	0	0
Alismatales	Hydrocharitaceae	6	3	-	-	6	3	50
Alismatales	Juncaginaceae	1	1	-	-	1	1	100
Alismatales	Potamogetonaceae	6	6	-	-	6	6	100
Alismatales	Tofieldiaceae	1	1	-	-	1	1	100
Alismatales	Zosteraceae	1	1	-	-	1	1	100
Apiales	Apiaceae	34	31	1	1	34	31	91
Apiales	Araliaceae	3	3	-	-	3	3	100
Apiales	Griseliniaceae	1	1	-	-	1	1	100
Apiales	Pittosporaceae	1	1	-	-	1	1	100
Aquifoliales	Aquifoliaceae	1	1	-	-	1	1	100
Asparagales	Amaryllidaceae	6	5	-	-	6	5	83
Asparagales	Asparagaceae	3	2	-	-	3	2	67
Asparagales	Hyacinthaceae	2	2	-	-	2	2	100
Asparagales	Iridaceae	2	2	-	-	2	2	100
Asparagales	Orchidaceae	19	15	-	-	19	15	79
Asparagales	Xanthorrhoeaceae	1	1	-	-	1	1	100
Asterales	Asteraceae	92	90	6	6	92	90	98
Asterales	Campanulaceae	9	9	-	-	9	9	100
Asterales	Menyanthaceae	2	2	-	-	2	2	100
Boraginales	Boraginaceae	17	17	-	-	17	17	100
Boraginales	Hydrophyllaceae	1	1	-	-	1	1	100
Brassicales	Brassicaceae	59	52	3	3	60	52	87
Brassicales	Resedaceae	1	1	-	-	1	1	100
Buxales	Buxaceae	1	1	-	-	1	1	100
Caryophyllales	Aizoaceae	1	1	-	-	1	1	100
Caryophyllales	Amaranthaceae	5	5	-	-	5	5	100
Caryophyllales	Caryophyllaceae	49	46	6	6	50	47	94

Table 3.2. Results of in silico analysis of primer matching for UniPlantF and UniPlantR for plant families within the two UK databases, at the species level. Primer matches are where primers fit with a maximum of 3bp mismatches and no mismatches in the last two bp at the 3 prime end. Data presented here are from sequences where both primer binding sites were available for analysis; details of species tested for forward and reverse primer matches separately are given in Appendix 1.3.

		UK database		Turtle Dove database		Overall		
Order	Family	No. tested	No. matches	No. tested	No. matches	No. tested	No. matches	% match
Caryophyllales	Chenopodiaceae	12	12	1	1	13	13	100
Caryophyllales	Droseraceae	2	2	-	-	2	2	100
Caryophyllales	Montiaceae	2	2	-	-	2	2	100
Caryophyllales	Phytolaccaceae	1	1	-	-	1	1	100
Caryophyllales	Plumbaginaceae	2	2	-	-	2	2	100
Caryophyllales	Polygonaceae	11	10	2	2	11	10	91
Caryophyllales	Portulacaceae	1	1	-	-	1	1	100
Caryophyllales	Tamaricaceae	1	1	-	-	1	1	100
Celastrales	Celastraceae	1	1	-	-	1	1	100
Ceratophyllales	Ceratophyllaceae	2	2	-	-	2	2	100
Cornales	Hydrangeaceae	1	1	-	-	1	1	100
Cucurbitales	Cucurbitaceae	3	3	-	-	3	3	100
Dipsacales	Adoxaceae	3	3	-	-	3	3	100
Dipsacales	Caprifoliaceae	5	5	-	-	5	5	100
Ericales	Balsaminaceae	1	1	-	-	1	1	100
Ericales	Diapensiaceae	1	1	-	-	1	1	100
Ericales	Ericaceae	16	15	-	-	17	15	88
Ericales	Primulaceae	6	6	1	1	6	6	100
Fabales	Fabaceae	52	49	5	5	55	52	95
Fabales	Polygalaceae	2	2	-	-	2	2	100
Fagales	Betulaceae	6	6	-	-	6	6	100
Fagales	Fagaceae	2	2	-	-	2	2	100
Fagales	Juglandaceae	1	1	-	-	1	1	100
Fagales	Myricaceae	1	1	-	-	1	1	100
Gentianales	Gentianaceae	7	7	-	-	7	7	100
Gentianales	Rubiaceae	4	4	1	1	4	4	100
Geraniales	Geraniaceae	13	13	1	1	13	13	100
Gunnerales	Gunneraceae	1	1	-	-	1	1	100
Lamiales	Acanthaceae	1	1	-	-	1	1	100
Lamiales	Calceolariaceae	1	1	-	-	1	1	100
Lamiales	Gesneriaceae	1	0	-	-	1	0	0
Lamiales	Lamiaceae	15	14	-	-	15	14	93
Lamiales	Lentibulariaceae	4	3	-	-	4	3	75

Table 3.2. Results of in silico analysis of primer matching for UniPlantF and UniPlantR for plant families within the two UK databases, at the species level. Primer matches are where primers fit with a maximum of 3bp mismatches and no mismatches in the last two bp at the 3 prime end. Data presented here are from sequences where both primer binding sites were available for analysis; details of species tested for forward and reverse primer matches separately are given in Appendix 1.3.

		UK database		Turtle Dove database		Overall		
Order	Family	No. tested	No. matches	No. tested	No. matches	No. tested	No. matches	% match
Lamiales	Oleaceae	3	3	-	-	3	3	100
Lamiales	Orobanchaceae	24	24	-	-	24	24	100
Lamiales	Plantaginaceae	23	22	2	2	25	24	96
Lamiales	Scrophulariaceae	5	5	-	-	5	5	100
Lamiales	Verbenaceae	1	1	-	-	1	1	100
Liliales	Liliaceae	5	4	-	-	5	4	80
Liliales	Melanthiaceae	1	1	-	-	1	1	100
Malpighiales	Euphorbiaceae	6	6	1	1	7	7	100
Malpighiales	Hypericaceae	7	7	-	-	7	7	100
Malpighiales	Linaceae	1	1	-	-	1	1	100
Malpighiales	Salicaceae	14	14	-	-	14	14	100
Malpighiales	Violaceae	6	6	2	2	8	8	100
Malvales	Cistaceae	1	1	-	-	1	1	100
Malvales	Malvaceae	13	11	-	-	13	11	85
Malvales	Thymelaeaceae	2	1	-	-	2	1	50
Myrtales	Lythraceae	1	1	-	-	1	1	100
Myrtales	Myrtaceae	3	2	-	-	3	2	67
Myrtales	Onagraceae	11	10	-	-	11	10	91
Nymphaeales	Cabombaceae	1	1	-	-	1	1	100
Nymphaeales	Nymphaeaceae	1	1	-	-	1	1	100
Oxalidales	Oxalidaceae	2	2	-	-	2	2	100
Pinales	Araucariaceae	1	1	-	-	1	1	100
Pinales	Cupressaceae	3	3	-	-	3	3	100
Pinales	Pinaceae	3	3	-	-	3	3	100
Pinales	Taxaceae	1	1	-	-	1	1	100
Piperales	Aristolochiaceae	1	0	-	-	1	0	0
Poales	Cyperaceae	44	0	-	-	44	0	0
Poales	Juncaceae	23	23	-	-	23	23	100
Poales	Poaceae	96	88	7	7	96	88	92
Poales	Typhaceae	4	4	-	-	4	4	100
Polypodiales	Aspleniaceae	1	0	-	-	1	0	0
Polypodiales	Pteridaceae	1	1	-	-	1	1	100
Proteales	Platanaceae	1	1	-	-	1	1	100

Table 3.2. Results of in silico analysis of primer matching for UniPlantF and UniPlantR for plant families within the two UK databases, at the species level. Primer matches are where primers fit with a maximum of 3bp mismatches and no mismatches in the last two bp at the 3 prime end. Data presented here are from sequences where both primer binding sites were available for analysis; details of species tested for forward and reverse primer matches separately are given in Appendix 1.3.

		UK database		Turtle Dove database		Overall		
Order	Family	No. tested	No. matches	No. tested	No. matches	No. tested	No. matches	% match
Ranunculales	Berberidaceae	1	1	-	-	1	1	100
Ranunculales	Papaveraceae	6	6	2	2	8	8	100
Ranunculales	Ranunculaceae	19	18	-	-	19	18	95
Rosales	Cannabaceae	2	2	-	-	2	2	100
Rosales	Moraceae	1	1	-	-	1	1	100
Rosales	Rhamnaceae	1	1	-	-	1	1	100
Rosales	Rosaceae	65	61	-	-	65	61	94
Rosales	Ulmaceae	2	2	-	-	2	2	100
Rosales	Urticaceae	3	3	1	1	3	3	100
Salviniales	Azollaceae	1	0	-	-	1	0	0
Santalales	Thesiaceae	1	1	-	-	1	1	100
Santalales	Viscaceae	1	0	-	-	1	0	0
Sapindales	Aceraceae	1	1	-	-	1	1	100
Sapindales	Anacardiaceae	1	1	-	-	1	1	100
Sapindales	Simaroubaceae	1	1	-	-	1	1	100
Saxifragales	Crassulaceae	6	4	-	-	6	4	67
Saxifragales	Haloragaceae	1	1	-	-	1	1	100
Saxifragales	Saxifragaceae	13	13	-	-	13	13	100
Selaginellales	Selaginellaceae	1	1	-	-	1	1	100
Solanales	Convolvulaceae	5	5	1	1	5	5	100
Solanales	Solanaceae	8	8	-	-	8	8	100
Vitales	Vitaceae	1	0	-	-	1	0	0
	Total	**972**	**868**	**43**	**43**	**986**	**880**	**89**

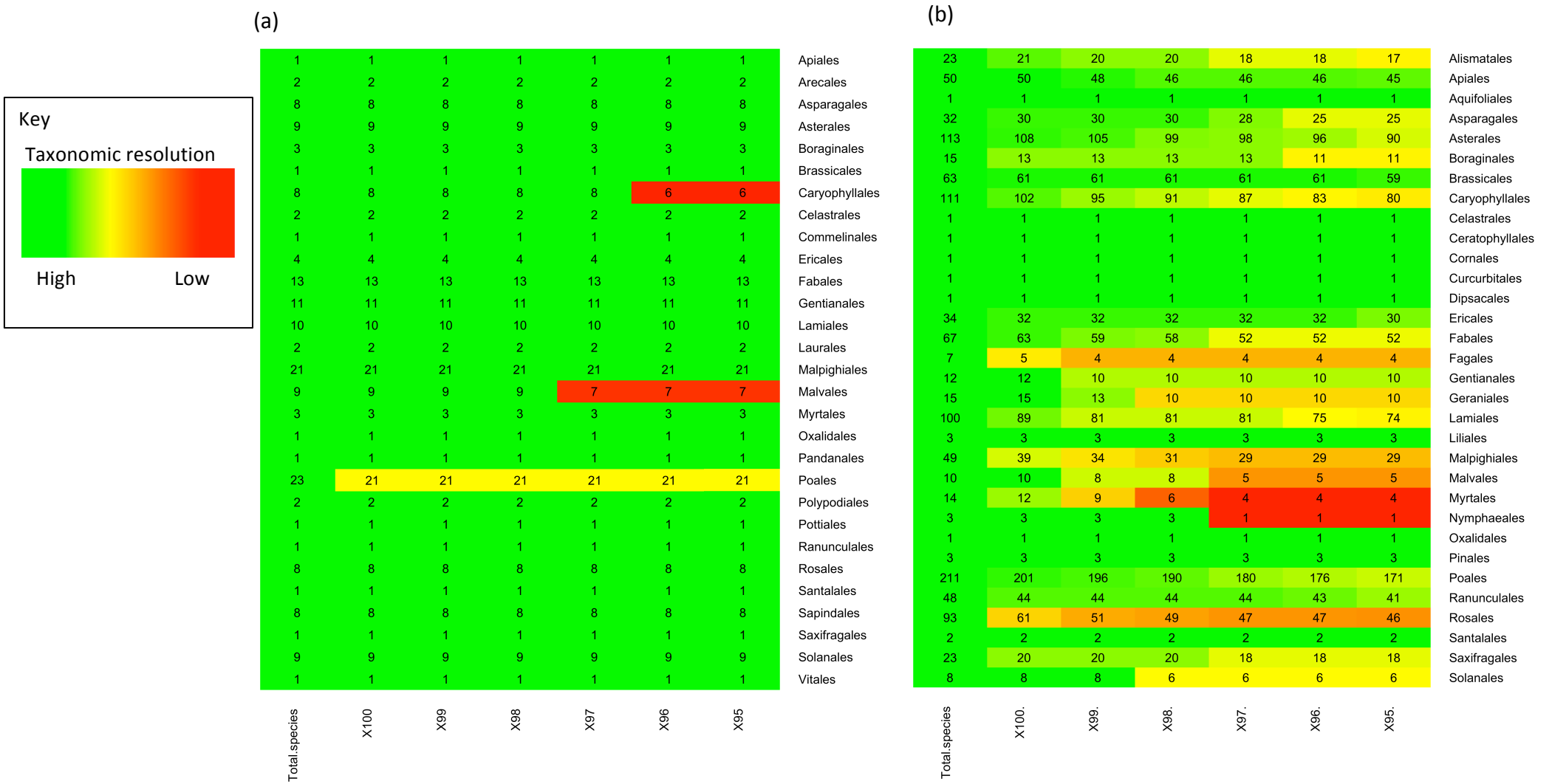


Figure 3.2. Order-level summary of clustering thresholds for the ITS2 region only between 95 and 100% for (a) Mauritius, n=165 species and (b) UK databases, n=1116 species. Order names are listed on the y-axis and clustering threshold forms the x-axis. The colour of the cells represents the percentage of species within an order that can be identified to species level at a given clustering threshold; numbers within cells show the number of species that can be resolved at each threshold. Colour gradient from green through to red signifies high species-level resolution moving towards poor species-level resolution. The figure shows the rate at which species-level taxonomic resolution is lost in each order as sequences are clustered at 95 – 100% clustering thresholds. For example, there were 9 species belonging to the order Malvales in the Mauritian database (a) and at a 100% clustering threshold (equivalent to no clustering) all 9 species can be differentiated. However, when the threshold is reduced to 97% (sequences that differ by no more than 3% are clustered together), only 7 of the 9 species can be differentiated. The figure illustrates that the rate of species-level identification loss across different clustering thresholds varies between orders.

3.5 Discussion

3.5.1 Taxonomic discrimination

Current approaches for molecular analysis of herbivory lack the taxonomic resolution to identify plants to the species level. Valentini *et al.* (2009) report that using the chloroplast *trn*L (UAA) gene, the most widely used DNA barcode to study herbivory, about 50% of taxa can be identified to species. However, other studies have demonstrated that this figure is difficult to attain. For example, Gebremedhin *et al*., (2016) found that 29.8% of species in their reference library could be identified to species level. Using *trn*L does, however, have the advantage of being able to work with particularly degraded DNA where short amplicons might be expected to be more reliably amplified (12–134 bp using primer pair g and h (Pompanon *et al.* 2012)). Taxonomic discrimination at the species level can be as high as 78% using the trnH-psbA region. However, amplicon lengths can be as long as 887 bp, which is highly problematic when working with degraded samples. By contrast our new ITS2 primers produce amplicons of 187–387 bp in length, with taxonomic discrimination at the species level as high as 86.1% across all three databases.

Such high taxonomic discrimination is only possible when the sequences for the species consumed by an animal are available in a DNA barcode library (Valentini *et al.* 2009). Indeed, a major criticism of ITS2 has been the lack of reference sequences available for this region. However, the latest update to the ITS2 database has doubled the number of reference sequences available to 711,172, of which 208,822 belong to the Chloroplastida (Ankenbrand *et al.* 2015). When sequences are not available for plant species within the study area in question, we suggest that building a bespoke DNA barcode library is crucial.

3.5.2 Overcoming the potential drawbacks of using ITS2 for metabarcoding

There are three further potential criticisms of the use of ITS2 as a DNA barcode (Hollingsworth 2011). Firstly, there are sometimes paralogous ITS copies present within an individual genome (Coleman 2003; Hollingsworth 2011; Li *et al.* 2011). However ITS is considered to behave as a single locus due to concerted evolution in most organisms (Han *et al.* 2013) and recent research

indicates that the impacts of incomplete concerted evolution may not be as severe as previously thought (Hollingsworth 2011; Li *et al.* 2011). Secondly, amplifying ITS can be difficult with universal primers (Li *et al.* 2011), however this problem can largely be overcome by amplifying ITS2 only, as demonstrated in Li *et al.* (2011) and Chen *et al*. (2010) in addition to this study. The final criticism is the risk of fungal contamination, given the similarity between plant and fungi universal primers for this region (Hollingsworth 2011). However, Li *et al.* (2011) found, with *in silico searches,* that only 2–3% of samples showed evidence of fungal contamination. When used for metabarcoding, the UniPlant primers do amplify both fungal and bacterial sequences (Chapter 4, Chapter 5) but they were filtered out in the bioinformatics pipeline (either due to low read depth, amplicon lengths falling outside of specified limits or identification as fungi or bacteria at the taxon assignment stage) and we retained more than sufficient plant read depth for our herbivory analyses. For example, after removing long or short amplicons and those with a low read depth, ITSx (Bengtsson-Palme *et al.* 2013) identified only 1063 unique fungal sequences in comparison to 16226 unique vascular plant sequences in one Illumina MiSeq run where dietary items were amplified from consumers in Mauritius (Chapter 4, Chapter 5).

3.5.3 Advice for downstream bioinformatics analyses

Given the findings from our threshold analysis, that intraspecific variation at the ITS2 region will not be removed by clustering at a greater rate than species loss, we recommend a closest species match approach to sequence identification such as in de Vere *et al*. (2017) and Hawkins *et al.* (2015), rather than a MOTU clustering approach. This also removes any issues caused by potential multiple ITS polymorphisms within an individual (Iwanowicz *et al.* 2016) but does emphasise the need for comprehensive reference barcode libraries for the study system. Iwanowicz *et al*. (2016) highlight that Sanger sequencing of multiple samples from individual species may not adequately represent total ITS diversity due to low-frequency polymorphisms (in, for example, Brassicaceae). In such cases it may be pertinent to include some single species samples in an NGS run alongside DNA samples for analysis.

3.5.4 Universality of UniPlant ITS2 primers

Our *in vitro* and *in silico* testing of the UniPlant primers proved that they can amplify a diverse assemblage of plants. The *in silico* PCR results were more conservative than the *in vitro* testing. For example, *in silico* testing revealed that the primers were a poor fit for species within the Orchidaceae and Cyperaceae families, but these were shown to amplify successfully *in vitro*. Illumina sequencing data using the UniPlant primers suggest that our *in silico* parameters were too conservative. For example, *Cyperus dubius* and *Fimbristylis sp.*, both of the Cyperaceae family, were retrieved as dietary items in the Mauritian system (Chapter 4, Chapter 5). Thus, in practice, our primers may be better than suggested by our *in silico* results. However, such species with potentially poor primer fit should be tested *in vitro* to confirm successful amplification before use for the examination of herbivory.

3.5.5 Final conclusions

Our novel primers amplify a fragment of 187–387 bp, which is suitable for use with NGS platforms and here we show that they are general enough to amplify the vast majority of a phylogenetically diverse array of plant species found in the UK and Mauritius. These primers are a much needed new molecular tool to study herbivory in tropical Mauritius and the temperate UK and are therefore highly likely to be equally useful in other parts of the globe. We recommend *in silico* followed by *in vitro* testing of likely dietary items, particularly if they are ferns or within the Cyperaceae, Orchidaceae, Hydrocharitaceae or Thymelaeaceae families. We also advise that a comprehensive DNA barcode reference library is essential to obtain high taxonomic resolution and to avoid the pitfall of setting a clustering threshold, permitting assignment of taxa based on a closest match approach.

3.6 Acknowledgements

This work was funded by the Natural Environment Research Council (NE/K00719X/1) in the form of an iCASE studentship awarded to Cardiff University, co-funded by CASE partners the Durrell Wildlife Conservation Trust with support from the Mauritian Wildlife Foundation. We thank the Mauritian National Parks and Conservation Service for their permission to carry out work in Mauritius. The Turtle Dove element of research was jointly funded by the RSPB and

Natural England through the Action for Birds in England partnership, and thanks go to multiple fieldworkers and landowners who assisted with collection of samples and access to land, respectively. Illumina library preparation, sequencing and data analyses were supported by the NERC Biomolecular Analysis Facility at the University of Sheffield (NBAF-S) and funded by NERC, UK (NBAF983 and NBAF943). Thank you to Rouben Mootoocurpen, Dany Vencatasamy, Abdullah Faisal Nuckcheddy, Nicolas Zuël, Issabelle Désiré and Jean Claude Sevathian for their assistance with plant tissue sample collection and/or plant identification in Mauritius, and to Eliza Leat, Kerry Skelhorn, Judit Mateos, Alexander Ball, Rebecca Thomas and Jenny Bright for assistance with sample collection in the UK. Thank you to the Mauritius Herbarium for their support with plant identification. Thank you to Frédéric Boyer and Pablo Orozco ter Wengel for their advice on using ecoPCR. Thank you also to Isa-Rita Russo and Silke Waap for sharing their expertise in primer design.

Chapter Four - Selection of native, alien and supplementary food by (re)introduced species on Ile aux Aigrettes: Pink Pigeons and Telfair's skinks

The Pink Pigeon (top, photo credit: Gabby Salazar) and the Telfair's skink (bottom)

4.1 Abstract

Ecosystems have been altered to such an extent that ecosystem dysfunction is commonplace. A myriad of conservation interventions have been implemented to facilitate the restoration of ecosystem functions, and these include threatened species recovery projects. An understanding of the trophic interactions within these ecosystems is necessary to inform, monitor and assess the success of such ambitious conservation strategies. Here, DNA metabarcoding of plant remains in faeces was used to analyse the herbivorous diet of two species endemic to Mauritius, the Telfair's skink (*Leiolopisma telfairii*) and Pink Pigeon (*Nesoenas mayeri*), which have been (re)introduced to Ile aux Aigrettes. Herbivory by the Telfair's skink as determined by metabarcoding was compared with the diet as determined by morphological analysis. Using metabarcoding, taxonomic discrimination at the species level was as high as 100% when all plant taxa consumed were present in a comprehensive DNA barcode library. Based on the metabarcoding data, it was concluded that both species are generalists with broad dietary niches and as such are likely to be resilient to disturbance within their ecological network. However, Pink Pigeons are reliant on introduced plant species, and managers should consider this when attempting to control introduced weeds. Pink Pigeons use supplementary feed infrequently, despite regularly visiting the sites where the feed is provided, and males use this resource more than females. Telfair's skink diet as determined by morphological analyses, detects fewer taxa and provides lower taxonomic resolution in comparison to metabarcoding. Using morphology alone, it would be concluded that the Telfair's skink specializes on a smaller number of plants. Morphological analyses, however, has the capacity to determine the plant tissue type consumed, which can provide information on seed dispersal in addition to diet. However, neither technique can provide information on the nutritional content of the plants consumed, which is necessary to determine the importance of each dietary resource to the fitness of the species. Here, the implications of these results is discussed in the context of threatened species recovery and ecological restoration.

4.2 Introduction

Ecosystems have been modified to such an extent by humans that anthropogenic activities are considered the main driver of global change (Sanderson *et al.* 2002; Barnosky *et al.* 2011; Dirzo *et al.* 2014). Such modifications include the decline and loss of native species, and the introduction of alien species and pollution (Mack *et al.* 2000;

Cole *et al.* 2005; Dirzo *et al.* 2014; Birnie-Gauvin *et al.* 2017). As a consequence, trophic interactions have been modified, which can lead to ecosystem dysfunction (Estes *et al.* 2011). Ecological restoration through rewilding aims to restore ecosystem functions (Griffiths *et al.* 2011; Svenning *et al.* 2016). Conservation interventions to restore ecosystems include reintroductions, translocations, and ecological replacement (Donlan *et al.* 2005; Donlan *et al.* 2006; Griffiths *et al.* 2010; Hansen *et al.* 2010; Hunter *et al.* 2013; Seddon *et al.* 2014; Fernandez *et al.* 2017). A good understanding of trophic interactions is fundamental to monitor and assess the health of the species that have been restored and guide conservation management strategies.

It is best practice to monitor trophic interactions, such as predation and herbivory, subsequent to (re)introductions (IUCN/SSC 2013). Such monitoring is necessary to detect dietary overlap and competition with both native (Jung *et al.* 2015) and non-native species (Brown *et al.* 2014a), to preempt or monitor human-wildlife conflict (Kowalczyk *et al.* 2011), monitor the need for supplementary feed (Edmunds *et al.* 2008), and understand seed dispersal and pollination mechanisms to inform ecosystem restoration (Pernetta *et al.* 2005). An understanding of trophic links also allows species at risk due to inflexible niches to be identified, it reveals particularly vulnerable interactions within networks, allows for suitable (re)introduction sites to be identified (Pernetta *et al.* 2005; Clare 2014; Soorae 2016), and provides a better understanding of the reasons for the successes and failures of endangered species recovery programs.

DNA metabarcoding is increasingly used to elucidate interactions between species and their environment through dietary (Pompanon *et al.* 2012) and pollination analyses (e.g. Clare *et al.* 2013; Hawkins *et al.* 2015; de Vere *et al.* 2017). In comparison to more traditional morphological analyses, metabarcoding provides information on more trophic links at a finer taxonomic resolution (Soininen *et al.* 2009; Ando *et al.* 2013; Alonso *et al.* 2014; Clare 2014). However, metabarcoding is unable to specify the type of tissue ingested (e.g. fruits, leaves or flowers for plants, or larval or adult stages for animals) and provides no information on the nutritional content of dietary items. Therefore, it is important to combine techniques to fully understand trophic links, and their importance to maximize the benefit for conservation programmes.

Ile aux Aigrettes is a 26 hectare coralline island nature reserve situated approximately 600 m from the south-eastern coast of the Mauritian mainland. The island has been degraded by the invasion of exotic species and partial clear-felling. Restoration work

began in 1965 with the weeding of invasive plants and the planting of native species (Jones & Hartley 1995; Cheke & Hume 2008). In 1991, two predatory species: the black rat (*Rattus rattus*) and the feral cat (*Felis catus*) were eradicated (Jones & Hartley 1995). Today, one exotic predatory mammal remains established: the Asian musk shrew (*Suncus murinus*), despite eradication attempts (Seymour *et al.* 2005). However, the island was recently invaded by the tailless tenrec (*Tenrec ecaudatus*) and efforts are currently underway to eradicate the species. Since the rat and cat eradication, several reintroduction and translocation programmes for endemic vertebrates have taken place on the island, including the Mauritius fody (*Foudia rubra*), the Mauritius Olive white-eye (*Zosterops chloronothos*), the Pink Pigeon (*Nesoenas mayeri*), the Telfair's skink (*Leiolopisma telfairii*), and the Günther's gecko (*Phelsuma guentheri*). With the exception of predation by the omnivorous Telfair's skink (Brown *et al.* 2014a), the diet of these (re)introduced birds and reptiles is yet to be comprehensively examined on Ile aux Aigrettes. A better understanding of trophic interactions on Ile aux Aigrettes could be used to inform management, for example the availability of preferred native species could be increased, which may also mitigate the need for supplementary feed.

The aim of this study was to use DNA metabarcoding to give the first detailed account of the diet of the Pink Pigeon and herbivory by Telfair's skinks on Ile aux Aigrettes. Specific aims were to (i) analyse which plants are most frequently eaten; (ii) compare the roles of native vs. introduced plant species in the diet; (iii) investigate whether Pink Pigeons and Telfair's skinks exhibit random herbivory (eating plant species in proportion to their availability) or show preference for/avoidance of certain species; (iv) assess the differences in Telfair's skink diet as determined by DNA metabarcoding and the morphological examination of faecal samples; and finally for Pink Pigeons only (v) determine whether the prevalence of supplementary feed varies with season or sex. The implications of these findings for conservation management are discussed alongside avenues for future research.

4.3 Methods

4.3.1 The Pink Pigeon

The Endangered Pink Pigeon (BirdLife International 2016) recovered from the brink of extinction when, in 1991, there were just ten birds remaining in the wild (Jones & Swinnerton 1997). Thanks to an intensive species recovery programme, the population now fluctuates at around 400 individuals (Concannon 2014). The majority of the birds

can be found in the Black River Gorges National Park on the Mauritian mainland, but a small sub-population has established on Ile aux Aigrettes following introduction in 1994 (Jones & Swinnerton 1997) (42 ringed individuals in 2014-15, but the population may have suffered further declines since). The Pink Pigeon is largely arboreal and is known to forage on the fruit, seeds, leaves, and flowers of trees (Jones *et al.* 2013), but supplementary feed (mostly consisting of wheat, but occasionally includes maize) is provided *ad libitum* throughout the year, both in the Black River Gorges National Park (Southwest Mauritius) and on Ile aux Aigrettes (Jones & Swinnerton 1997).

4.3.2 The Telfair's skink

The Telfair's skink is a Vulnerable (Madagascar Reptile & Amphibian Specialist Group 1996) reptile, once present on the Mauritian mainland and islands (Round Island, Gunner's Quoin and Flat island) before the introduction of mammalian predators reduced its range to Round Island (Vinson & Vinson 1969; Arnold 1980; Bullock 1986; Pernetta *et al.* 2005). However, thanks to successful translocations subsequent to the eradication of most mammalian predators, Telfair's skinks are now present on Round Island, Gunner's Quoin and Ile aux Aigrettes (Cole *et al.* 2009; Cole *et al.* 2013; Cole *et al.* 2014). Skinks are primarily terrestrial and diurnal, but are known to climb on boulders, palms and other vegetation, and also hunt cockroaches and nocturnal geckos at night (Bullock 1986; Jones 1993). Morphological dietary analysis and feeding observations on Round Island, Ile aux Aigrettes and Gunner's Quoin have shown that skinks are omnivorous and have the potential to disperse both native and exotic seeds (Bullock 1986; Jones 1993; Pernetta *et al.* 2005; Cole *et al.* 2009; Zuel 2009). Thorough investigations into the diet of Telfair's skink in both their native and (re)introduced ranges were carried out by the morphological examination of remains in faecal samples (Cole *et al.* 2009). Predation by Telfair's skinks on Ile aux Aigrettes has also been examined by metabarcoding (Brown *et al.* 2014a) but there have been no molecular studies on herbivory by this species within its (re)introduced range. Current threats to Telfair's skinks on Ile aux Aigrettes include dietary competition with exotic Asian musk shrews (Cole *et al.* 2009; Brown *et al.* 2014a) and Tailless tenrecs, but also predation by these two exotic mammals and the exotic House crow (*Corvus splendens*).

4.3.3 Pink Pigeon faecal sample collection

All faecal sampling took place between July 2014 and July 2015. The majority of Pink Pigeon faecal samples were collected at the Pink Pigeon aviary on Ile aux Aigrettes. Pink

Pigeons regularly visit this location to access water and supplementary feed provided in hoppers. Prior to sample collection, flat platforms around the aviary were cleaned and plastic sheeting was laid out underneath where pigeons were likely to perch. The aviary was then observed through binoculars 1h up to three times a day and when a Pink Pigeon visited and was seen to defecate, the location of the faecal sample and the identity of the Pink Pigeon was recorded. All adult pigeons on Ile aux Aigrettes can be identified by their unique combination of coloured rings. Once the observation period was over, faecal samples were located and collected into polythene bags. A small number of faecal samples were collected opportunistically when a Pink Pigeon was seen to defecate elsewhere on the island. DNA within each sample was preserved by drying over silica gel; a method widely used to preserve plant DNA in faeces (e.g. Kowalczyk *et al.* 2011; Raye *et al.* 2011; Baamrane *et al.* 2012).

4.3.4 Telfair's skink faecal sample collection

Telfair's s skink faecal samples were collected for DNA metabarcoding between July 2014 and June 2015. Skinks were caught by hand or noose. Each individual's unique PIT (Passive Integrated Transponder)-tag number was read, skinks were sexed and an abdominal massage applied to induce defecation. Faecal samples were collected in polythene bags and dried over silica gel.

To compare the diet as determined by DNA metabarcoding to a more traditional morphological method, a dataset of 142 faecal samples collected between 2008 and 2011 (Cole *et al.* 2009) was used. Here, samples were collected when a Telfair's skink defecated during handling (including in clean cloth holding bags) or after applying an abdominal massage. Samples were placed in individually labeled 50 ml tubes with 70% ethanol. The faecal samples were separated into matching dietary items. Both plant and animal material were identified visually, but the focus here is on the plant component of the diet. From this dataset, the percentage of samples in which each dietary item was recorded were calculated.

4.3.5 Plant food availability

Forest plant food availability data were collected to test for deviations from random herbivory. Pink Pigeons forage on leaves, seeds, fruits and flowers (Jones *et al.* 2013) and Telfair's skinks are known to consume fruits and flowers in addition to invertebrates (Cole *et al.* 2009; Zuel 2009). Therefore, the overall percentage cover of each plant was recorded as a proxy for all available plant tissue types. Grid squares

were randomly selected for surveying in both the wet and dry season (n = 172 and 130, respectively; see 5.3.2 for details) using the research tools plugin in QGIS version 2.4.0-Chugiak (QGISDevelopmentTeam 2014). The percentage cover of each plant species at the ground (<0.5 m), understory (0.5 – 2 m) and canopy (>2 m) levels in each of the 12.5 m^2 grid squares was estimated. Although Pink Pigeons are largely arboreal (Jones *et al.* 2013), they have access to forage on the ground, in addition to the understory and canopy. Therefore, for Pink Pigeon food availability the percentage cover of each plant species at each canopy level in each grid square surveyed was summed. This provides the availability of each plant species, relative to all other species present. Telfair's skinks primarily forage within the ground layer of vegetation (Cole *et al.* 2009), so the sum of only the ground vegetation (<0.5 m) was used to obtain a measure of food availability.

4.3.6 DNA extraction, PCR amplification and next generation sequencing

DNA was extracted from 36 – 44 mg of faecal material using the QIAamp DNA stool mini kit (Qiagen, Manchester, UK), with the modifications outlined in Appendix 2.1. At least one DNA extraction negative was included in each DNA extraction session. PCRs were carried out in 20 µL reaction volumes containing 4 µL DNA template, 10 µL of multiplex PCR mix alongside 2 µL of Q solution (both Qiagen, Manchester, UK), and 0.2 µM of each UniPlant primer (see Chapter 3) where both forward and reverse were labeled with MID-tags (Multiplex Identifiers in the form of unique DNA tags) following Brown *et al* (2014a). PCR reaction conditions were initial denaturation at 95°C for 15 minutes, 40 cycles of 95°C for 30 s, 56°C for 90 s, 72°C for 90 s followed by a final extension of 72°C for 10 min. Each sample in each Illumina Miseq run had a unique MID-tag combination to allow for DNA sequences to be traced back to individual faecal samples. All PCR products were run on a 2% agarose gel stained with SYBR®Safe (ThermoFisher Scientific, Paisley, UK). At least two PCR negatives were included in each PCR reaction. If any DNA extraction or PCR negatives produced a band after gel electrophoresis, those extraction sessions and/or PCRs were repeated until all negatives were clean. All products from a single PCR plate were sorted into categories based on the brightness of the band after gel electrophoresis (e.g. very faint, faint, medium, bright, very bright). The DNA concentration in at least two representative PCR products from each category was quantified using a broad range assay with a Qubit Flourometer (ThermoFisher Scientific, Paisley, UK), to confirm that estimating relative DNA concentration by eye from a gel photo was accurate. Only those samples with the lowest DNA concentrations (<15 ng/µL) were purified using a QIAquick PCR purification kit (Qiagen, Manchester, UK) prior to DNA quantification for a second time. These samples were also concentrated

during purification by applying more initial product than final elute. All PCR products, including both PCR and DNA extraction negatives, from each PCR plate were then pooled ensuring that the concentration of all samples in the pool was approximately equal. The DNA concentration in these pools were then quantified once more before the concentration across all pools was equalised and all pools were combined into one of two final pools: one for each Illumina Miseq run. Each final pool was run on an Agilent 2200 TapeStation with a D1000 ScreenTape (Agilent Technologies, Waldbronn), which revealed that there was insignificant primer dimer so no further purification steps were required. The final two pools of individually tagged amplicons were used for library preparation with the NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA). Each library was sequenced separately using 250 bp paired end reads on a MiSeq desktop sequencer (Illumina, San Diego, CA). Library preparation and sequencing was carried out by the NERC Biomolecular Analysis Facility at the University of Sheffield.

4.3.7 Bioinformatics

The scripts used in the metabarcoding bioinformatics pipeline are available in Appendix 2.2. Paired-end Illumina reads were first filtered for quality using Trimmomatic v0.32 (Bolger *et al.* 2014) specifying a minimum length of 135 bp and a minimum base quality score of 20 over a sliding window of 4 bp. Filtered reads were subsequently aligned using FLASH (Magoc & Salzberg 2011). Mothur (Schloss *et al.* 2009) was used to assign reads to their respective sample ID's based on MID tag sequence combinations prior to MID tag and primer removal. Reads were then subsequently demultiplexed into one file per sample ID. Usearch (Edgar 2010) was used to remove chimeric sequences alongside those reads represented fewer than ten times in a single sample. The header for each read was then annotated with sample ID before concatenating all non-chimeric reads into a single file. ITS2 sequences were extracted from all reads using ITSx (Bengtsson-Palme *et al.* 2013) and Usearch (Edgar 2010) was used once again to extract all unique ITS2 sequences. A presence/absence matrix of sample ID against unique ITS2 sequence was created in R (R Core Team 2016). All unique sequences were subsequently numbered. The Blastn algorithm (Altschul *et al.* 1990) was used in Blast+ (Camacho *et al.* 2009) for taxonomic assignment, comparing all sequences to the comprehensive ITS2 DNA barcode library (Chapter 2). Sequences were assigned to taxa based on BIT score (as in Hawkins et al., and de Vere et al., 2017): if the highest BIT score was reserved to a match with a single species then species-level identification was achieved and the same rule was applied to genus-level matches. If a sequence failed to match a plant in the

barcode library, the blastn algorithm (Altschul *et al.* 1990) was used, as above, to search for matches on NCBI GenBank. A suite of scripts (Appendix 2.2) were used to (i) fill in the presence/absence matrix with the plant taxa assigned from the DNA barcode library, (ii) calculate the number of reads of each unique sequence in each sample (iii) extract read numbers found in PCR and DNA extraction negatives and (iv) remove plant species detections from those samples where the read number was not higher than that found in the negative samples; and finally (v) collapse the matrix so that all plant species detections for each haplotype of a species are represented by one species entry. This final dataset was cleaned further by first removing those species which are absent on the island (those species known to be present in the supplementary feed of other species, those known to be cooked and composted by the field staff or dropped by tour groups, other grains and bio control agents suspected to be present at low-levels in the Pink Pigeon supplementary feed from silo contamination, and finally British species suspected to be low-level contamination from pollen in the UK lab).

4.3.8 Statistical analyses

The percentage of samples in which a particular dietary item occurred was calculated and further broken down by sex, age (all skinks sampled were adult) and season (wet, dry or the border between wet and dry)

To determine whether native or introduced plant species richness in the diet was greater, the total number of native and introduced taxa consumed by each species was calculated. Where an individual was sampled more than once, the mean species richness of native and introduced plants was calculated for that individual to avoid pseudo replication. The data were not normally distributed and could not be normalised via data transformations so a Wilcoxon matched-pairs test was carried out in R (R Core Team 2016) to test for a significant difference in the median species richness of native and introduced plant species in the diet.

What an animal eats may be strongly influenced by food availability. However, it is possible to test for specific feeding preferences by generating a null model based on food availability data (see *4.3.5*), followed by testing for significant differences from this null model. This allows us to differentiate between those species that are eaten in greater, lesser or equal proportions to their availability. This approach was applied in the econullnetr R package (Vaughan *et al.* 2017). Here, 20,000 iterations of the model

were run to produce frequency distributions of expected rates of herbivory based on the plant food available following (King *et al.* 2010). Observed herbivory rates were then compared to those expected by chance. When observed herbivory rates fell outside of the central 95% of simulated values, this indicated deviations from random herbivory. To account for repeated measures (multiple faecal samples collected from the same individual Pink Pigeon or Telfair's skink), the observed and modeled diet for each individual was averaged prior to combining all individuals to give population-level herbivory rates. This ensures that each individual has equal weight in the analysis even if it has been sampled multiple times. These analyses were carried out for the dry and wet seasons separately to detect seasonal variation in feeding preferences. Plant species that were not detected during the food availability surveys were excluded from these analyses alongside those plant species that were never detected in the diet. The fern *Phymatodes scolopendria* was also excluded from these analyses because it is very abundant on the island but only detected in two samples each for Pink Pigeons and Telfair's skink. The inclusion of this fern in the analyses for feeding preferences strongly skews the results so that the majority of other plant species are preferred.

For pigeons, binomial generalised linear mixed effects models were run in R (R Core Team 2016) to determine whether the prevalence of supplementary food in the diet was influenced by season or sex. Models were fit by maximum likelihood using the lme4 package (Bates *et al.* 2015). The relationship between supplementary food and age-class was not investigated due to small sample sizes for fledglings and squabs (n=8 and n=6 respectively). For this analysis, the dietary data for wheat and maize was combined into a new supplementary feed dependent variable. Pink Pigeon ID was included as a random effect to account for repeated measures.

4.4 Results

A total of 170 and 274 faecal samples were collected from pigeons and skinks respectively. Twenty-four pigeon samples consisted of multiple samples produced by the same individual on the same day and so were combined with the first sample, giving 146 samples for molecular analysis. ITS2 DNA sequences were recovered from 141 (96.6%) and 246 (89.8%) of the pigeon and skink samples respectively.

4.4.1 Pink Pigeon diet

After data cleaning, 63 dietary items remained in the diet of the Pink Pigeon (Table 4.1). Of these, 93.7% were identified to species and 100% to genus. Of the dietary taxa that were also present in the comprehensive DNA barcode library (Chapter 2), 100% were identified to species. Details of dietary items that were removed from the dataset can be found in Appendix 2.3. Dietary items most frequently detected were the introduced *Leucaena leucocephala* and *Passiflora suberosa* (75.9% and 61.7% of samples respectively, n=141). These were followed by the native *Hilsenbergia petiolaris* and *Premna serratifolia* (56.0% and 52.5% of samples respectively, n=141). Both wheat and maize (supplementary feed) were consumed and appeared in 27.0% and 5.7% of samples, respectively (n=141). Table 4.1 provides a comprehensive breakdown (by sex, age class, and season) of the percentage of samples in which dietary items were detected.

A Wilcoxon matched-pairs test revealed that the species richness of native plant species in Pink Pigeon diet was significantly higher than that of exotic species ($V = 874.5$, $p = <0.01$) (Figure 4.1a).

Testing for deviations from a null model of random herbivory revealed that Pink Pigeons have feeding preferences in both the wet and dry seasons (Fig. 4.2. standardised effect sizes are presented in Table 5.2). Accounting for repeated measures, in each season the pigeons had significant preferences for five introduced and six native species, although the specific taxa differed between seasons. *Leucaena leucocephala* and *P. suberosa* (both introduced), and *Ipomoea violacea* and *P. serratifolia* (both native) are consumed by the highest proportion of individuals and also eaten in significantly higher frequencies than expected given their availability across the seasons (Fig. 4.2, Table 4.1).

Binomial generalized linear mixed modeling revealed that the prevalence of supplementary feed in the diet is greater in males in comparison to females (estimate = 0.6805 ± 0.2555, $z = 2.664$, $p = 0.0073$), but this prevalence is not influenced by season (estimate = -0.3680 ± 0.2497, $z = -1.474$, $p = 0.140545$).

4.4.2 Telfair's Skink diet

Using DNA metabarcoding, 76 plant species were detected in the diet of skinks. *Ficus rubra, F. reflexa,* and *I. violacea* (all native) were the most frequently occurring dietary items (74.4%, 57.7% and 52.8% of samples respectively, Table 4.2). Of the dietary items

detected, 94.7% were identified to species and 100% to genus. Of the dietary taxa that were also present in the comprehensive DNA barcode library (Chapter 2), 100% were identified to species. Details of dietary items that were removed from the dataset can be found in Appendix 2.3.

Using the morphological identification of plant tissue in faecal samples, 16 different dietary items were identified (fruits, seeds and leaves from the same species would be classified as three dietary items). Combining various plant tissue types into their respective taxa, 11 dietary items were identified, where 72.7% were identified to species-level, 9.1% to genus, 9.1% to family, and 9.1% to kingdom (Table 4.3).

Using only the metabarcoding data, after calculating the mean species richness of native and exotic plants for those individual skinks that were sampled more than once, 176 samples were used for the Wilcoxon matched-pairs test. Native species richness in the diet was significantly higher than introduced species richness ($V = 12735$, $p = <0.001$) (Fig 4.1b).

Skinks have feeding preferences in both the dry and wet seasons. Accounting for repeated measures, skinks had preferences for four introduced and ten native species in the dry season and five introduced and 15 native species in the wet season (Fig. 4.3, standardised effect sizes presented in Table 5.2). *Ficus rubra*, *F. reflexa, I. violacea* (all native), and *P. suberosa* (introduced) were consumed by the highest proportion of individuals and also consumed in significantly higher proportions than expected given their availability across the seasons (Table 4.2, Fig. 4.3).

Table 4.1 The percentage of Pink Pigeon faecal samples testing positive for dietary items broken down by sex, age-class and season. The status (endemic, native or introduced) of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item							
Dietary item	Common name	Present in DNA barcode library	Status	All (n=141)	Female (n=52)	Male (n=79)	Adult (n=126)	Fledgling (n=8)	Squab (n=6)	Dry season (n=80)	Wet season (n=61)
Leucaena leucocephala	Acacia	TRUE	introduced	75.9	78.8	73.4	75.4	75.0	83.3	70.00	83.61
Passiflora suberosa	Liane poc poc	TRUE	introduced	61.7	75.0	54.4	61.9	50.0	83.3	46.25	81.97
Hilsenbergia petiolaris	Bois de pipe	TRUE	native	56.0	53.8	58.2	56.3	50.0	50.0	43.75	72.13
Premna serratifolia	Bois sureau	TRUE	native	52.5	50.0	54.4	53.2	37.5	50.0	50.00	55.74
Ipomoea violacea	-	TRUE	native	48.9	48.1	49.4	48.4	50.0	50.0	33.75	68.85
Triticum sp.	wheat	FALSE	sup.feed*	27.0	13.5	32.9	26.2	25.0	33.3	30.00	22.95
Ficus reflexa	La fouche bâtard, Affouche á petites feuilles	TRUE	native	23.4	28.8	19.0	22.2	37.5	16.7	13.75	36.07
Ficus rubra	Affouche rouge, La fouche, Affouche á grandes feuilles	TRUE	native	17.0	21.2	16.5	18.3	12.5	0.0	13.75	21.31
Cyperus dubius	-	TRUE	native	14.2	17.3	13.9	15.1	12.5	0.0	10.00	19.67
Coptosperma borbonica	Bois de rat	TRUE	endemic	13.5	7.7	17.7	14.3	0.0	16.7	20.00	4.92
Eragrostis amabilis	-	TRUE	native	12.8	19.2	8.9	12.7	25.0	0.0	7.50	19.67
Morinda citrifolia	Bois tortue	TRUE	introduced	12.8	5.8	15.2	11.1	12.5	33.3	11.25	14.75
Euphorbia hirta	Jean Robert	TRUE	introduced	12.1	11.5	11.4	11.9	0.0	16.7	13.75	9.84
Solanum americanum	Brède martin	TRUE	introduced	8.5	7.7	10.1	8.7	12.5	0.0	7.50	9.84
Eugenia lucida	Bois clou, Bois de clous	TRUE	endemic	7.8	13.5	3.8	6.3	25.0	16.7	7.50	8.20
Hibiscus tiliaceus	Var, Vaur	TRUE	native	7.8	7.7	8.9	7.9	12.5	0.0	11.25	3.28
Margaritaria anomala	Bois chenille	TRUE	endemic	7.1	1.9	11.4	7.9	0.0	0.0	12.50	0.00

Table 4.1 The percentage of Pink Pigeon faecal samples testing positive for dietary items broken down by sex, age-class and season. The status (endemic, native or introduced) of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item							
Dietary item	Common name	Present in DNA barcode library	Status	All (n=141)	Female (n=52)	Male (n=79)	Adult (n=126)	Fledgling (n=8)	Squab (n=6)	Dry season (n=80)	Wet season (n=61)
Zea mays	Maize	FALSE	sup.feed*	5.7	5.8	5.1	5.6	0.0	16.7	7.50	3.28
Thespesia populnea	Mahoe, Ste Marie, Porcher	TRUE	native	5.0	9.6	1.3	4.8	12.5	0.0	3.75	6.56
Asystasia gangetica	Herbe á pistache	TRUE	introduced	5.0	9.6	2.5	4.8	12.5	0.0	1.25	9.84
Scaevola taccada	Veloutier vert	TRUE	native	5.0	1.9	7.6	5.6	0.0	0.0	8.75	0.00
Digitaria horizontalis	Gros Meinki	TRUE	introduced	3.5	3.8	3.8	4.0	0.0	0.0	6.25	0.00
Gagnebina pterocarpa	Acacia indigene	TRUE	native	3.5	0.0	6.3	4.0	0.0	0.0	6.25	0.00
Polyscias maraisiana	Bois de Boeuf, Bois d'éponge	TRUE	endemic	3.5	3.8	3.8	4.0	0.0	0.0	2.50	4.92
Ipomoea obscura	Amourette	TRUE	introduced	2.8	1.9	3.8	3.2	0.0	0.0	1.25	4.92
Poupartia borbonica	Bois Poupart	TRUE	endemic	2.8	3.8	2.5	3.2	0.0	0.0	1.25	4.92
Agrostis sp	-	FALSE	unknown	2.1	0.0	2.5	1.6	12.5	0.0	3.75	0.00
Asparagus setaceus	Liane asperge	TRUE	introduced	2.1	3.8	1.3	2.4	0.0	0.0	3.75	0.00
Phyllanthus casticum	Bois castique, Castique, Bois de demoiselle	TRUE	native	2.1	1.9	2.5	2.4	0.0	0.0	0.00	4.92
Phymatodes scolopendria	Fougère polypode	TRUE	native	2.1	0.0	2.5	1.6	12.5	0.0	2.50	1.64
Pithecellobium dulce	Cassie de Manille	TRUE	introduced	2.1	3.8	1.3	2.4	0.0	0.0	2.50	1.64
Stachytarpheta jamaicensis	-	TRUE	introduced	2.1	1.9	2.5	2.4	0.0	0.0	0.00	4.92
Stenotaphrum dimidiatum	Herbe bourique	TRUE	native	2.1	3.8	1.3	2.4	0.0	0.0	2.50	1.64
Turnera angustifolia	-	TRUE	introduced	2.1	0.0	3.8	2.4	0.0	0.0	2.50	1.64

Table 4.1 The percentage of Pink Pigeon faecal samples testing positive for dietary items broken down by sex, age-class and season. The status (endemic, native or introduced) of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

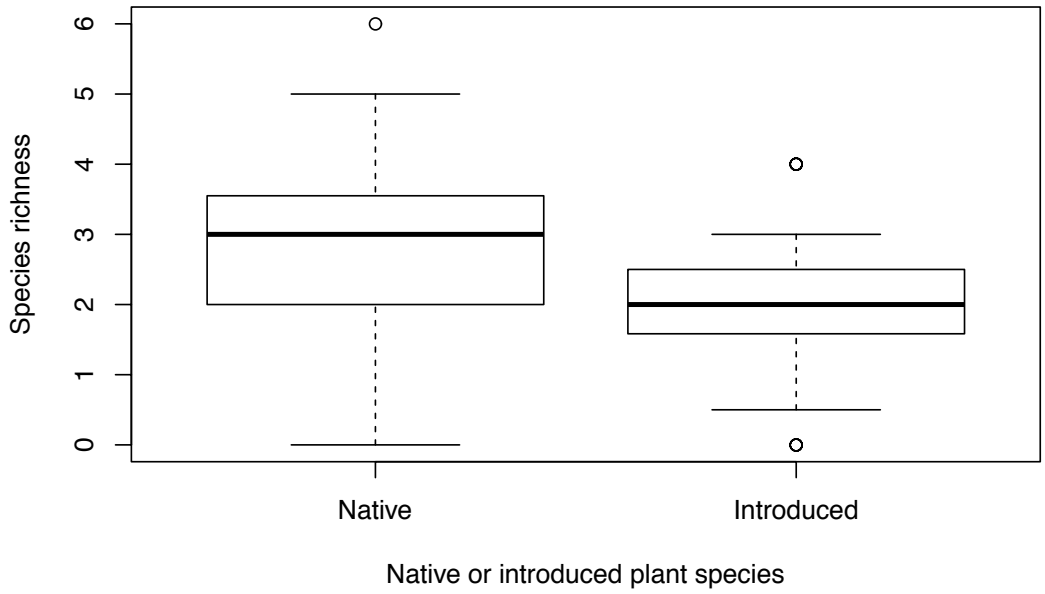
				Percentage of samples testing positive for a dietary item							
Dietary item	Common name	Present in DNA barcode library	Status	All (n=141)	Female (n=52)	Male (n=79)	Adult (n=126)	Fledgling (n=8)	Squab (n=6)	Dry season (n=80)	Wet season (n=61)
Turraea thouarsiana	Bois quivi	TRUE	endemic	1.4	1.9	1.3	1.6	0.0	0.0	2.50	0.00
Chloris barbata	-	TRUE	introduced	1.4	0.0	2.5	1.6	0.0	0.0	2.50	0.00
Clerodendrum heterophyllum	Bois cabris	TRUE	endemic	1.4	1.9	1.3	1.6	0.0	0.0	0.00	3.28
Dodonaea viscosa	Bois de reinette	TRUE	native	1.4	0.0	1.3	0.8	0.0	16.7	2.50	0.00
Holcus sp.	-	FALSE	unknown	1.4	0.0	2.5	1.6	0.0	0.0	1.25	1.64
Phyllanthus tenellus	-	TRUE	introduced	1.4	1.9	0.0	0.8	12.5	0.0	1.25	1.64
Plantago sp.	-	FALSE	unknown	1.4	1.9	1.3	1.6	0.0	0.0	2.50	0.00
Portulaca oleracea	Pourpier rouge, Pourpier	TRUE	introduced	1.4	0.0	2.5	1.6	0.0	0.0	2.50	0.00
Pteris vittata	Ptéris rubané (Reunion Island)	FALSE	native	1.4	0.0	2.5	1.6	0.0	0.0	2.50	0.00
Santalum album	Bois de santal	TRUE	introduced	1.4	0.0	0.0	0.0	0.0	33.3	1.25	1.64
Sida pussila	-	TRUE	native	1.4	3.8	0.0	1.6	0.0	0.0	2.50	0.00
Tylophora coriacea	Ipéca du Pays	TRUE	native	1.4	1.9	1.3	1.6	0.0	0.0	2.50	0.00
Abutilon indicum	Mauve du pays	TRUE	introduced	0.7	1.9	0.0	0.8	0.0	0.0	1.25	0.00
Amaranthus dubius	Brède malabar	TRUE	introduced	0.7	0.0	1.3	0.8	0.0	0.0	1.25	0.00
Asparagus umbellatus	Asperge sauvage	TRUE	native	0.7	1.9	0.0	0.8	0.0	0.0	0.00	1.64
Caesalpinia bonduc	Cadoque, Cadoc, Bonduc	TRUE	native	0.7	1.9	0.0	0.8	0.0	0.0	1.25	0.00
Cenchrus echinatus	Herbe á cateaux	TRUE	introduced	0.7	1.9	0.0	0.8	0.0	0.0	1.25	0.00
Flacourtia indica	Prune malgache	TRUE	introduced	0.7	0.0	1.3	0.8	0.0	0.0	0.00	1.64
Gouania tiliifolia	Liane charretier	TRUE	native	0.7	0.0	0.0	0.0	0.0	16.7	1.25	0.00

Table 4.1 The percentage of Pink Pigeon faecal samples testing positive for dietary items broken down by sex, age-class and season. The status (endemic, native or introduced) of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item							
Dietary item	Common name	Present in DNA barcode library	Status	All (n=141)	Female (n=52)	Male (n=79)	Adult (n=126)	Fledgling (n=8)	Squab (n=6)	Dry season (n=80)	Wet season (n=61)
Heteropogon contortus	Herbe polisson	TRUE	introduced	0.7	0.0	0.0	0.0	0.0	0.0	0.00	1.64
Litsea glutinosa	Bois d'oiseaux	TRUE	introduced	0.7	0.0	1.3	0.8	0.0	0.0	1.25	0.00
Lolium perenne	-	FALSE	introduced	0.7	1.9	0.0	0.8	0.0	0.0	0.00	1.64
Maytenus pyria	Bois à poudre	TRUE	introduced	0.7	1.9	0.0	0.8	0.0	0.0	0.00	1.64
Millettia pinnata	Pongame, Coqueluche	TRUE	introduced	0.7	0.0	1.3	0.8	0.0	0.0	1.25	0.00
Poa annua	Annual meadow grass	FALSE	introduced	0.7	0.0	0.0	0.0	0.0	16.7	0.00	1.64
Poa infirma	Early meadow grass	FALSE	introduced	0.7	0.0	0.0	0.0	0.0	16.7	0.00	1.64
Tridax procumbens	Herbe Caille	TRUE	introduced	0.7	0.0	1.3	0.8	0.0	0.0	1.25	0.00
Triphasia trifolia	Orangine	TRUE	introduced	0.7	0.0	1.3	0.8	0.0	0.0	1.25	0.00
Wikstroemia indica	Herbe tourterelle	TRUE	introduced	0.7	1.9	0.0	0.8	0.0	0.0	0.00	1.64

*Sup. feed is supplementary feed provided to Pink Pigeons

(a)



(b)

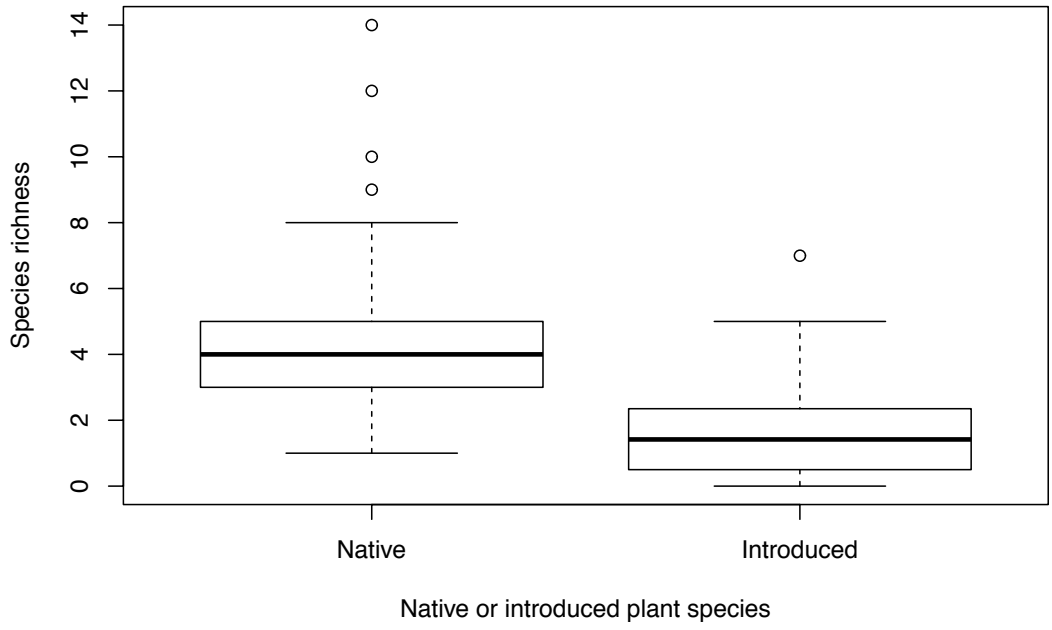
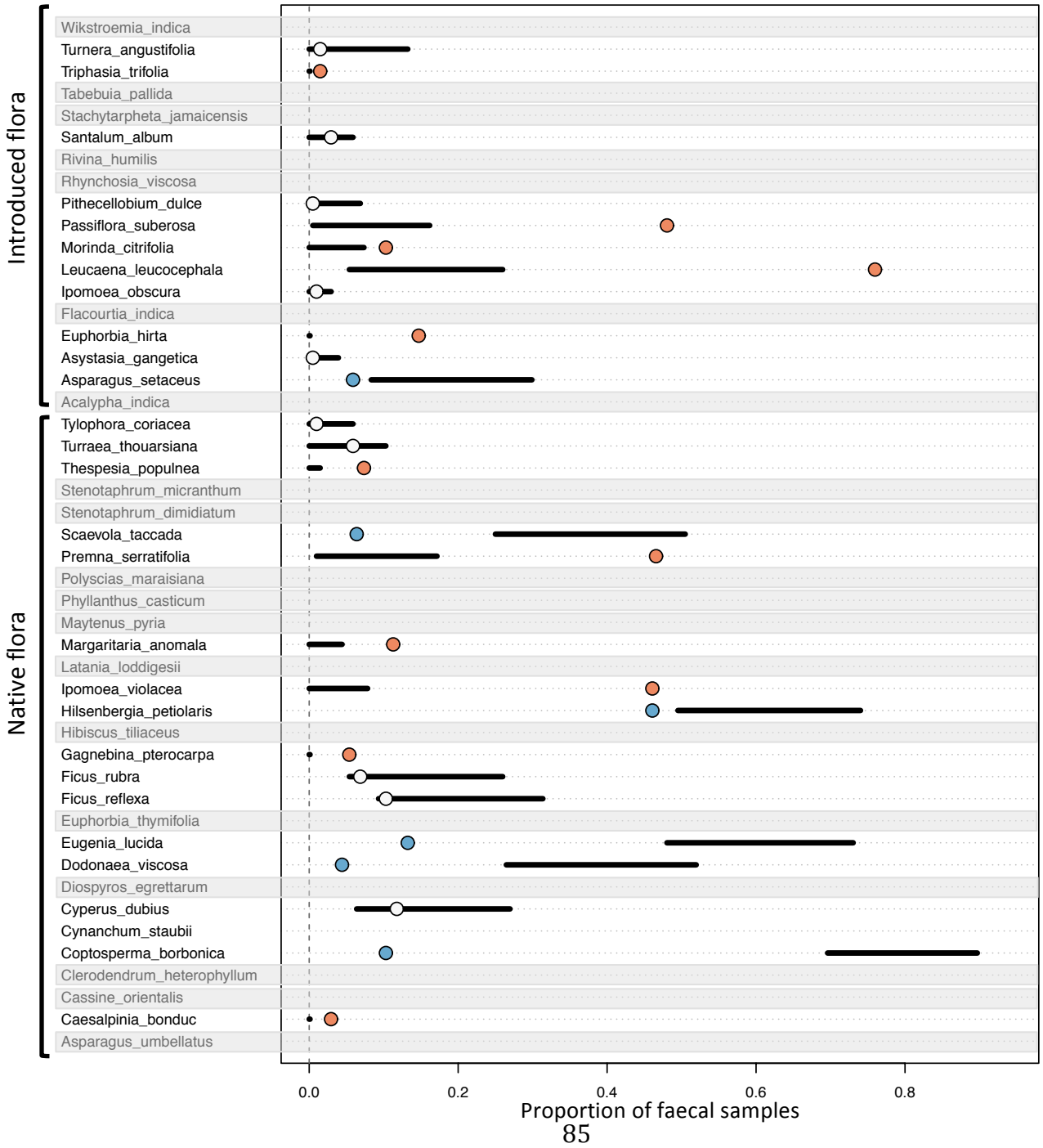


Figure 4.1. Box and whisker plot showing the difference in species richness of native and introduced plant species in the diets of (a) Pink Pigeons, and (b) Telfair's skinks.

(a) Pink Pigeon
dry season



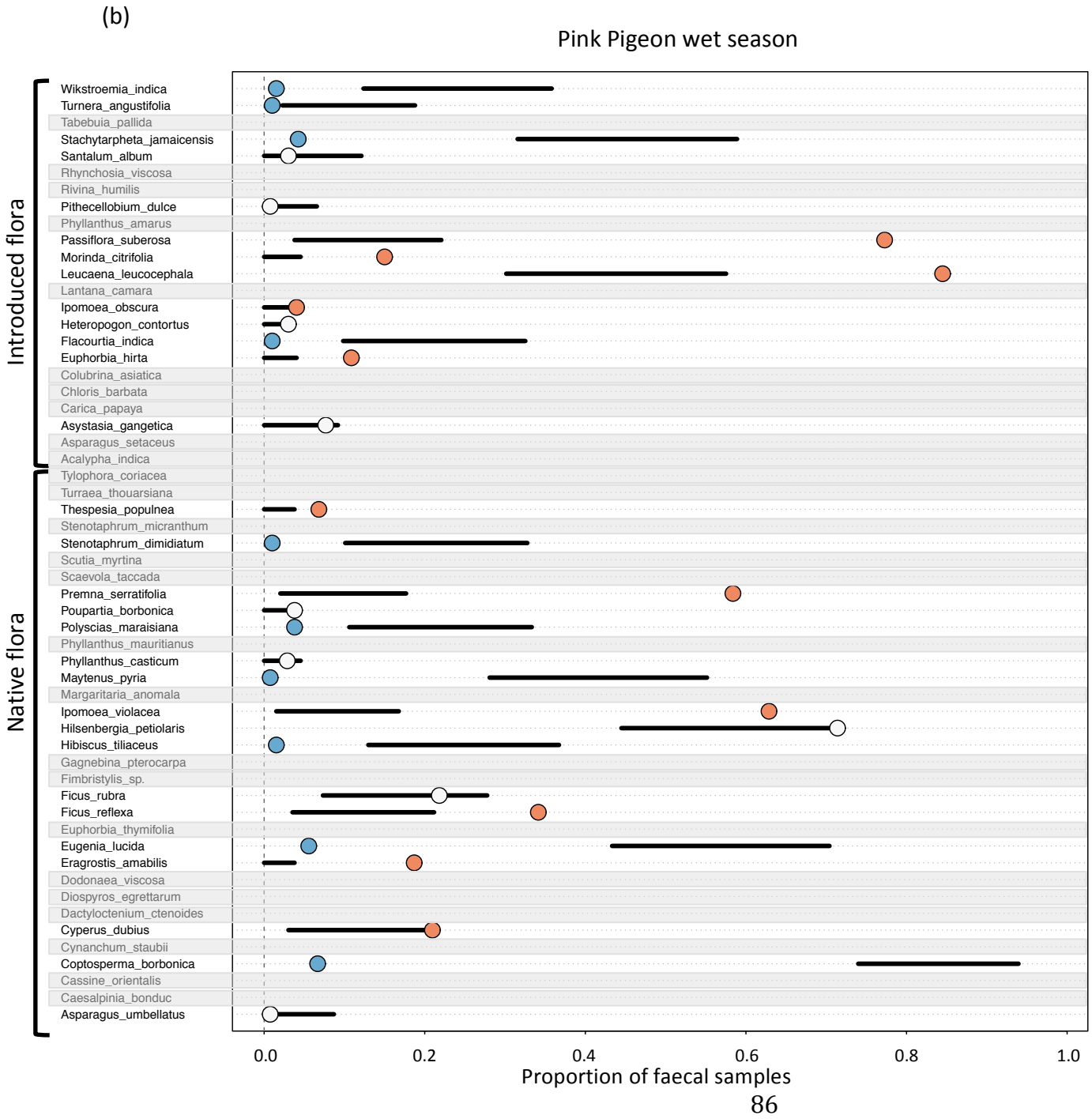


Figure 4.2. Dietary preferences of Pink Pigeons on Ile aux Aigrettes in both the (a) dry season, and (b) wet season. Thick black lines: predictions from the null model with 95% confidence limits; white circles: plant species eaten in proportion to their availability; blue circles: species eaten in lower proportions than expected; orange circles: species eaten at a greater proportion than expected. Plant species that are absent in pigeon diet but present in the diet of either Telfair's skinks or Aldabra giant tortoises (see Chapter 5 for tortoises) are listed and highlighted in grey but their predictions are absent.

Table 4.2 The diet of the Telfair's skink as determined by metabarcoding alone. The percentage of skink faecal samples from Ile aux Aigrettes testing positive for dietary items broken down by sex and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item					
Dietary item	Common name	Present in DNA barcode library	Status	All (n=246)	Female (n=123)	Male (n=115)	Unknown sex (n=8)	Dry season (n=65)	Wet season (n=181)
Ficus rubra	Affouche rouge, La fouche, Affouche á grandes feuilles	TRUE	native	74.4	75.6	72.2	87.5	76.9	73.5
Ficus reflexa	La fouche bâtard, Affouche á petites feuilles	TRUE	native	57.7	61.0	53.9	62.5	58.5	57.5
Ipomoea violacea	-	TRUE	native	52.8	55.3	47.8	87.5	70.8	46.4
Passiflora suberosa	Liane poc poc	TRUE	introduced	43.9	36.6	49.6	75.0	50.8	41.4
Hilsenbergia petiolaris	Bois de pipe	TRUE	native	38.2	40.7	32.2	87.5	56.9	31.5
Margaritaria anomala	Bois chenille	TRUE	endemic	36.2	37.4	34.8	37.5	13.8	44.2
Leucaena leucocephala	Acacia	TRUE	introduced	31.7	29.3	32.2	62.5	23.1	34.8
Hibiscus tiliaceus	Var, Vaur	TRUE	native	17.9	20.3	16.5	0.0	4.6	22.7
Morinda citrifolia	Bois tortue	TRUE	introduced	17.9	15.4	21.7	0.0	10.8	20.4
Premna serratifolia	Bois sureau	TRUE	native	17.1	17.9	13.0	62.5	13.8	18.2
Cyperus dubius	-	TRUE	native	16.3	15.4	18.3	0.0	16.9	16.0
Eugenia lucida	Bois clou, Bois de clous	TRUE	endemic	15.0	14.6	15.7	12.5	10.8	16.6
Eragrostis amabilis	-	TRUE	native	13.8	17.1	10.4	12.5	16.9	12.7
Thespesia populnea	Mahoe, Ste Marie, Porcher	TRUE	native	11.8	8.9	14.8	12.5	15.4	10.5
Solanum americanum	Brède martin	TRUE	introduced	10.2	10.6	8.7	25.0	9.2	10.5
Scaevola taccada	Veloutier vert	TRUE	native	7.7	8.1	7.8	0.0	0.0	10.5
Euphorbia hirta	Jean Robert	TRUE	introduced	7.7	7.3	7.8	12.5	9.2	7.2
Polyscias maraisiana	Bois de Boeuf, Bois d'éponge	TRUE	endemic	6.5	6.5	4.3	37.5	9.2	5.5

Table 4.2 The diet of the Telfair's skink as determined by metabarcoding alone. The percentage of skink faecal samples from Ile aux Aigrettes testing positive for dietary items broken down by sex and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item					
Dietary item	Common name	Present in DNA barcode library	Status	All (n=246)	Female (n=123)	Male (n=115)	Unknown sex (n=8)	Dry season (n=65)	Wet season (n=181)
Stachytarpheta jamaicensis	-	TRUE	introduced	5.3	6.5	3.5	12.5	4.6	5.5
Asystasia gangetica	Herbe á pistache	TRUE	introduced	4.9	6.5	2.6	12.5	6.2	4.4
Santalum album	Bois de santal	TRUE	introduced	4.5	4.1	4.3	12.5	3.1	5.0
Scutia myrtina	Liane bambara, Bambara	TRUE	native	4.1	3.3	5.2	0.0	0.0	5.5
Ipomoea obscura	Amourette	TRUE	introduced	3.7	5.7	1.7	0.0	4.6	3.3
Digitaria horizontalis	Gros Meinki	TRUE	introduced	3.3	3.3	3.5	0.0	0.0	4.4
Pithecellobium dulce	Cassie de Manille	TRUE	introduced	3.3	2.4	3.5	12.5	1.5	3.9
Tylophora coriacea	Ipéca du Pays	TRUE	native	2.8	1.6	4.3	0.0	4.6	2.2
Turnera angustifolia	-	TRUE	introduced	2.8	1.6	3.5	12.5	3.1	2.8
Clerodendrum heterophyllum	Bois cabris	TRUE	endemic	2.8	2.4	3.5	0.0	3.1	2.8
Cynanchum staubii	Liane calle	TRUE	endemic	2.8	3.3	2.6	0.0	0.0	3.9
Asparagus setaceus	Liane asperge	TRUE	introduced	2.8	3.3	2.6	0.0	9.2	0.6
Coptosperma borbonica	Bois de rat	TRUE	endemic	2.8	4.1	1.7	0.0	4.6	2.2
Diospyros egrettarum	Bois d'ébène Ile aux Aigrettes	TRUE	endemic	2.8	0.0	5.2	12.5	7.7	1.1
Gouania tiliifolia	Liane charretier	TRUE	native	2.8	0.8	5.2	0.0	4.6	2.2
Gagnebina pterocarpa	Acacia indigene	TRUE	native	2.4	2.4	2.6	0.0	0.0	3.3
Stenotaphrum dimidiatum	Herbe bourique	TRUE	native	2.4	1.6	3.5	0.0	1.5	2.8
Poupartia borbonica	Bois Poupart	TRUE	endemic	2.4	3.3	1.7	0.0	7.7	0.6
Dodonaea viscosa	Bois de reinette	TRUE	native	2.0	2.4	1.7	0.0	3.1	1.7

Table 4.2 The diet of the Telfair's skink as determined by metabarcoding alone. The percentage of skink faecal samples from Ile aux Aigrettes testing positive for dietary items broken down by sex and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item					
Dietary item	Common name	Present in DNA barcode library	Status	All (n=246)	Female (n=123)	Male (n=115)	Unknown sex (n=8)	Dry season (n=65)	Wet season (n=181)
Maytenus pyria	Bois à poudre	TRUE	introduced	2.0	0.8	3.5	0.0	1.5	2.2
Triticum sp.	wheat	FALSE	sup. feed*	2.0	0.8	2.6	12.5	4.6	1.1
Phyllanthus casticum	Bois castique, Castique, Bois de demoiselle	TRUE	native	2.0	1.6	2.6	0.0	0.0	2.8
Rivina humilis	Petite groseille	TRUE	introduced	2.0	2.4	1.7	0.0	0.0	2.8
Wikstroemia indica	Herbe tourterelle	TRUE	introduced	2.0	0.8	3.5	0.0	0.0	2.8
Cenchrus echinatus	Herbe á cateaux	TRUE	introduced	1.6	1.6	1.7	0.0	0.0	2.2
Plantago sp.	-	FALSE	unknown	1.6	2.4	0.9	0.0	0.0	2.2
Caesalpinia bonduc	Cadoque, Cadoc, Bonduc	TRUE	native	1.6	0.8	2.6	0.0	1.5	1.7
Flacourtia indica	Prune malgache	TRUE	introduced	1.6	0.0	3.5	0.0	1.5	1.7
Tridax procumbens	Herbe Caille	TRUE	introduced	1.2	2.4	0.0	0.0	0.0	1.7
Sida pussila	-	TRUE	native	1.2	1.6	0.9	0.0	0.0	1.7
Chloris barbata	-	TRUE	introduced	1.2	1.6	0.9	0.0	0.0	1.7
Cassine orientalis	Bois d'olive	TRUE	endemic	1.2	2.4	0.0	0.0	3.1	0.6
Portulaca oleracea	Pourpier rouge, Pourpier	TRUE	introduced	1.2	0.8	1.7	0.0	0.0	1.7
Turraea thouarsiana	Bois quivi	TRUE	endemic	1.2	0.0	2.6	0.0	1.5	1.1
Acalypha indica	Herbe chatte	TRUE	introduced	1.2	0.8	1.7	0.0	0.0	1.7
Asparagus umbellatus	Asperge sauvage	TRUE	native	1.2	0.0	2.6	0.0	1.5	1.1
Holcus sp.	-	FALSE	unknown	1.2	1.6	0.9	0.0	0.0	1.7
Zea mays	Maize	FALSE	sup. feed*	1.2	0.8	1.7	0.0	1.5	1.1

Table 4.2 The diet of the Telfair's skink as determined by metabarcoding alone. The percentage of skink faecal samples from Ile aux Aigrettes testing positive for dietary items broken down by sex and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

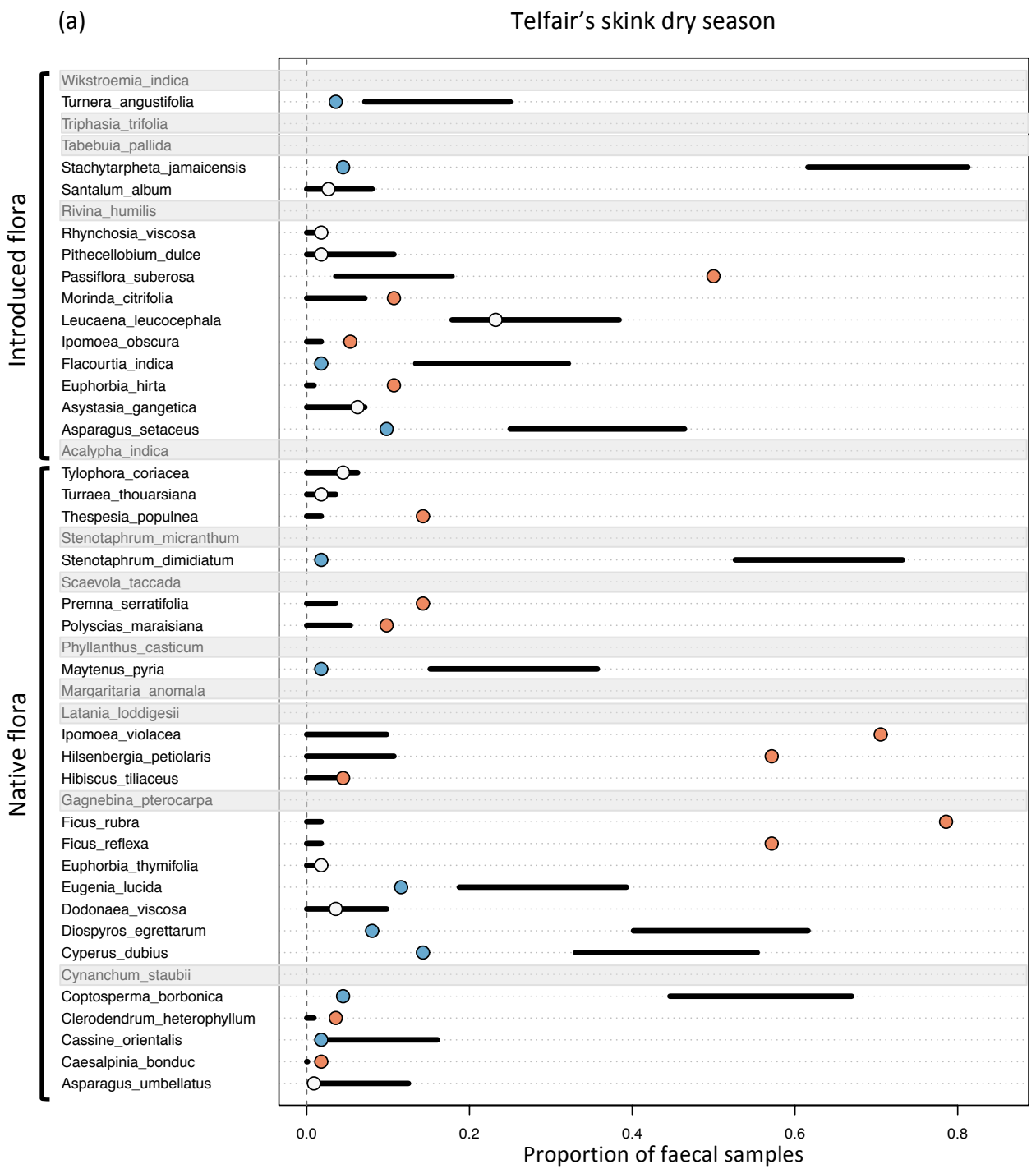
				Percentage of samples testing positive for a dietary item					
Dietary item	Common name	Present in DNA barcode library	Status	All (n=246)	Female (n=123)	Male (n=115)	Unknown sex (n=8)	Dry season (n=65)	Wet season (n=181)
Abutilon indicum	Mauve du pays	TRUE	introduced	0.8	0.0	1.7	0.0	0.0	1.1
Achyranthes aspera	-	TRUE	introduced	0.8	1.6	0.0	0.0	0.0	1.1
Euphorbia thymifolia	Petite rougette	TRUE	Cryptogenic	0.8	0.8	0.9	0.0	1.5	0.6
Phymatodes scolopendria	Fougère polypode	TRUE	native	0.8	0.8	0.9	0.0	0.0	1.1
Nicotiana tabacum	Tabak	TRUE	introduced	0.8	0.0	1.7	0.0	0.0	1.1
Dactyloctenium ctenoides	-	TRUE	native	0.4	0.8	0.0	0.0	0.0	0.6
Bidens pilosa	Herbe Villebague	TRUE	introduced	0.4	0.8	0.0	0.0	0.0	0.6
Phyllanthus amarus	Petit tamarin blanc	TRUE	introduced	0.4	0.8	0.0	0.0	0.0	0.6
Desmanthus virgatus	Petit acacia	TRUE	introduced	0.4	0.0	0.9	0.0	0.0	0.6
Agrostis sp.	-	FALSE	unknown	0.4	0.0	0.9	0.0	0.0	0.6
Phyllanthus mauritianus	-	TRUE	native	0.4	0.0	0.9	0.0	0.0	0.6
Rhynchosia viscosa	Liane lastic	TRUE	introduced	0.4	0.0	0.9	0.0	1.5	0.0
Sesuvium ayresii	Pourpier marin	TRUE	native	0.4	0.0	0.9	0.0	0.0	0.6
Carica papaya	Papayer, Papaye, Pawpaw	TRUE	introduced	0.4	0.0	0.9	0.0	0.0	0.6
Colubrina asiatica	-	TRUE	introduced	0.4	0.0	0.9	0.0	0.0	0.6
Dichondra repens	-	TRUE	introduced	0.4	0.0	0.9	0.0	0.0	0.6
Diospyros tesselaria	Bois d'ébène noir, ébenier	TRUE	endemic	0.4	0.0	0.0	12.5	1.5	0.0
Millettia pinnata	Pongame, Coqueluche	TRUE	introduced	0.4	0.0	0.9	0.0	0.0	0.6
Poa trivialis	Rough meadow grass	FALSE	introduced	0.4	0.0	0.9	0.0	1.5	0.0

Table 4.2 The diet of the Telfair's skink as determined by metabarcoding alone. The percentage of skink faecal samples from Ile aux Aigrettes testing positive for dietary items broken down by sex and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item					
Dietary item	Common name	Present in DNA barcode library	Status	All (n=246)	Female (n=123)	Male (n=115)	Unknown sex (n=8)	Dry season (n=65)	Wet season (n=181)
Terminalia bentzoe	Bois benjoin	TRUE	endemic	0.4	0.0	0.9	0.0	0.0	0.6

Table 4.3 The diet of the Telfair's skink as determined by morphology alone. The percentage of skink faecal samples from Ile aux Aigrettes testing positive for dietary items broken down by sex. The status of each dietary item is also indicated.

				Percentage of samples testing positive for a dietary item			
Name	Common name	Tissue type	Status	All samples (n=131)	Female samples (n=64)	Male samples (n=55)	Unknown sex (n=12)
Diospyros egrettarum	Bois d'ébène Ile aux Aigrettes	fruit	endemic	50.4	57.8	50.9	8.3
Ficus sp.	-	fruit	native	40.5	31.3	47.3	58.3
Hilsenbergia petiolaris	Bois de pipe	seed	native	15.3	15.6	7.3	50.0
Diospyros egrettarum	Bois d'ébène Ile aux Aigrettes	leaf	endemic	3.1	0.0	7.3	0.0
Dracaena concinna	Bois de chandelle	fruit	endemic	3.1	3.1	3.6	0.0
Ficus sp.	-	seed	native	2.3	3.1	1.8	0.0
Coptosperma borbonica	Bois de rat	flower	endemic	1.5	3.1	0.0	0.0
Diospyros egrettarum	Bois d'ébène Ile aux Aigrettes	seed	endemic	1.5	1.6	0.0	8.3
Hilsenbergia petiolaris	Bois de pipe	fruit	native	1.5	3.1	0.0	0.0
Passiflora suberosa	Liane poc poc	fruit	introduced	1.5	3.1	0.0	0.0
Twig	-	twig	-	1.5	1.6	1.8	0.0
Aloe tormentorii	Mazambron	seed	endemic	0.8	0.0	1.8	0.0
Asparagus setaceus	Liane asperge	leaf	introduced	0.8	0.0	1.8	0.0
Coptosperma borbonica	Bois de rat	fruit	endemic	0.8	1.6	0.0	0.0
Poaceae	-	leaf	-	0.8	1.6	0.0	0.0
Scaevola taccada	Veloutier vert	seed	native	0.8	0.0	1.8	0.0


(a)
Telfair's skink dry season
Introduced flora
Native flora
Wikstroemia_indica
Turnera_angustifolia
Triphasia_trifolia
Tabebuia_pallida
Stachytarpheta_jamaicensis
Santalum_album
Rivina_humilis
Rhynchosia_viscosa
Pithecellobium_dulce
Passiflora_suberosa
Morinda_citrifolia
Leucaena_leucocephala
Ipomoea_obscura
Flacourtia_indica
Euphorbia_hirta
Asystasia_gangetica
Asparagus_setaceus
Acalypha_indica
Tylophora_coriacea
Turraea_thouarsiana
Thespesia_populnea
Stenotaphrum_micranthum
Stenotaphrum_dimidiatum
Scaevola_taccada
Premna_serratifolia
Polyscias_maraisiana
Phyllanthus_casticum
Maytenus_pyria
Margaritaria_anomala
Latania_loddigesii
Ipomoea_violacea
Hilsenbergia_petiolaris
Hibiscus_tiliaceus
Gagnebina_pterocarpa
Ficus_rubra
Ficus_reflexa
Euphorbia_thymifolia
Eugenia_lucida
Dodonaea_viscosa
Diospyros_egrettarum
Cyperus_dubius
Cynanchum_staubii
Coptosperma_borbonica
Clerodendrum_heterophyllum
Cassine_orientalis
Caesalpinia_bonduc
Asparagus_umbellatus
0.0
0.2
0.4
0.6
0.8
Proportion of faecal samples

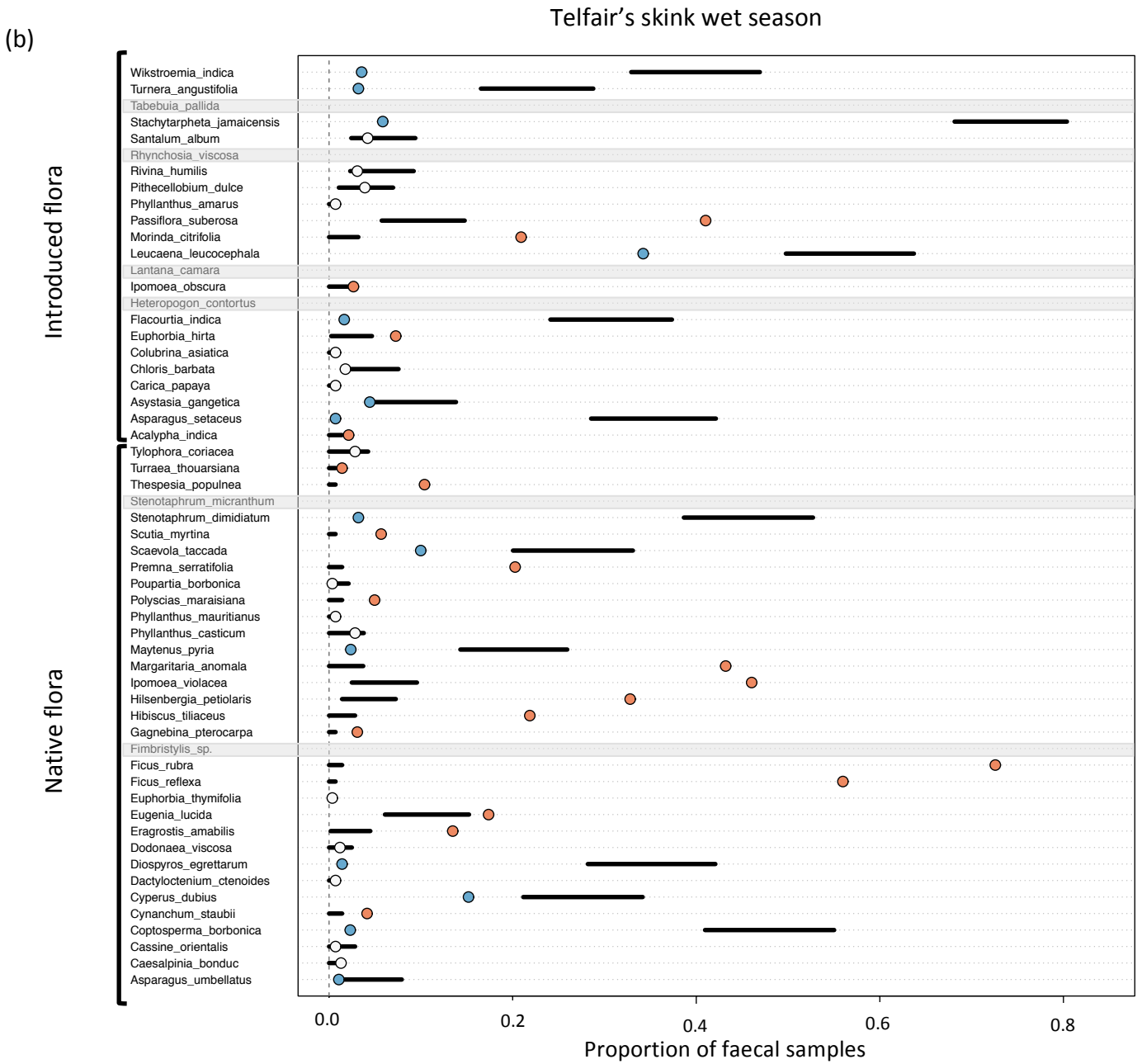


Figure 4.3. Dietary preferences of Telfair's skinks on Ile aux Aigrettes in both the (a) dry season, and (b) wet season. Thick black lines: predictions from the null model with 95% confidence limits; white circles: plant species eaten in proportion to their availability; blue circles: species eaten in lower proportions than expected; orange circles: species eaten at a greater proportion than expected. Plant species that are absent in skink diet but present in the diet of either Pink Pigeons or Aldabra giant tortoises (see Chapter 5 for tortoises) are listed and highlighted in grey but their predictions are absent.

4.5 Discussion

This study represents the first comprehensive account of herbivory by Pink Pigeons and Telfair's skinks as determined by DNA metabarcoding. This study demonstrates that both species are generalists, occupying a broad dietary niche. As such, these species are likely to be robust to moderate disturbance within the system: changes in food availability are likely to result in dietary switching to mitigate negative effects (van Baalen *et al.* 2001; Clare 2014). Of the plants eaten, both species select a number of species in a greater proportion to their availability in the environment. This indicates that Pink Pigeons and Telfair's skinks exhibit dietary preferences. However, whether or not these preferences translate into dietary importance is dependent on the plant tissue type consumed and its nutritional value. This was not investigated here but encouraged for future research. Such nutritional information would also better inform how robust these species are to change. A further consideration to keep in mind is that Telfair's skinks are omnivores and the carnivorous component of their diet was not investigated here. To capture a clear and comprehensive picture of the diet of this species it is important to determine which animals, in addition to which plants, they consume. Predation by Telfair's skinks on Ile aux Aigrettes has previously been investigated by metabarcoding (Brown *et al.* 2014a), but taxonomic discrimination at the species level was not possible. This was at least in part due to the absence of a comprehensive DNA barcode of the island's invertebrates. Identifying and DNA barcoding the invertebrate community on Ile aux Aigrettes is recommended to further our understanding of Telfair's skink diet and other predators on the island.

4.5.1 The importance of native and introduced plant species in the diet

Overall, both pigeons and skinks consumed a broader range of native plants in comparison to introduced plants. This may indeed be a reflection of what is available in the environment: thanks to extensive habitat restoration efforts the number of native species outnumbers those that are introduced (77 native and 59 introduced, see Chapter 2).

Comparing what was consumed to what was available in the environment revealed that Telfair's skinks had preferences for up to three times as many native plants in comparison to introduced species. In addition, the most frequently occurring dietary items were the native *F. rubra, F. reflexa* and *I. violacea.* This is a good indication that the

skinks' diet is dominated by native species and that ongoing work to restore the native plant community and reduce the abundance of introduced species is likely to be beneficial for the recovery of this Vulnerable reptile.

The Pink Pigeons, however, have dietary preferences for a similar number of native and introduced plant species. Additionally, the most frequently occurring species across all samples were the introduced *L. leucocephala* and *P. suberosa*. However, without knowing the nutritional importance of these species, their importance for the fitness of the consumer is unknown. Indeed, *L. leucocephala* is known to be poisonous to some ruminants despite being palatable and nutritious (Peixoto *et al.* 2008), but its toxicity to wild bird species is unknown. However, the data provide an indication that Pink Pigeons may be reliant on introduced species and managers must be mindful of this when carrying out restoration work (i.e. weeding of introduced species) on Ile aux Aigrettes. This is in line with previous research that showed that restoration activities on Ile aux Aigrettes are associated with reduced Pink Pigeon survival (Concannon 2014). Previous work on a Critically Endangered columbid also revealed the frequent consumption of introduced plant species by metabarcoding and warn of the risks associated with the rapid removal of introduced plants (Ando *et al.* 2013).

Despite an overall reliance on introduced plants, pigeons also exhibited preferences for native plant species. For example, *P. serratifolia* and *I. violacea* both had a high frequency of occurrence in the diet and were preferred by the pigeons. Therefore increasing the abundance of preferred native species on Ile aux Aigrettes could potentially mitigate the loss of important introduced plant species through restoration activities.

4.5.2 The use of supplementary feed by Pink Pigeons

Supplementary feed was infrequently detected in Pink Pigeon faecal samples. This is surprising since it is provided *ad libitum* all year and the vast majority of the faecal samples for this study were collected at the Pink Pigeon Aviary, which is where the supplementary feed is provided. It is clear from this study and from earlier work (Edmunds *et al.* 2008) that the majority of the Ile aux Aigrettes' Pink Pigeon subpopulation visit the feeding hoppers. However, this study suggests that they infrequently consume the supplementary feed. Thus, visits to the hoppers and aviary may be carried out for other reasons, such as social interaction or for water.

Edmunds *et al* (2008) suggested that supplementary feed may not be a significant component of Pink Pigeon diet, but may be used to make up any shortfall in any natural food availability. The metabarcoding data support that the feed is not a major component of the diet and although there was no significant seasonal variation in the use of supplementary food, there was an indication that use in the dry season is slightly higher than in the wet season. Earlier work indicates that there may be a reduction of natural food on Ile aux Aigrettes in July and August (at the onset of the dry season) (Atkinson and Sawmy 2003, cited in Edmunds *et al.* 2008). In the analysis, the dry season samples were collected from July until December. This broad grouping of samples may mask more subtle monthly variation.

The metabarcoding data also provide information on the usage of natural food items. These data can be used in combination with plant phenology information to identify periods when there is a shortfall in natural food. This information can guide the management of the subpopulation by both limiting the provision of supplementary food to times when it is required and working towards increasing the abundance of natural food on the island that would be available to the pigeons when there is a shortfall in other species. A reduction in supplementary food provisioning has economic advantages and may also increase the fitness of the species. For example, providing supplementary feed is thought to increase disease transmission (Murray *et al.* 2016) and aggression (Edmunds *et al.* 2008; Birnie-Gauvin *et al.* 2017). Pink Pigeons are known to suffer from three diseases, the most serious being Trichomonosis (Swinnerton *et al.* 2005a; Swinnerton *et al.* 2005b). Exotic columbids on Ile aux Aigrettes are known reservoirs of this disease and they also use the supplemental feed provided to the Pink Pigeons. Therefore, providing supplementary food may increase disease transmission risk to Pink Pigeons by both providing a space for conspecifics to aggregate and also by increasing opportunities for interspecific disease transmission.

The metabarcoding data show that males use supplementary feed more frequently than females. The nutritional requirements of each sex may be different, as has been shown in other bird species (e.g. Louzao *et al.* 2006; Houston *et al.* 2007; Navarro *et al.* 2009). Thus the feed provided to the Pink Pigeons may not be optimal for females. The energetic and nutritional demands of wild Pink Pigeons have not been quantified (Edmunds *et al.* 2008), nor is it known to what extent these demands are met by both natural and supplemental feed. To better understand the need for supplementary food and to ultimately reduce provisioning, these knowledge gaps must be filled.

4.5.3 Lessons learned from comparing DNA metabarcoding and the morphological examination of dietary remains

This study also validates the use of novel ITS2 primers detailed in Chapter Three for metabarcoding studies. Taxonomic discrimination at the species level was 100% when all plants consumed were present in the bespoke DNA barcode library presented in Chapter Two. This value dropped to 93.65% and 94.74% for the Pink Pigeon and Telfair's skinks, respectively, when GenBank was used to assign taxonomy to sequences that did not match to the bespoke library. This compares favorably other barcoding regions that are used for herbivory (Taberlet *et al.* 2007; Valentini *et al.* 2009; Pompanon *et al.* 2012) and also highlights the importance of comprehensive DNA barcode libraries in metabarcoding studies.

Using Telfair's skink faecal samples as an example, DNA metabarcoding outperforms traditional morphological methods of diet assessment in terms of sensitivity (the number of different taxa identified) and taxonomic resolution. Based on the metabarcoding data, it was concluded that Telfair's skinks are generalists that eat a broad range of both native and exotic plants. However, if conclusions were based on morphological identification alone, it would appear that the plant component of Telfair's skink diet is relatively limited and veering towards specialism.

However, using morphological analyses the plant tissue types being consumed can be determined (leaf, fruit, seed, flower etc.), whereas this is not possible using metabarcoding alone. Knowing what part of a plant is being consumed in addition to which plant species allows for questions around seed dispersal and pollination to be addressed and for the impacts of a consumer on the plant community to be more clearly understood. Based on the morphological data, Telfair's skinks are likely to disperse the seeds of three native and two endemic species. However, due to the inherent biases in morphological techniques (as discussed in Chapter 3), it is likely that other seeds (including those from introduced species) were missed. Indeed, the fruits of the introduced *P. suberosa* were found in the morphological study, which means that seeds were likely to be present too. *Passiflora suberosa* seeds, amongst the seeds of other introduced species, were also noted in the faecal samples used for DNA metabarcoding

in this study and were also identified in Telfair's skink faecal samples collected from Round Island (Cole *et al.* 2009; Zuel 2009; Zuel *et al.* 2012).

4.5.4 Conclusions and recommendations for conservation management and future research

Dietary analysis by metabarcoding indicates that both Telfair's skinks and Pink Pigeons are generalists with broad dietary niches. However, there is evidence to suggest that Pink Pigeons are reliant on introduced species and managers must be mindful of this when rapidly removing introduced weeds.

Understanding trophic interactions can directly benefit the fitness of restored species by assessing the need for supplementary feed, that when provided can have unintended effects such as increased aggression (Edmunds *et al.* 2008; Birnie-Gauvin *et al.* 2017) and disease prevalence in addition to the provided food often being nutrient poor (Murray *et al.* 2016). Supplementary feed is infrequently used, and is used more often by males. Combining the metabarcoding data on the use of natural food with phenology information to identify gaps in natural food availability is a recommendation for future work. Restoration work can then focus on filling these gaps by planting alternative plant species. In the meantime, only providing supplementary feed during these gaps may reduce disease transmission and project costs.

To fully understand the importance of the plant component of Telfair's skink diet, herbivory must be considered alongside predation. DNA barcoding the invertebrate community on Ile Aux Aigrettes may allow for the taxon assignments in Brown *et al.* (2014b) to be reassigned at a finer taxonomic resolution and the Telfair's skink faecal samples in this study to be analysed for invertebrates. This is an encouraged area of future research.

To maximize the power of dietary analyses for conservation management, using a combination of methodologies is recommended. DNA metabarcoding can initially be used to determine the dietary breadth of a species to a fine taxonomic resolution. Subsequently, morphological analyses can be used to better understand the plant tissue types that are being consumed, and such data can be used to inform seed dispersal networks, for example. Finally, determining the nutritional composition of those tissue types for each species is recommended to better understand the importance of each dietary item for the fitness of the consumer.

4.6 Acknowledgements

Thank you to Ian Vaughan for help with applying econullnetr and to Helen Hipperson and the NBAF team at the University of Sheffield for carrying out the Illumina library preparation, sequencing and for training in bioinformatics. Thank you to the Mauritian Wildlife Foundation Pink Pigeon field staff and the Islands Restoration Team for assistance collecting faecal samples.

Chapter Five – Impacts and interactions of an analogue species: how does tortoise grazing affect the plant communities and endemic fauna of Ile aux Aigrettes?

An Aldabra giant tortoise enjoying Dracaena concinna leaf litter. The heterophyllic seedlings of Coptosperma borbonica are seen in the foreground.

5.1 Abstract

Island extinctions can have complex cascading effects and lead to dysfunctional ecosystems, especially when keystone species are lost. When a keystone species is globally extinct, a surrogate species can be introduced in a bid to restore ecosystem functioning (ecological replacement). In Mauritius, exotic giant tortoises, *Aldabrachelys gigantea,* have been introduced to restore dysfunctional ecosystems after the loss of their endemic counterparts, which were keystone grazers. Dietary analysis is essential to understand the impact that tortoises have on the plant communities, food webs and ecosystem functions. Metabarcoding of plant DNA from faecal samples provides us with an invaluable tool to recover detailed dietary information, but has yet to be applied to an ecological replacement experiment. In this study the diet of introduced tortoises and two endemic species, Telfair's skink (*Leiolopisma telfairii*) and the Pink Pigeon (*Nesoenas mayeri)* were compared. In parallel a tortoise exclusion experiment was undertaken to reveal the impact of ecological replacement on food webs on an island undergoing restoration. Tortoises both consume and prefer a wide range of both native and introduced plant species. This grazing reduces the height of the vegetation, particularly in open areas, leading to a mosaic habitat. Tortoise grazing also reduced the biomass of introduced weeds. However, potentially negative effects arise because the Telfair's skinks and Pink Pigeons also showed strong preferences for some of these exotic species.

5.2 Introduction

The extinction of keystone species can have cascading effects on communities of plants and animals (Paine 1980; Estes *et al.* 2011). Such trophic cascades can be triggered by the loss of apex predators or seed dispersers, diminished nutrient cycling, and a change in grazing or browsing pressure that would otherwise modulate plant communities (Kaiser-Bunbury *et al.* 2010; Dirzo *et al.* 2014). The loss of these ecological functions can have substantial negative impacts, which ultimately lead to ecosystem dysfunction and further biodiversity loss.

Arguably, the best-known example of a trophic cascade comes from the Yellowstone National Park. Here, aspen (*Populus tremuloides*) regeneration was suppressed by elk (*Cervus elaphus*) grazing until wolves (*Canis lupus*) were reintroduced, which altered elk movement and grazing pressure (Ripple *et al.* 2001; Ripple & Beschta 2007). Other examples include the classic experimental exclusion of Pisaster, an apex predator, from a rocky shore (Paine 1969), the lost Pleistocene megafauna from the Americas (Galetti 2004; Donlan *et al.* 2005; Donlan *et al.* 2006) and Siberia (Zimov 2005), and white rhinoceros (*Ceratotherium simum*) in South Africa (Waldram *et al.* 2008).

The reintroduction of locally extinct fauna, for example wolves in Yellowstone, is one of a suite of potential conservation tools, collectively known as rewilding, to restore lost species interactions and key ecological functions. Rewilding ranges from passive management that may include reintroductions, to the targeted introduction of ecological analogues, to Pleistocene rewilding (the introduction of megafauna to replace those species lost by the onset of the Holocene (Zimov *et al.* 1995; Donlan *et al.* 2005; Zimov 2005; Donlan *et al.* 2006; Fernandez *et al.* 2017). Whilst rewilding remains a contentious topic in conservation biology (e.g. Jorgensen 2015; Prior & Ward 2016), there have been calls for a rewilding approach to play a central role in long-term and broad-scale visions for the conservation of biodiversity (Fernandez *et al.* 2017).

With more intense rewilding interventions, there is increased ecological uncertainty and conservation conflicts are more likely (Fernandez *et al.* 2017). However, when a species is globally extinct, it may be worth considering the introduction of a proxy species that is likely to perform the same ecological functions as its extinct counterpart – an ecological analogue. When choosing an analogue species, it is important to consider conservation

priorities in addition to taxonomic similarity and ecological equivalence to the extinct counterpart. Taxonomic similarity does not necessarily mean ecological similarity since many closely related taxa differ markedly in their ecology. Since the role of ecological analogues is to restore ecological function, ecological rather than taxonomic similarity may be the more important criterion (Jones 2002). However, it can be difficult to predict what interactions between analogues and the native biota will emerge and what their effects will be (Rubenstein *et al.* 2006; Ricciardi & Simberloff 2009; Hunter *et al.* 2013).

Large herbivores are often considered keystone species, shaping the structure of landscapes and ecosystem dynamics (Owen-Smith 1987, 1988; Dirzo & Miranda 1991; Estes *et al.* 2011; Hunter *et al.* 2013; Bakker *et al.* 2016). In the tropics, megafaunal extinctions involve mostly, but not exclusively, large herbivores (Corlett 2013). Islands may be particularly sensitive to the loss of their megafauna since these ecosystems are relatively depauperate and simple. Here, the effects of megafaunal loss may result in a more impactful trophic cascade in comparison to continental systems where there may be more functional redundancy (Hansen & Galetti 2009). These simpler systems may also provide an opportunity to assess the impacts of ecological replacement, where impacts may be more apparent than in more complex systems. Recently, non-native giant tortoises have been introduced to tropical island ecosystems as ecological analogues to replace extinct giant tortoises, reinstating lost plant-herbivore interactions (e.g. Griffiths *et al.* 2010; Hunter *et al.* 2013). However, the extent to which such rewilding interventions have restored lost ecosystem functions, and the knock-on effects on numerous other species, remains largely unexplored (Corlett 2013; Fernandez *et al.* 2017).

Ile aux Aigrettes is a 26 ha island nature reserve located approximately 800 m from the mainland of Mauritius. Between the years 2000 and 2011, 26 Aldabra giant tortoises, *Aldabrachelys gigantea*, were introduced as ecological analogues to restore lost plant-herbivore interactions in a reversible rewilding experiment. This simple island system is a useful case study for understanding the role of an analogue grazer in a degraded island ecosystem. It has been shown that introduced *A. gigantea* have increased seed dispersal and improved seedling success in a critically endangered endemic ebony (*Diospyros egrettarum*) (Griffiths *et al.* 2011). Giant tortoises can also influence plant community composition and structure by promoting some species while suppressing others through selective grazing. Although the community composition of the lost tortoise-

grazed plant community is unknown, the prevalence of herbivory defenses such as heterophylly and prostrate growth forms among the native Mauritian flora suggest that tortoises provided a strong evolutionary selection pressure (Eskildsen *et al.* 2004; Hansen *et al.* 2004; Griffiths *et al.* 2010). Furthermore, in their native range, *A. gigantea,* are ecosystem engineers that maintain tortoise lawns through their grazing (Gibson & Hamilton 1983). Such modification of plant community composition and structure is likely to have knock-on effects across the food web. To fully understand the impact of tortoise grazing, their dietary preferences must be determined.

It has been suggested that introduced giant tortoises avoid native plants due to the availability of exotic plants that may not be as strongly defended against herbivory. Thus these tortoises may contribute to the control of palatable exotic plants (Griffiths *et al.* 2010; Griffiths *et al.* 2013). However, previous assessments of introduced giant tortoise diet have been limited by small sample sizes and inherent difficulties in delineating diet from feeding observations and the morphological identification of dietary items in faecal samples (Holechek *et al.* 1982; Pompanon *et al.* 2012; Griffiths *et al.* 2013). However, advances in molecular methods of diet assessment have the capacity to rapidly generate larger volumes of data of a greater precision (Symondson 2002; King *et al.* 2008). Today, DNA metabarcoding is largely accepted to be the accurate and sensitive method for dietary analysis (Soininen *et al.* 2009; Pompanon *et al.* 2012; Ando *et al.* 2013; Alonso *et al.* 2014, and discussed in detail in 3.2).

This study aimed to investigate the diet, through DNA metabarcoding, of introduced Aldabra giant tortoises (hereinafter referred to as tortoises) on Ile aux Aigrettes to assess how their grazing is changing the plant community and whether this has the capacity to benefit restoration of the island. Furthermore, tortoise dietary preferences were compared to those of Pink Pigeons (*Nesoenas mayeri*) and Telfair's skinks (*Leiolopisma telfairii*) (presented in Chapter 4) to begin to understand the knock-on effects that tortoise grazing is having on the endemic vertebrate fauna. Specific aims were to determine whether: (i) tortoises exhibit feeding preferences for introduced plant species and avoidance for native species; (ii) due to these dietary preferences, tortoise grazing reduces the height, abundance, species richness and diversity of introduced species but not of native species; and finally (iii) there is dietary overlap between the tortoises, Pink Pigeons and Telfair's skinks.

5.3 Materials and Methods

5.3.1 Tortoise exclusion experiment

To determine the impact of tortoise grazing on the plant community, an exclusion experiment was designed. Tortoises do not range across the entire island so tortoise home-ranges were calculated to ensure that tortoises were excluded from areas where they are normally present. Tortoise movement patterns have been recorded on Ile aux Aigrettes since 2006 (MWF, unpublished data). This dataset was supplemented by intensive searches for tortoises in the 2014 dry season and 2015 wet season. These combined datasets (n=2711 relocations) were used to calculate the mimimum convex polygon (MCP) home-ranges for each tortoise using the adehabitat package (Calenge 2006) in R Version 3.0.3 (R Core Team, 2014) . To determine which relocations may be the result of exceptional activities (areas of the island not usually visited by tortoises) and discounted in home-range estimates, home-range sizes were calculated including a range of extreme relocations. The percentage of relocations found after an asymptote of home-range size was reached were classified as extreme relocations and removed (Appendix 3.1).

Within the combined MCPs, 20 GPS locations were randomly selected using the Research Tools Plugin in QGIS version 2.4.0-Chugiak (QGIS development team, 2014) which were at least 40 m apart (Fig. 5.1a). Half of the sites were in open or semi-open areas and half were in forested areas. Both habitat types are exploited by the tortoises. At each of these 20 locations a 2 m^2 tortoise exclosure made from 3 ft. high wire fencing was constructed (Fig. 5.1b). A 1 m^2 quadrat was laid in the center of each exclosure, allowing a 0.5m buffer zone to mitigate against edge effects caused by the fencing. Each exclosure was paired with a control (unfenced) quadrat placed in a random direction from the exclosure. The distance of the control quadrat varied from 1 – 3 m: the exact location was determined by the terrain and the habitat type (e.g. tree trunks and large exposed rocks were avoided). At least two corners (diagonally opposite) of the 1 m^2 control quadrats were marked with metal stakes to aid relocating quadrats in subsequent surveys.

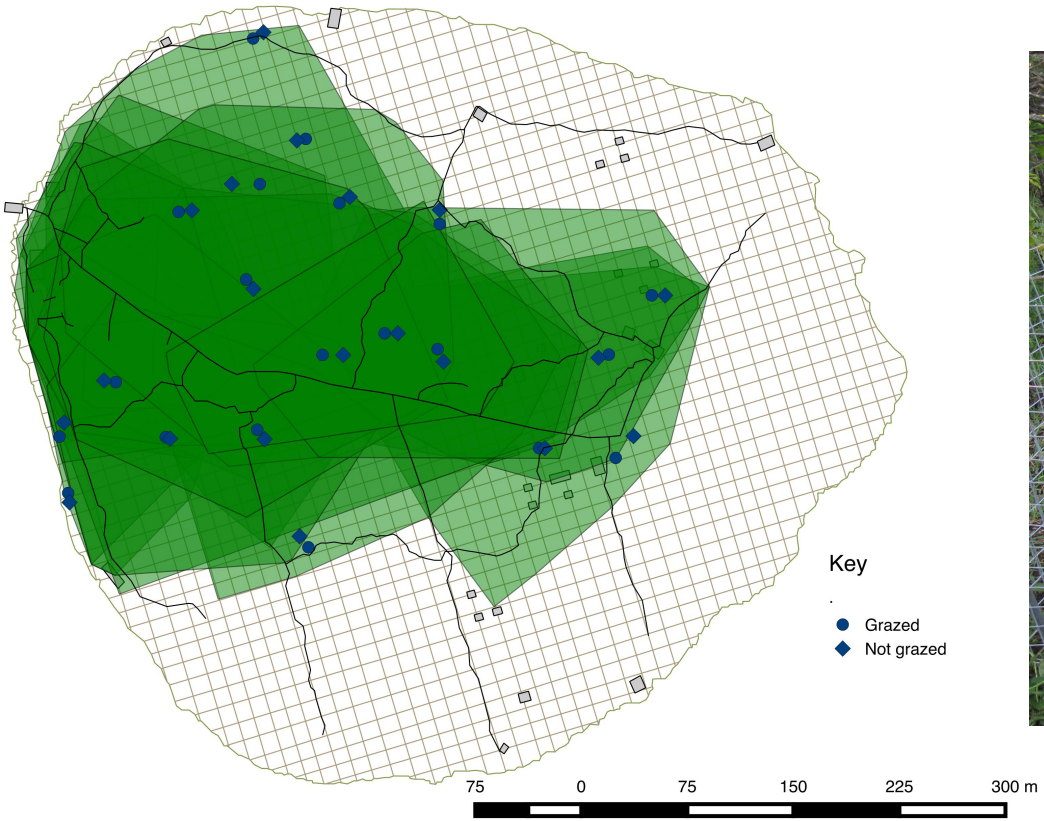
All plants in each of the 40 quadrats were identified to species, counted and the height of each plant was measured. Each grass tuft was recorded as a single plant. The percentage cover of each plant species was estimated. Data collection was assisted by placing a quadrat divided into nine grids over the fixed quadrat. For grasses, percentage cover

was recorded and the height measured at a maximum of 25 points within the quadrat (at all intersecting and central points in the grid). Photographs were taken directly above each quadrat and the percentage cover of all vegetation overall was estimated by eye. The exclosures were constructed in May 2015 and all plant surveys were carried out within ten days of them being built. Plant surveys were repeated one year later, in May 2016.

The change in abundance, species richness, Shannon diversity index and mean height between 2015 and 2016 were calculated for each plot and also separately for native and introduced plants. These data were then used as dependent variables in twelve separate generalised linear mixed models (GLMMs) using the lme4 package (Bates *et al.* 2015) in R version 3.3.1 (R Core Team 2016). Treatment (grazed or not grazed) and habitat type (forest or open) and their interaction were included as independent variables alongside exclosure pair ID as a random effect. If the interaction between treatment and habitat type was not significant, this term was removed from the model to examine the effects of treatment and habitat type in isolation. All models were run using the Gaussian family of errors and the identity link function on normalized data. All models were validated checking for equal variances and that residuals are normally distributed.

Two common introduced species that were known to be particularly invasive and targeted by manual weeding (Newfield *et al.* 2003), *Leucaena leucocephala* and *Passiflora suberosa,* were selected for further analyses. For each species, the change in abundance and mean plant height per plot between 2015 and 2016 was calculated. These data were then used as the dependent variable in four separate GLMMs with the same independent variables and model structures as described above.

(a)



(b)

Figure 5.1. Tortoise exclosure experiment on Ile aux Aigrettes. (a) Exclosure locations and their paired grazed plots plotted on top of Aldabra giant tortoise home-ranges estimated by minimum convex polygons (MCPs). Buildings (grey), paths and island grid squares are shown. (b) Tortoise exclosure containing a 1 m^2 quadrat used for the vegetation survey.

5.3.2 Food availability

Ile aux Aigrettes is divided into an alphanumeric grid system composed of 1,637 12.5 X 12.5 m grid squares. Tortoises only inhabit a portion of the island. The scarcity of water and the inaccessible terrain in some areas of the island are likely to prevent their expansion. In addition, their low population density may mean that they have no need to roam further. Thus, to determine the food that is available to the tortoises, the approximate area that tortoises could access was calculated by computing 100% MCPs (see above) for each of the 20 adult tortoises included in the study (one tortoise was excluded because it was supplementary fed due to ill health, which may have influenced its ranging behaviour). To incorporate any potential seasonal variation in tortoise movements, separate MCPs were computed for 792 relocations collected between July and August 2014 for the dry season and 415 relocations collected between January and May 2015 for the wet season. For each season, grid squares were randomly selected but stratified across the tortoises' home-range using the Research Tools Plugin in QGIS version 2.4.0-Chugiak (QGIS development team, 2014). One hundred and seventy-two and 130 grid squares were selected in the dry and wet season respectively for vegetation surveys. The percentage cover of each species present at the ground layer, and thus directly accessible to the tortoises, in each of the grid squares was estimated. Fruit and leaf litter may have supplemented food availability by falling from higher levels in the forest but these were not quantified.

5.3.3 Tortoise faecal sample collection for molecular analysis

Faecal samples were collected from both adult and hatchling Aldabra giant tortoises. At the time of the study, there were 21 adult free-roaming tortoises on Ile aux Aigrettes and an unknown number of hatchlings. When found, hatchlings were taken into captivity after a faecal sample had been obtained and thus individuals were only sampled once. All adult tortoises could be identified by both a unique identifier painted on their carapace and by a PIT-tag located in one of the hind legs, and their sex was known. Hatchlings received an identifier number when they were placed into captivity, but gender could not be determined. A faecal sample was only collected when the identity of the tortoise that produced the sample was known. This was achieved by searching for tortoises soon after sunrise when they begin to move and are more likely to defecate (Moorhouse-Gann, personal observation). Adult faeces were sampled by collecting the entire dung pile in a single-use biodegradable bag. The dung sample was then homogenized within the bag and three small (approximately 3 cm^3) aliquots from

different areas of the homogenized dung. For hatchling tortoises the entire sample was collected. DNA in the aliquots was preserved by drying with self-indicating silica gel. The silica gel was replaced once it was saturated, until the sample was completely dry (approximately 24 hours on average). The dried samples were then stored at -20˚C.

5.3.4 Molecular and bioinformatic analyses

Tortoise dung contains large fibrous pieces of poorly digested plant material. To further homogenize the samples, they were shredded with sterile scissors before DNA was extracted from 36-44 mg of the sample. DNA extraction, PCR amplification, sequencing and bioinformatics were carried out as described in Chapter Four, and Appendix 2.1 and 2.2. All sequences were compared against the comprehensive DNA barcode library presented in Chapter 2 using the BLASTn algorithm in BLAST plus (Camacho *et al.* 2009).

5.3.5 Tortoise dietary analysis

Tortoise diet was summarised by calculating the percentage of samples in which a particular dietary item occurred. This was broken down by sex, age and season (wet or dry).

To determine whether native or introduced plant species richness in the diet was greater, the total number of native and introduced taxa consumed by each tortoise was calculated. Where an individual was sampled more than once, the mean species richness of native and introduced plants was calculated for that individual to avoid pseudo replication. The data were not normally distributed and could not be normalised via a data transformation so a Wilcoxon matched-pairs test was carried out in R (R Core Team 2016) to test for differences in the median species richness of native and introduced plant species in the diet.

What a tortoise eats may be strongly influenced by food availability. However, it is possible to test for specific feeding preferences by generating a null model based on food availability data (see 5.3.2) followed by testing for departure from this null model. This allows us to differentiate between those species that are eaten in greater, lesser or equal proportions to their availability. This approach was applied in the econullnetr R package (Vaughan *et al.* 2017). Here, 20,000 iterations of the model were run (following King *et al.* 2010) to produce frequency distributions of expected rates of herbivory based on the plant food available. Observed herbivory rates were then compared to those expected by chance. When observed herbivory rates fell outside of the central 95% of simulated

values, this indicated deviations from random herbivory. To account for repeated measures (multiple faecal samples collected from the same individual tortoise), the observed and modeled diet for each individual was averaged prior to combining all individuals to give population-level herbivory rates. This ensures that each individual has equal weight in the analysis. These analyses were carried out for the dry and wet seasons separately to detect seasonal variation in feeding preferences. Plant species that were not detected during the food availability surveys were excluded from these analyses alongside those plant species that were never detected in the diet.

5.3.6 Food webs

To visualize the interactions between floral resources and Aldabra giant tortoises, Pink Pigeons, and Telfair's skinks, bipartite food webs were created using the nullnetr package (Vaughan *et al.* 2017). Here, the results from the feeding preference tests (see 5.3.5 for tortoises, and Chapter 4 section 4.3.8 for Telfair's skinks and Pink Pigeons) were incorporated into the webs, so each interaction is stronger, weaker or as expected given the availability of a particular resource. Separate food availability values were provided for the Pink Pigeons since they have access to the understory and canopy in addition to the ground vegetation layer (see Chapter 4 section 4.3.8).

5.4 Results

5.4.1 Tortoise exclusion experiment

Overall, the change in the mean height of plants was significantly more positive in ungrazed plots in comparison to grazed plots (conditional R-Squared = 0.36, t = 2.511, P = <0.05) (Fig. 5.2a). There was no significant effect of habitat type (open vs. forested) or the interaction between treatment and habitat type.

There was a significant association between the change in the mean height of native plants and the interaction between treatment and habitat type (conditional R-squared = 0.54, t = 2.980, P = <0.01). Specifically, in open areas the mean height change of native plants was greater in ungrazed plots than in grazed plots. However, in forested areas the mean height change was relatively similar between grazed and ungrazed plots (Fig. 5.2b). For introduced plants, there was no significant association between treatment and habitat type. Once this term was removed from the model, there was a significant association between treatment and the change in mean height of introduced plants and no significant association between the latter and habitat type (conditional R-squared = 0.34). Specifically, the change in the mean height of introduced plants was significantly

more positive in those plots where tortoise grazing was excluded in comparison to the grazed plots in both forested and open areas (mean difference in height change $\pm$ SE = 5.386 $\pm$ 2.614, t = 2.06, P = <0.05) (Fig. 5.2c).

Treatment, habitat or their interaction did not significantly influence the change in all, native, or introduced plant abundance, species richness or Shannon diversity from 2015 to 2016.

The change in the mean height of *L. leucocephala* was significantly associated with the interaction between treatment and habitat type (conditional R-squared = 0.32, t = 2.495, P = <0.05). There was very little change in the height of *L. leucocephala* in both grazed and ungrazed plots in forested areas. In open areas however, there was a marked positive height change in ungrazed plots and very little change in height in the grazed plots (Fig. 5.3a). The change in abundance of *L. leucocephala* was greater in ungrazed plots in comparison to grazed plots in forested areas only, but this association was not significant (conditional R-squared = 0.48, t = 1.635, P = <0.1). The removal of the interaction between treatment and habitat type revealed that neither of these variables had a significant effect on the change in abundance of *L. leucocephala.*

There was no significant association between the change in the mean height of *P. suberosa* with neither habitat type or treatment, nor their interaction. However, in plots where tortoises were excluded, the change in the abundance of *P. suberosa* was significantly higher than in grazed plots in both forested and open areas (mean difference in height change $\pm$ SE = 1.7285 $\pm$ 0.7258, t = 2.234, P = <0.05) (Fig. 5.3b).

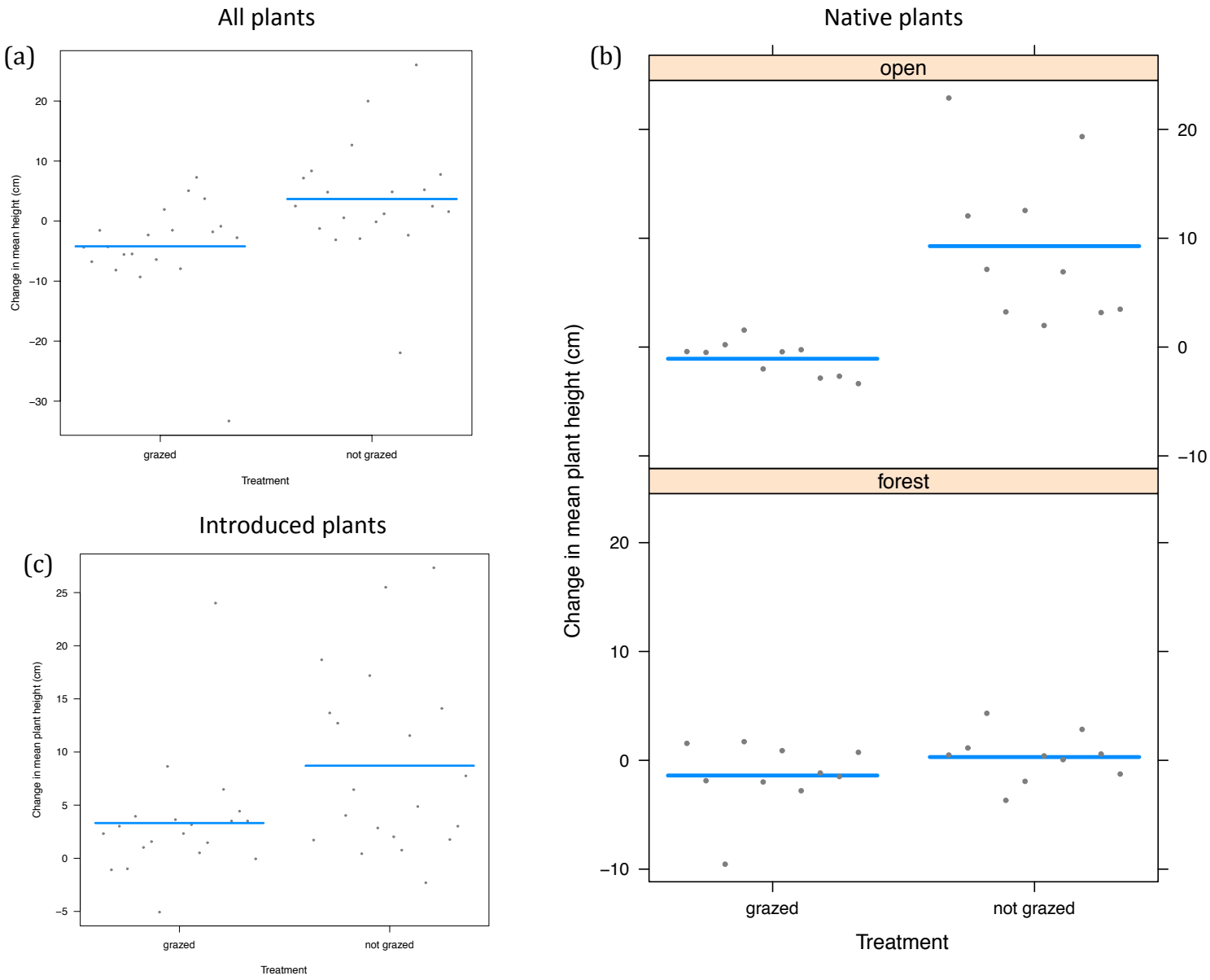


Figure 5.2 The effect of tortoise grazing on the change in mean plant height from 2015 to 2016. (a) All plants, (b) native plant species, and (c) introduced plant species. Blue lines represent predictions from generalised linear mixed models, raw data points are shown. Plots created in visreg (Breheny & Burchett 2013).

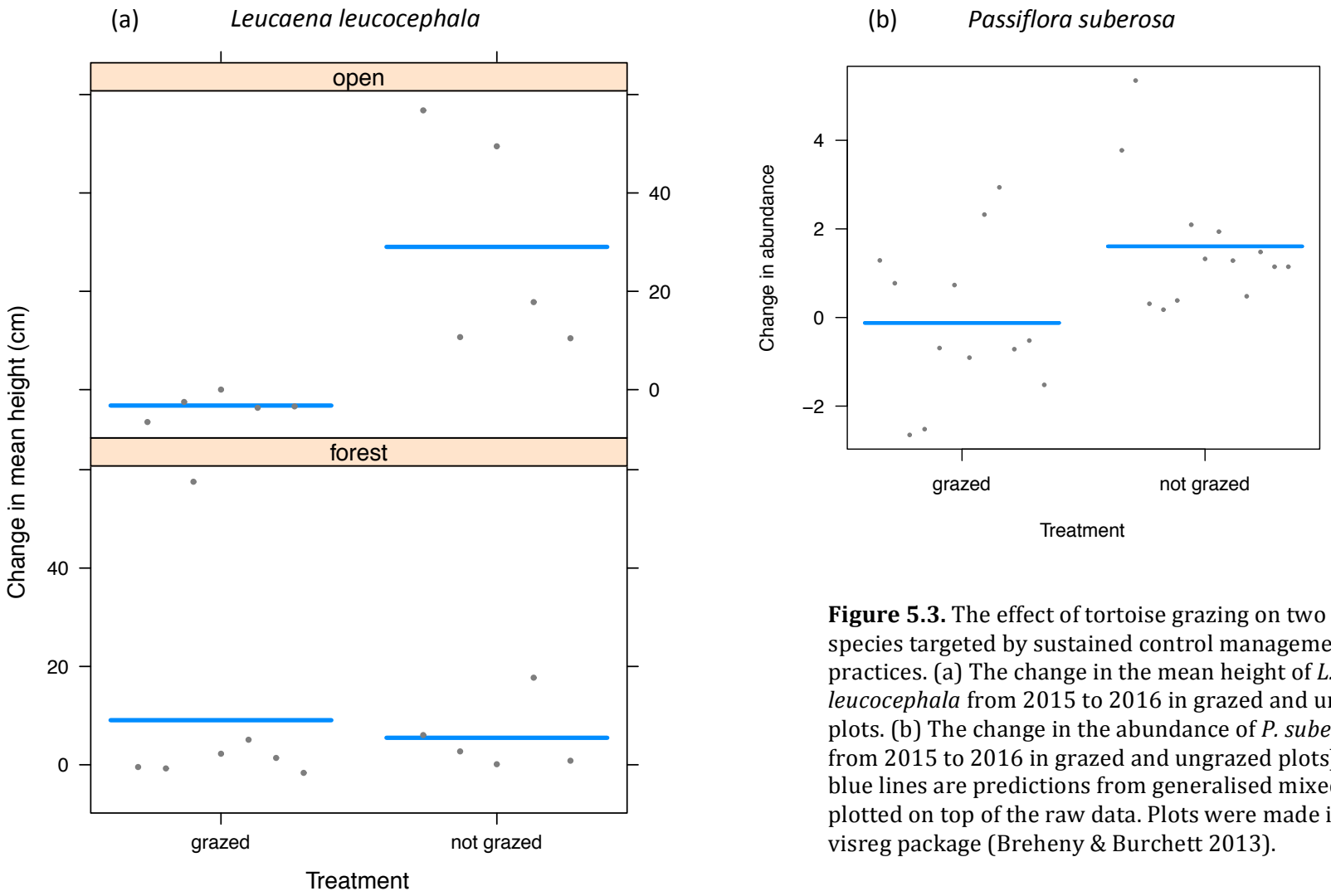


Figure 5.3. The effect of tortoise grazing on two weed species targeted by sustained control management practices. (a) The change in the mean height of *L. leucocephala* from 2015 to 2016 in grazed and ungrazed plots. (b) The change in the abundance of *P. suberosa* from 2015 to 2016 in grazed and ungrazed plots). The blue lines are predictions from generalised mixed models plotted on top of the raw data. Plots were made in the visreg package (Breheny & Burchett 2013).

5.4.2 Food availability

In the wet season, the most available plant food items for the tortoises were *Phymatodes scolopendria* (a native fern)*, Stachytarpheta jamaicensis, and L. leucocephala* (both invasive introduced species)*.* In the dry season, the most available food items are *P. scolopendria, Stenotaphrum dimidiatum* (a native grass), and *S. jamaicensis.* For a comprehensive breakdown of the plant food availability in each season, which also details the plant species never eaten by the tortoises, refer to Appendix 3.2. *P. scolopendria* was widely abundant on Ile aux Aigrettes but only seven samples (from three tortoises, two Telfair's skinks, two Pink Pigeons) tested positive for this species in the diet. The inclusion of this species in the analyses for feeding preferences strongly skews the results so that the majority of other plant species are preferred. Thus, this species was excluded from all econullnetr (Vaughan *et al.* 2017) analyses.

5.4.3 Tortoise faecal sample collection, labwork and bioinformatics

A total of 339 faecal samples were collected from tortoises. Of these, 197 were collected in the dry season (90 females, 95 males, and 12 hatchlings) and 142 in the wet season (59 females, 59 males and 24 hatchlings). Forty samples were discarded either because they were repeats (collected within two days of another sample from the same tortoise) or the ID of the tortoise was not known with certainty. Of these 299 samples, DNA was successfully extracted and sequenced from 88% (Table 5.1). For information regarding the number of reads in each Illumina miseq run, refer to Chapter Four section 4.4.

5.4.4 Tortoise dietary analysis

The most frequently occurring dietary items across all tortoise faecal samples were two creepers: *Ipomoea violacea* (native) and *P. suberosa* (introduced), occurring in 74.4% and 73.1% of samples respectively (n=264). Four tree species were also frequently eaten, *Hibiscus tiliaceus* (native), *Hilsenbergia petiolaris* (native), *L. leucocephala* (introduced), and *Ficus reflexa* (native) (occurring in 50.8%, 43.9%, 41.3% and 37.1% of samples respectively, n=264). Plant food items occurred in similar frequencies in both adult male and female faecal samples. Hatchling samples showed a slightly different composition with 100% of samples testing positive for *P. suberosa* and 77.8% contained *L. leucocephala* (n=18). There was also evidence of seasonal variation in the diet: for example *H. tiliaceus* and *L. leucocephala* were eaten more frequently in the wet season in

comparison to the dry season (34.4% and 36.8% difference respectively, n=264). A comprehensive breakdown of the diet of the Aldabra giant tortoise is found in Table 5.1.

There were 31 plant species that were recorded on Ile aux Aigrettes during plant availability surveys but were never detected in the faecal samples of the tortoises. Four of these species were detected in the diets of either Telfair's skinks or Pink Pigeons, meaning that there were 27 plant species recorded on the island but never detected in any faecal samples (Appendix 3.3).

The species richness of native plants eaten was significantly higher than that of introduced plant species ($V = 541.5$, $P = <0.001$, Fig 5.4).

In both the wet and dry seasons there was evidence of positive selection, random herbivory and avoidance of both native and introduced plant species (Fig. 5.5).

A list of the taxa removed from the Aldabra giant tortoise metabarcoding dietary dataset, and an explanation for removal, can be found in Appendix 3.4.

5.4.5 Food webs

Visualisation of the dietary preferences of the tortoises, Pink Pigeons and Telfair's Skinks in bipartite food webs revealed that there is considerable dietary overlap, in both the wet and dry seasons, and all three species appear to be generalists. Tortoises also consume the vast majority of those species preferred by the Pink Pigeons and Telfair's skinks (Fig. 5.6-7, Table 5.2).

Table 5.1 The percentage of Aldabra giant tortoise faecal samples from Ile aux Aigrettes testing positive for different plant species broken down by sex, age-class and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item						
Dietary item	Common name	Present in DNA barcode library	Status	All samples (n=264)	Adult female samples (n=100)	Adult male samples (n=146)	Adult samples (n=246)	Hatchling samples (n=18)	Dry season samples (n=152)	Wet season samples (n=112)
Ipomoea violacea	-	TRUE	native	74.2	72.0	76.7	74.8	66.7	69.7	80.4
Passiflora suberosa	Liane poc poc	TRUE	introduced	73.1	72.0	70.5	71.1	100.0	75.0	70.5
Hibiscus tiliaceus	Var, Vaur	TRUE	native	50.8	52.0	52.1	52.0	33.3	36.2	70.5
Hilsenbergia petiolaris	Bois de pipe	TRUE	native	43.9	45.0	40.4	42.3	66.7	45.4	42.0
Leucaena leucocephala	Acacia	TRUE	introduced	41.3	40.0	37.7	38.6	77.8	25.7	62.5
Ficus reflexa	La fouche bâtard, Affouche á petites feuilles	TRUE	native	37.1	48.0	32.2	38.6	16.7	36.8	37.5
Stenotaphrum dimidiatum	Herbe bourique	TRUE	native	32.2	33.0	34.9	34.1	5.6	21.7	46.4
Cyperus dubius	-	TRUE	native	29.9	28.0	28.1	28.0	55.6	18.4	45.5
Ficus rubra	Affouche rouge, La fouche, Affouche á grandes feuilles	TRUE	native	28.8	22.0	35.6	30.1	11.1	45.4	6.3
Premna serratifolia	Bois sureau	TRUE	native	25.4	23.0	26.7	25.2	27.8	27.0	23.2
Eragrostis amabilis	-	TRUE	native	25.0	22.0	25.3	24.0	38.9	17.1	35.7
Euphorbia hirta	Jean Robert	TRUE	introduced	23.9	20.0	24.7	22.8	38.9	7.9	45.5
Coptosperma borbonica	Bois de rat	TRUE	endemic	21.6	21.0	24.7	23.2	0.0	27.6	13.4
Thespesia populnea	Mahoe, Ste Marie, Porcher	TRUE	native	20.5	29.0	14.4	20.3	22.2	23.7	16.1
Scaevola taccada	Veloutier vert	TRUE	native	18.2	25.0	13.7	18.3	16.7	13.2	25.0
Turnera angustifolia	-	TRUE	introduced	15.9	19.0	14.4	16.3	11.1	14.5	17.9
Margaritaria anomala	Bois chenille	TRUE	endemic	14.0	16.0	13.7	14.6	5.6	18.4	8.0

Table 5.1 The percentage of Aldabra giant tortoise faecal samples from Ile aux Aigrettes testing positive for different plant species broken down by sex, age-class and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item						
Dietary item	Common name	Present in DNA barcode library	Status	All samples (n=264)	Adult female samples (n=100)	Adult male samples (n=146)	Adult samples (n=246)	Hatchling samples (n=18)	Dry season samples (n=152)	Wet season samples (n=112)
Eugenia lucida	Bois clou, Bois de clous	TRUE	endemic	12.5	10.0	13.0	11.8	22.2	12.5	12.5
Morinda citrifolia	Bois tortue	TRUE	introduced	12.5	14.0	13.0	13.4	0.0	9.9	16.1
Asystasia gangetica	Herbe á pistache	TRUE	introduced	11.0	12.0	8.9	10.2	22.2	3.9	20.5
Asparagus setaceus	Liane asperge	TRUE	introduced	10.6	11.0	9.6	10.2	16.7	11.8	8.9
Solanum americanum	Brède martin	TRUE	introduced	9.8	9.0	10.3	9.8	11.1	9.9	9.8
Santalum album	Bois de santal	TRUE	introduced	9.8	12.0	6.8	8.9	22.2	15.1	2.7
Dodonaea viscosa	Bois de reinette	TRUE	native	9.5	12.0	7.5	9.3	11.1	10.5	8.0
Stachytarpheta jamaicensis	-	TRUE	introduced	8.0	8.0	8.2	8.1	5.6	5.3	11.6
Polyscias maraisiana	Bois de Boeuf, Bois d'éponge	TRUE	endemic	7.2	3.0	10.3	7.3	5.6	6.6	8.0
Acalypha indica	Herbe chatte	TRUE	introduced	6.8	7.0	6.2	6.5	11.1	2.0	13.4
Ipomoea obscura	Amourette	TRUE	introduced	6.1	7.0	5.5	6.1	5.6	5.3	7.1
Pithecellobium dulce	Cassie de Manille	TRUE	introduced	4.9	6.0	4.1	4.9	5.6	6.6	2.7
Phyllanthus casticum	Bois castique, Castique, Bois de demoiselle	TRUE	native	4.5	4.0	4.8	4.5	5.6	3.3	6.3
Digitaria horizontalis	Gros Meinki	TRUE	introduced	4.2	1.0	6.8	4.5	0.0	0.7	8.9
Clerodendrum heterophyllum	Bois cabris	TRUE	endemic	4.2	8.0	1.4	4.1	5.6	5.3	2.7
Poupartia borbonica	Bois Poupart	TRUE	endemic	4.2	5.0	4.1	4.5	0.0	2.0	7.1
Tylophora coriacea	Ipéca du Pays	TRUE	native	3.0	6.0	1.4	3.3	0.0	5.3	0.0
Asparagus umbellatus	Asperge sauvage	TRUE	native	3.0	3.0	1.4	2.0	16.7	3.3	2.7

Table 5.1 The percentage of Aldabra giant tortoise faecal samples from Ile aux Aigrettes testing positive for different plant species broken down by sex, age-class and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item						
Dietary item	Common name	Present in DNA barcode library	Status	All samples (n=264)	Adult female samples (n=100)	Adult male samples (n=146)	Adult samples (n=246)	Hatchling samples (n=18)	Dry season samples (n=152)	Wet season samples (n=112)
Phyllanthus mauritianus	-	TRUE	native	3.0	1.0	4.1	2.8	5.6	0.7	6.3
Bothriochloa pertusa	-	TRUE	introduced	2.7	1.0	4.1	2.8	0.0	0.0	6.3
Wikstroemia indica	Herbe tourterelle	TRUE	introduced	2.7	2.0	1.4	1.6	16.7	1.3	4.5
Plantago sp.	-	FALSE	unknown	2.3	3.0	2.1	2.4	0.0	2.6	1.8
Cenchrus echinatus	Herbe á cateaux	TRUE	introduced	1.9	2.0	2.1	2.0	0.0	3.3	0.0
Abutilon indicum	Mauve du pays	TRUE	introduced	1.5	2.0	1.4	1.6	0.0	2.0	0.9
Euphorbia thymifolia	Petite rougette	TRUE	cryptogenic	1.5	1.0	2.1	1.6	0.0	0.7	2.7
Phyllanthus amarus	Petit tamarin blanc	TRUE	introduced	1.5	1.0	1.4	1.2	5.6	0.7	2.7
Maytenus pyria	Bois à poudre	TRUE	introduced	1.5	0.0	2.1	1.2	5.6	2.0	0.9
Agrostis sp.	-	FALSE	unknown	1.5	2.0	1.4	1.6	0.0	2.0	0.9
Cassine orientalis	Bois d'olive	TRUE	endemic	1.5	2.0	1.4	1.6	0.0	2.6	0.0
Pteris vittata	Ptéris rubané (Reunion Island)	TRUE	native	1.5	0.0	2.7	1.6	0.0	2.6	0.0
Tridax procumbens	Herbe Caille	TRUE	introduced	1.1	0.0	2.1	1.2	0.0	0.7	1.8
Chloris barbata	-	TRUE	introduced	1.1	1.0	1.4	1.2	0.0	1.3	0.9
Desmanthus virgatus	Petit acacia	TRUE	introduced	1.1	2.0	0.7	1.2	0.0	0.0	2.7
Phymatodes scolopendria	Fougère polypode	TRUE	native	1.1	1.0	1.4	1.2	0.0	1.3	0.9
Triticum sp.	wheat	FALSE	sup. feed*	1.1	2.0	0.7	1.2	0.0	1.3	0.9
Cynanchum staubii	Liane calle	TRUE	endemic	1.1	2.0	0.7	1.2	0.0	1.3	0.9

Table 5.1 The percentage of Aldabra giant tortoise faecal samples from Ile aux Aigrettes testing positive for different plant species broken down by sex, age-class and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item						
Dietary item	Common name	Present in DNA barcode library	Status	All samples (n=264)	Adult female samples (n=100)	Adult male samples (n=146)	Adult samples (n=246)	Hatchling samples (n=18)	Dry season samples (n=152)	Wet season samples (n=112)
Diospyros tesselaria	Bois d'ébène noir, ébenier	TRUE	endemic	1.1	1.0	1.4	1.2	0.0	0.0	2.7
Eleusine indica	-	TRUE	introduced	1.1	1.0	1.4	1.2	0.0	0.0	2.7
Gouania tiliifolia	Liane charretier	TRUE	native	1.1	2.0	0.7	1.2	0.0	1.3	0.9
Heteropogon contortus	Herbe polisson	TRUE	introduced	1.1	0.0	2.1	1.2	0.0	0.0	2.7
Latania loddigesii	Latanier bleu, Latanier de Maurice, Latanier de l'Ile Ronde	TRUE	endemic	1.1	2.0	0.7	1.2	0.0	2.0	0.0
Poa trivialis	Rough meadow grass	FALSE	introduced	1.1	2.0	0.7	1.2	0.0	0.7	1.8
Stenotaphrum micranthum	-	TRUE	native	1.1	2.0	0.7	1.2	0.0	1.3	0.9
Terminalia bentzoe	Bois benjoin	TRUE	endemic	1.1	1.0	1.4	1.2	0.0	2.0	0.0
Turraea thouarsiana	Bois quivi	TRUE	endemic	1.1	1.0	1.4	1.2	0.0	1.3	0.9
Amaranthus viridis	-	TRUE	introduced	0.8	1.0	0.0	0.4	5.6	1.3	0.0
Caesalpinia bonduc	Cadoque, Cadoc, Bonduc	TRUE	native	0.8	2.0	0.0	0.8	0.0	1.3	0.0
Cordia curassavica	Herbe Condé	TRUE	introduced	0.8	0.0	1.4	0.8	0.0	0.0	1.8
Flacourtia indica	Prune malgache	TRUE	introduced	0.8	0.0	0.7	0.4	5.6	0.7	0.9
Rivina humilis	Petite groseille	TRUE	introduced	0.8	1.0	0.7	0.8	0.0	1.3	0.0
Tabebuia pallida	Técoma	TRUE	introduced	0.8	1.0	0.0	0.4	5.6	0.7	0.9
Achyranthes aspera	-	TRUE	introduced	0.4	1.0	0.0	0.4	0.0	0.0	0.9
Gagnebina pterocarpa	Acacia indigene	TRUE	native	0.4	0.0	0.7	0.4	0.0	0.7	0.0
Portulaca oleracea	Pourpier rouge, Pourpier	TRUE	introduced	0.4	1.0	0.0	0.4	0.0	0.0	0.9

Table 5.1 The percentage of Aldabra giant tortoise faecal samples from Ile aux Aigrettes testing positive for different plant species broken down by sex, age-class and season. The status of each dietary item and presence in the bespoke plant DNA barcode library (details in Chapter 2) are also indicated.

				Percentage of samples testing positive for a dietary item						
Dietary item	Common name	Present in DNA barcode library	Status	All samples (n=264)	Adult female samples (n=100)	Adult male samples (n=146)	Adult samples (n=246)	Hatchling samples (n=18)	Dry season samples (n=152)	Wet season samples (n=112)
Anthoxanthum sp.	-	FALSE	introduced	0.4	0.0	0.0	0.0	5.6	0.0	0.9
Bryophyllum pinnatum	Soudefafe	TRUE	introduced	0.4	1.0	0.0	0.4	0.0	0.0	0.9
Diospyros egrettarum	Bois d'ébène lie aux Aigrettes	TRUE	endemic	0.4	0.0	0.7	0.4	0.0	0.0	0.9
Euphorbia heterophylla	-	TRUE	introduced	0.4	1.0	0.0	0.4	0.0	0.0	0.9
Fimbristylis sp.	-	TRUE	native	0.4	0.0	0.7	0.4	0.0	0.0	0.9
Saccharum sp	Sugar Cane	TRUE	introduced	0.4	0.0	0.7	0.4	0.0	0.0	0.9
Lantana camara	Vieille fille	TRUE	introduced	0.4	0.0	0.7	0.4	0.0	0.0	0.9
Lobelia cliffortiana	Brède mamzelle	TRUE	introduced	0.4	1.0	0.0	0.4	0.0	0.0	0.9
Ludia mauritiana	Bois mozambique	TRUE	endemic	0.4	1.0	0.0	0.4	0.0	0.7	0.0
Millettia pinnata	Pongame, Coqueluche	TRUE	introduced	0.4	1.0	0.0	0.4	0.0	0.7	0.0
Phyllanthus niruroides	Petite castique, Curanellie blanche	TRUE	introduced	0.4	0.0	0.0	0.0	5.6	0.7	0.0
Protium obtusifolium	Colophane bâtard	TRUE	endemic	0.4	0.0	0.7	0.4	0.0	0.7	0.0
Rhynchosia viscosa	Liane lastic	TRUE	introduced	0.4	1.0	0.0	0.4	0.0	0.0	0.9
Triphasia trifolia	Orangine	TRUE	introduced	0.4	1.0	0.0	0.4	0.0	0.7	0.0
Turnera orientalis	-	FALSE	introduced	0.4	1.0	0.0	0.4	0.0	0.7	0.0
Zea mays	Maize	FALSE	sup. feed*	0.4	0.0	0.7	0.4	0.0	0.0	0.9

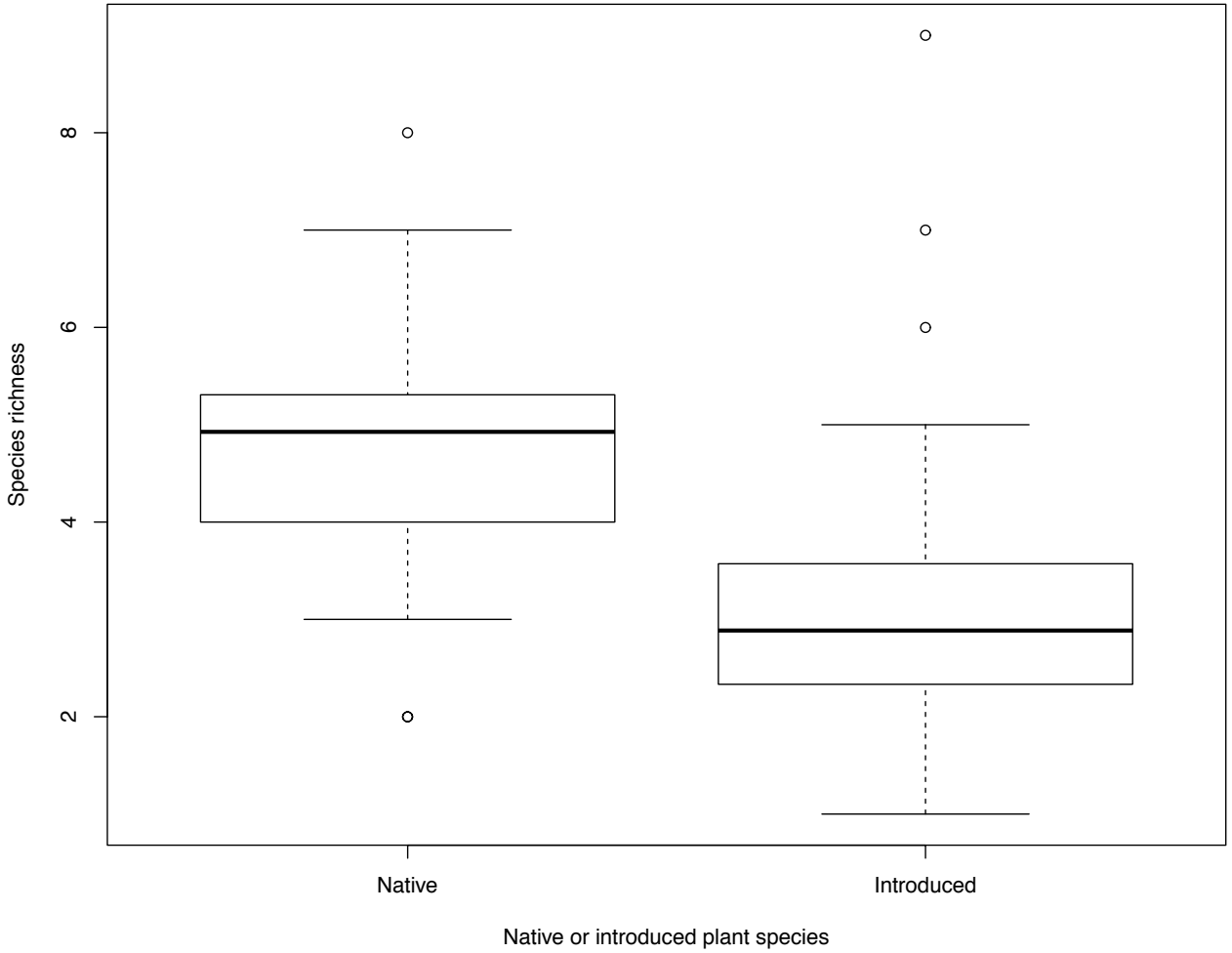
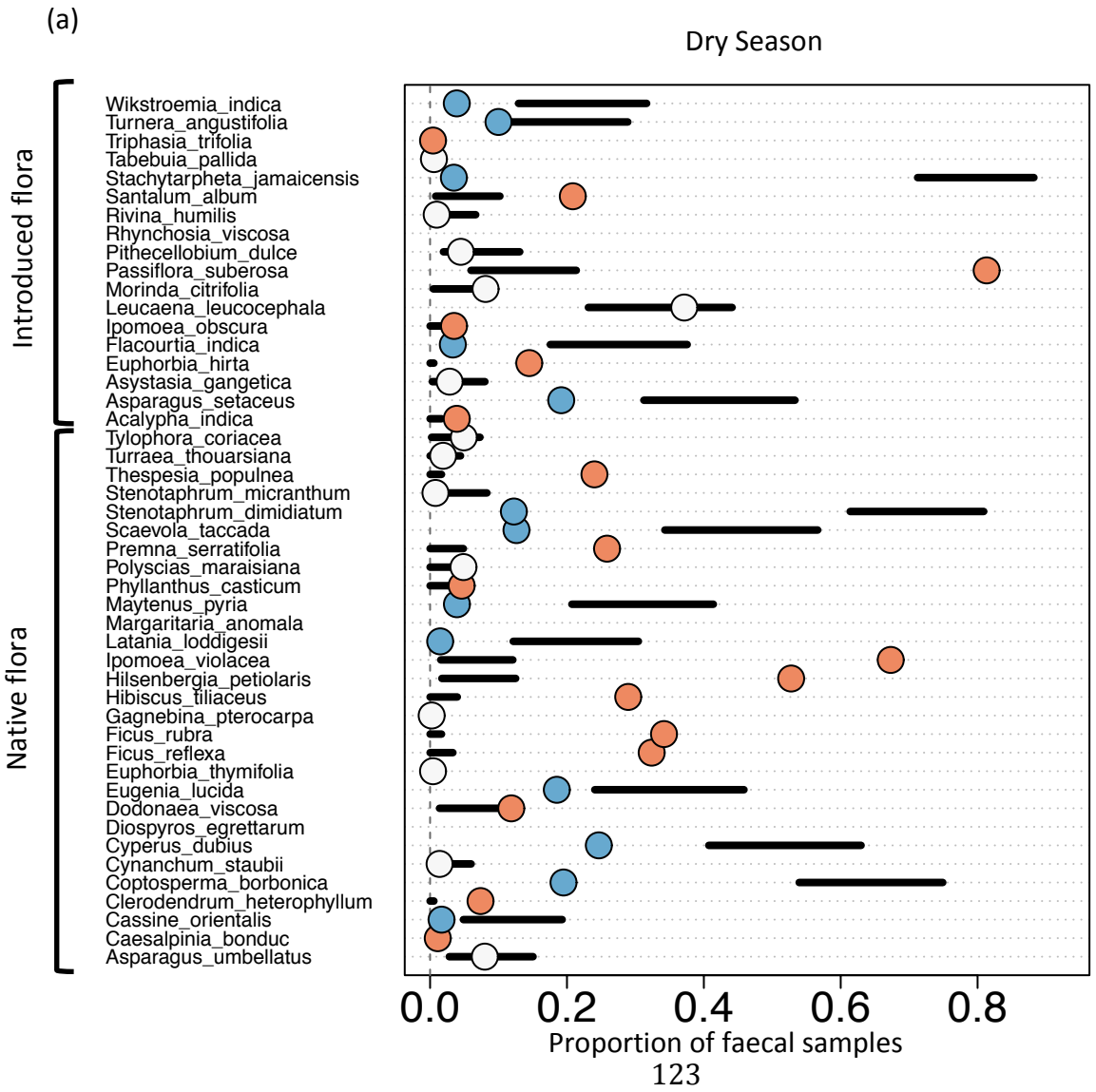


Figure 5.4. Box and whisker plot showing the difference in species richness of native and introduced plant species in tortoise diet. Wilcoxon signed rank test: V = 541.5, P = 3.049e-05.


(a)
Dry Season
Introduced flora
Native flora
Wikstroemia_indica
Turnera_angustifolia
Triphasia_trifolia
Tabebuia_pallida
Stachytarpheta_jamaicensis
Santalum_album
Rivina_humilis
Rhynchosia_viscosa
Pithecellobium_dulce
Passiflora_suberosa
Morinda_citrifolia
Leucaena_leucocephala
Ipomoea_obscura
Flacourtia_indica
Euphorbia_hirta
Asystasia_gangetica
Asparagus_setaceus
Acalypha_indica
Tylophora_coriacea
Turraea_thouarsiana
Thespesia_populnea
Stenotaphrum_micranthum
Stenotaphrum_dimidiatum
Scaevola_taccada
Premna_serratifolia
Polyscias_maraisiana
Phyllanthus_casticum
Maytenus_pyria
Margaritaria_anomala
Latania_loddigesii
Ipomoea_violacea
Hilsenbergia_petiolaris
Hibiscus_tiliaceus
Gagnebina_pterocarpa
Ficus_rubra
Ficus_reflexa
Euphorbia_thymifolia
Eugenia_lucida
Dodonaea_viscosa
Diospyros_egrettarum
Cyperus_dubius
Cynanchum_staubii
Coptosperma_borbonica
Clerodendrum_heterophyllum
Cassine_orientalis
Caesalpinia_bonduc
Asparagus_umbellatus
0.0
0.2
0.4
0.6
0.8
Proportion of faecal samples

123

(b)

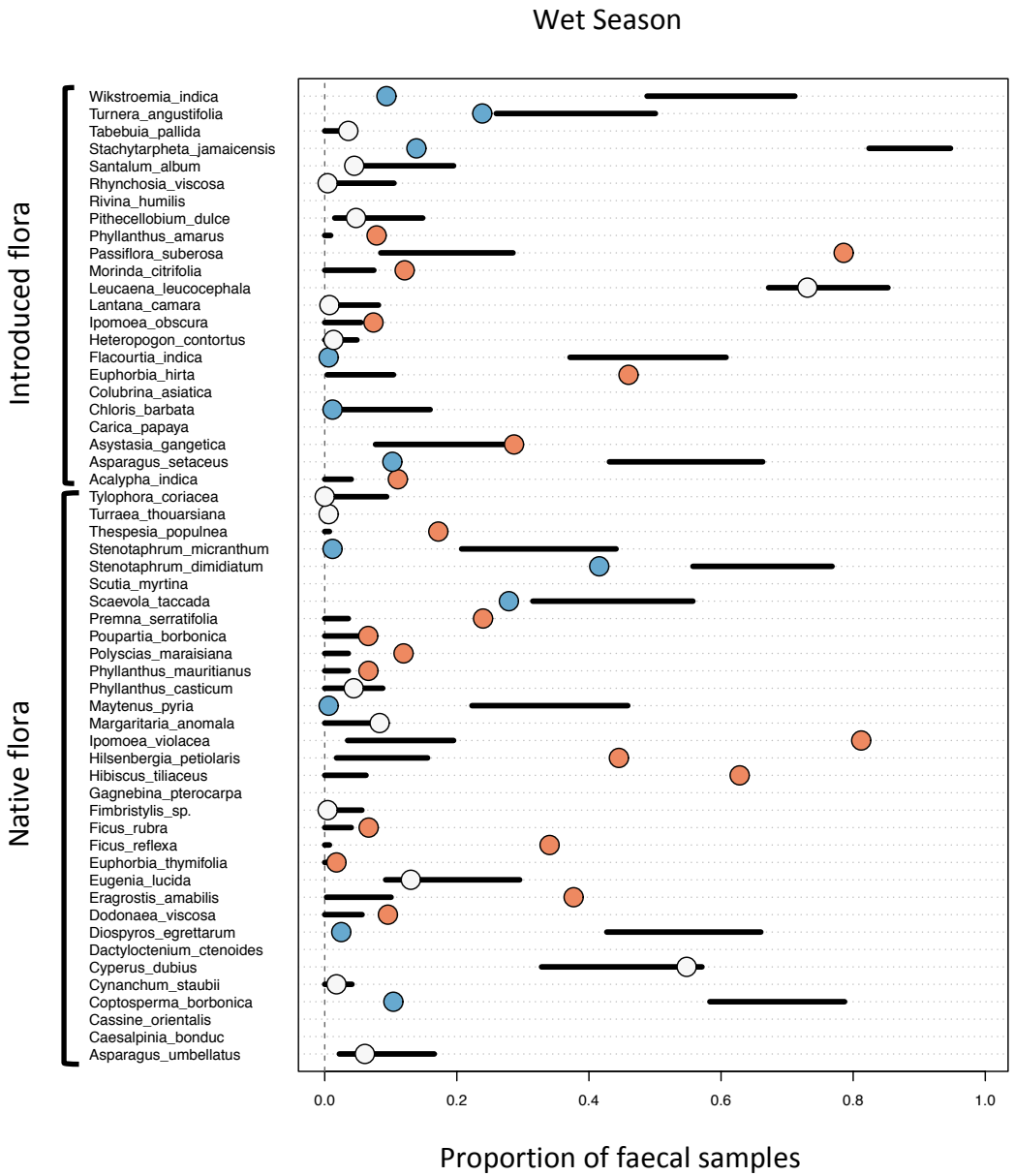


Figure 5.5. Dietary preferences of Aldabra giant tortoises on Ile aux Aigrettes in both the (a) dry season, and (b) wet season. Thick black lines: predictions from the null model with 95% confidence limits; white circles: plant species eaten in proportion to their availability; blue circles: species eaten in lower proportions than expected; orange circles: species eaten at a greater proportion than expected. Plant species that are absent in tortoise diet but present in the diet of either Telfair's skinks or Pink Pigeons are listed but their predictions are absent.

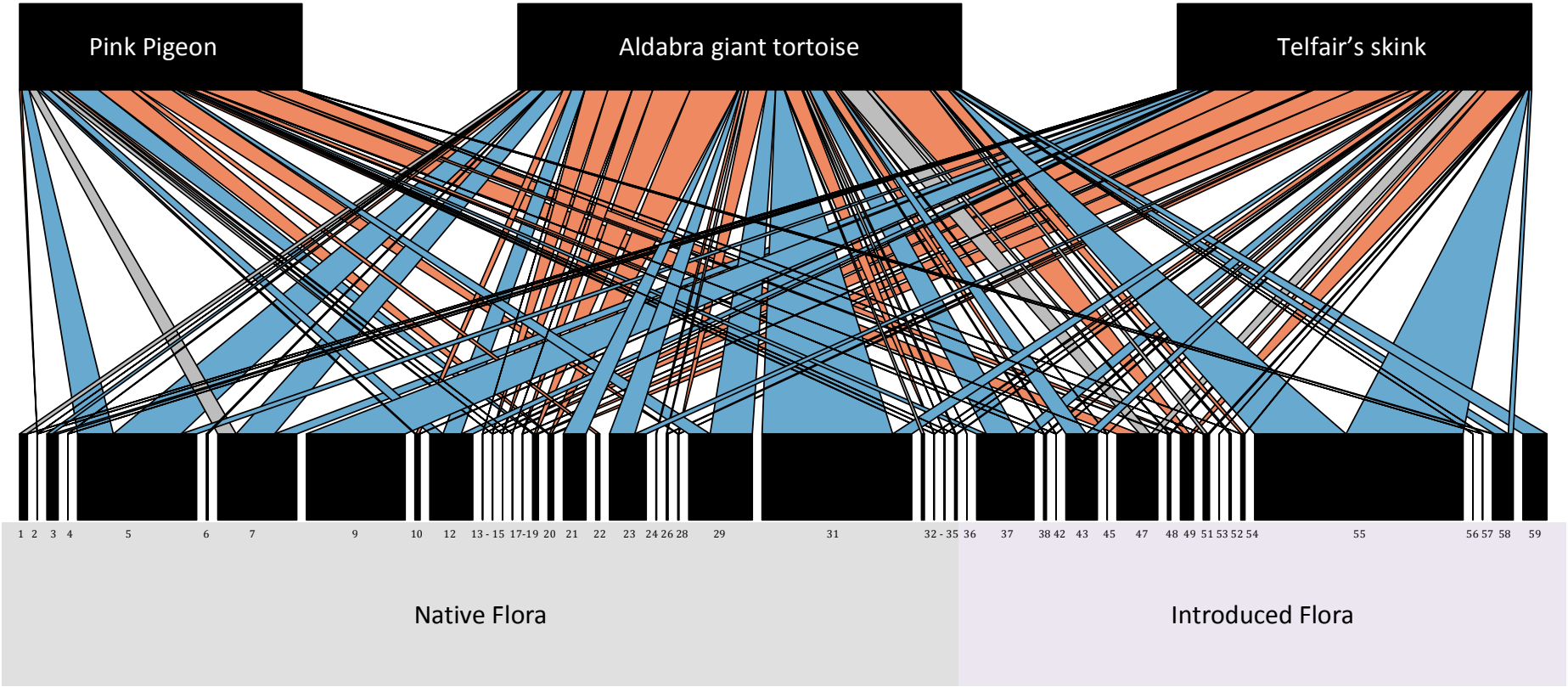


Figure 5.6. Bipartite food web illustrating the interactions, and their strength, between the plant community and Aldabra giant tortoises, Pink Pigeons and Telfair's skinks in the **dry season**. Grey, blue and orange connections illustrate those interactions that occur at equal, lower and higher proportions, respectably, than expected given the availability of the plant dietary items. The upper width of connections is proportional to the number of individuals having consumed a particular plant. Width of the lower boxes is proportional to the availability of a particular plant taxon at the ground level. Exceptions to this are when only Pink Pigeons were found to eat a particular plant, in this case the food availability is the sum of what is available at the ground, understory and canopy levels (Chapter 3). Width of the upper boxes represent the sample sizes of Telfair's skinks, Pink Pigeons and Aldabra giant tortoises. Numbering = *1: Asparagus umbellatus, 2: Caesalpinia bonduc, 3: Cassine orientalis, 4: Clerodendrum heterophyllum, 5: Coptosperma borbonica, 6: Cynanchum staubii, 7: Cyperus dubius, 9: Diospyros egrettarum, 10: Dodonaea viscosa, 12: Eugenia lucida, 13: Euphorbia thymifolia, 14: Ficus reflexa, 15: Ficus rubra, 17: Gagnebina pterocarpa, 18: Hibiscus tiliaceus, 19: Hilsenbergia petiolaris, 20: Ipomoea violacea, 21: Latania loddigesii, 22: Margaritaria anomala, 23: Maytenus pyria, 24: Phyllanthus casticum, 26: Polyscias maraisiana, 28: Premna serratifolia, 29: Scaevola taccada, 31: Stenotaphrum dimidiatum, 32: Stenotaphrum micranthum, 33: Thespesia populnea, 34: Turraea thouarsiana, 35: Tylophora coriacea, 36: Acalypha indica, 37: Asparagus setaceus, 38: Asystasia gangetica, 42: Euphorbia hirta, 43: Flacourtia indica, 45: Ipomoea obscura, 47: Leucaena leucocephala, 48: Morinda citrifolia, 49: Passiflora suberosa, 51: Pithecellobium dulce, 53: Rhynchosia viscosa, 52: Rivina humilis, 54: Santalum album, 55: Stachytarpheta jamaicensis, 56: Tabebuia pallida, 57: Triphasia trifolia, 58: Turnera angustifolia, 59: Wikstroemia indica. Phymatodes scolopendria* has been excluded from this analysis.

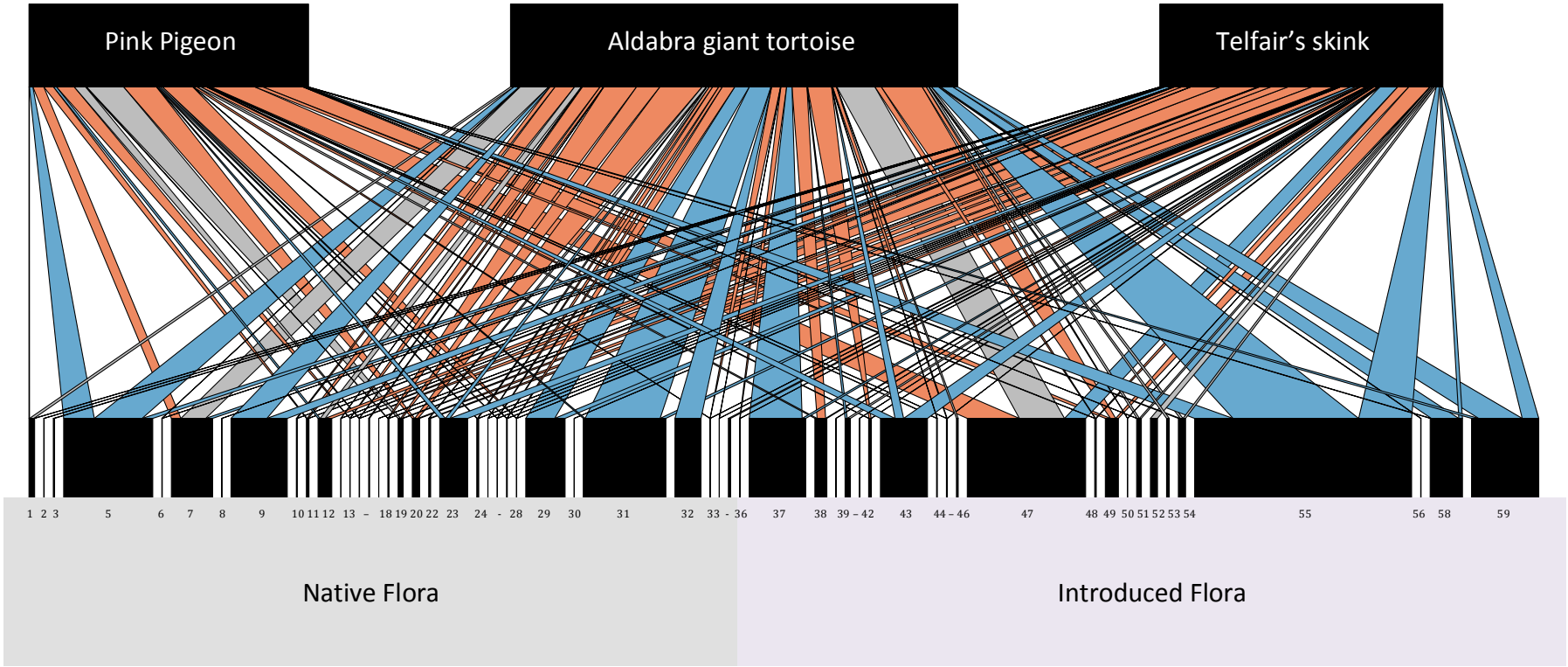


Figure 5.7. Bipartite food web illustrating the interactions, and their strength, between the plant community and Aldabra giant tortoises, Pink Pigeons and Telfair's skinks in the **wet season**. Grey, blue and orange connections illustrate those interactions that occur at equal, lower and higher proportions, respectably, than expected given the availability of the plant dietary items. The upper width of connections is proportional to the number of individuals having consumed a particular plant. Width of the lower boxes is proportional to the availability of a particular plant taxon at the ground level. Exceptions to this are when only Pink Pigeons were found to eat a particular plant, in this case the food availability is the sum of what is available at the ground, understory and canopy levels (Chapter 3). Width of the upper boxes represent the sample sizes of Telfair's skinks, Pink Pigeons and Aldabra giant tortoises. Numbering = *1: Asparagus umbellatus, 2: Caesalpinia bonduc, 3: Cassine orientalis, 5: Coptosperma borbonica, 6: Cynanchum staubii, 7: Cyperus dubius, 8: Dactyloctenium ctenoides, 9: Diospyros egrettarum, 10: Dodonaea viscosa, 11: Eragrostis amabilis, 12: Eugenia lucida, 13: Euphorbia thymifolia, 14: Ficus reflexa, 15: Ficus rubra, 16: Fimbristylis sp., 17: Gagnebina pterocarpa, 18: Hibiscus tiliaceus, 19: Hilsenbergia petiolaris, 20: Ipomoea violacea, 22: Margaritaria anomala, 23: Maytenus pyria, 24: Phyllanthus casticum, 25: Phyllanthus mauritianus, 26: Polyscias maraisiana, 27: Poupartia borbonica, 28: Premna serratifolia, 29: Scaevola taccada, 30: Scutia myrtina, 31: Stenotaphrum dimidiatum, 32: Stenotaphrum micranthum, 33: Thespesia populnea, 34: Turraea thouarsiana, 35: Tylophora coriacea, 36: Acalypha indica, 37: Asparagus setaceus, 38: Asystasia gangetica, 39: Carica papaya, 40: Chloris barbata, 41: Colubrina asiatica, 42: Euphorbia hirta, 43: Flacourtia indica, 44: Heteropogon contortus, 45: Ipomoea obscura, 46: Lantana camara, 47: Leucaena leucocephala, 48: Morinda citrifolia, 49: Passiflora suberosa, 50: Phyllanthus amarus, 51: Pithecellobium dulce, 52: Rivina humilis, 53: Rhynchosia viscosa, 54: Santalum album, 55: Stachytarpheta jamaicensis, 56: Tabebuia pallida, 58: Turnera angustifolia, 59: Wikstroemia indica. Phymatodes scolopendria* has been excluded from this analysis.

Table 5.2. Summary of the interactions, and their strength, between the plant community and Aldabra giant tortoises, Pink Pigeons and Telfair's skinks in both the dry and the wet season. Values are standardised effect sizes. Grey, blue and orange cells illustrate those interactions that occur at equal, lower and higher proportions, respectably, than expected given the availability of the plant dietary items. Dashes are those species not detected in the diet.

		Dry Season			Wet Season		
Resource	Status	Pink Pigeon	Aldabra Giant Tortoise	Telfair's Skink	Pink Pigeon	Aldabra Giant Tortoise	Telfair's Skink
Acalypha indica	introduced	-	7.665113813	-	-	9.266755512	3.880603384
Asparagus setaceus	introduced	-2.270177216	-4.075384445	-4.591789206	-	-7.522082735	-9.865945975
Asparagus umbellatus	native	-	0.039303824	-1.761584277	-0.910193338	-0.572713813	-2.105193019
Asystasia gangetica	introduced	-0.139278746	-0.037341231	2.048756431	1.711873132	2.431577	-2.113604091
Caesalpinia bonduc	native	28.17296803	5.878998979	10.50095192	-	-	1.805399419
Carica papaya	introduced	-	-	-	-	-	4.974515691
Cassine orientalis	native	-	-2.4999569	-1.978191574	-	-	-0.411899368
Chloris barbata	introduced	-	-	-	-	-1.774276592	-1.561744595
Colubrina asiatica	introduced	-	-	-	-	-	4.448144793
Clerodendrum heterophyllum	native	-	24.44437896	13.13713193	-	-	-
Coptosperma borbonica	native	-13.55228816	-8.341368096	-9.159544874	-15.345685	-11.24733637	-12.75946859
Cynanchum staubii	native	-	-0.303245104	-	-	0.94238823	7.939332215
Cyperus dubius	native	-0.773998698	-4.807712915	-5.24095064	2.207608606	1.563198711	-3.721524564
Dactyloctenium ctenoides	native	-	-	-	-	-	1.674837558
Diospyros egrettarum	native	-	-	-7.554394495	-	-8.742378978	-9.538594193
Dodonaea viscosa	native	-5.243986698	2.398549829	-0.25693847	-	4.817768538	0.572280527
Eragrostis amabilis	native	-	-	-	14.97399111	12.85747865	10.3737884
Eugenia lucida	native	-7.562792958	-2.882890229	-3.176801467	-7.416087296	-1.071189965	2.96352636
Euphorbia hirta	introduced	70.4404506	45.90824663	35.53968332	8.106703114	15.46756801	4.431072893
Euphorbia thymifolia	native*	-	0.457546145	3.495453899	-	5.131713705	2.300582836

Table 5.2. Summary of the interactions, and their strength, between the plant community and Aldabra giant tortoises, Pink Pigeons and Telfair's skinks in both the dry and the wet season. Values are standardised effect sizes. Grey, blue and orange cells illustrate those interactions that occur at equal, lower and higher proportions, respectably, than expected given the availability of the plant dietary items. Dashes are those species not detected in the diet.

		Dry Season			Wet Season		
Resource	Status	Pink Pigeon	Aldabra Giant Tortoise	Telfair's Skink	Pink Pigeon	Aldabra Giant Tortoise	Telfair's Skink
Ficus reflexa	native	-1.632305841	45.0703421	83.25171504	5.013749717	99.35479969	391.8273722
Ficus rubra	native	-1.520934536	65.44633564	161.4674003	0.963394503	5.129860632	152.295338
Fimbristylis sp.	native	-	-	-	-	-0.59901083	-
Flacourtia indica	introduced	-	-4.623699446	-4.080207316	-3.364278876	-7.989393918	-8.537914346
Gagnebina pterocarpa	native	27.0492636	0.472075752	-	-	-	15.39818023
Heteropogon contortus	introduced	-	-	-	4.167922351	0.154444117	-
Hibiscus tiliaceus	native	-	26.04879522	3.994975995	-3.762787543	33.44644252	27.19809527
Hilsenbergia petiolaris	native	-2.537627963	16.41041045	19.70240452	1.916584827	10.23459577	18.825265
Ipomoea obscura	introduced	0.711823486	5.425222172	9.183411326	4.678795384	3.628555839	2.791642477
Ipomoea violacea	native	18.91432758	22.07744658	25.23356685	13.88013402	16.90245299	22.0740692
Lantana camara	introduced	-	-	-	-	-0.867031078	-
Latania loddigesii	native	-	-4.063480315	-	-	-	-
Leucaena leucocephala	introduced	11.62990987	0.736131921	-0.816652622	5.806478168	-0.821611104	-6.350994039
Margaritaria anomala	native	7.080095507	-	-	-	2.368180111	42.91045613
Maytenus pyria	native	-	-5.020367162	-4.53981507	-5.869358834	-5.488277453	-5.887257275
Morinda citrifolia	introduced	3.732908456	2.149207348	3.942328224	10.06754446	4.799986769	22.57109641
Passiflora suberosa	introduced	10.2315453	17.22606803	10.65886495	14.01450992	11.7334966	13.35933748
Phyllanthus amarus	introduced	-	-	-	-	19.68541087	4.157875338
Phyllanthus casticum	native	-	3.77836556	-	1.503979659	0.624638011	1.282280799
Phyllanthus mauritianus	native	-	-	-	-	9.146303063	1.954911043

Table 5.2. Summary of the interactions, and their strength, between the plant community and Aldabra giant tortoises, Pink Pigeons and Telfair's skinks in both the dry and the wet season. Values are standardised effect sizes. Grey, blue and orange cells illustrate those interactions that occur at equal, lower and higher proportions, respectably, than expected given the availability of the plant dietary items. Dashes are those species not detected in the diet.

		Dry Season			Wet Season		
Resource	Status	Pink Pigeon	Aldabra Giant Tortoise	Telfair's Skink	Pink Pigeon	Aldabra Giant Tortoise	Telfair's Skink
Pithecellobium dulce	introduced	-0.640347657	-0.65391411	-1.184659549	-0.541644771	-0.624780318	0.101547534
Polyscias maraisiana	native	-	1.77216397	5.436323896	-2.990590969	11.47525766	11.44424327
Poupartia borbonica	native	-	-	-	2.818647925	3.625908783	-0.440575088
Premna serratifolia	native	9.309296767	18.11013829	10.5698254	12.2677246	25.2099123	50.4272952
Rhynchosia viscosa	introduced	-	-	3.623689154	-	-1.325932091	-
Rivina humilis	introduced	-	-0.697547475	-	-	-	-1.374063648
Santalum album	introduced	0.847122575	6.685565377	-0.287787103	-0.586640586	-1.406262329	-0.815759883
Scaevola taccada	native	-4.76100756	-5.76334065	-	-	-2.540509393	-4.965120805
Scutia myrtina	native	-	-	-	-	-	38.00924504
Stachytarpheta jamaicensis	introduced	-	-17.62674403	-13.57803343	-5.871077287	-23.55804732	-21.93774428
Stenotaphrum dimidiatum	native	-	-11.7847154	-11.3667405	-3.394763628	-4.70316168	-11.85076975
Stenotaphrum micranthum	native	-	-1.075908731	-	-	-5.123977931	-
Tabebuia pallida	introduced	-	1.050505437	-	-	2.846381257	-
Thespesia populnea	native	15.30150381	47.63093002	30.85875001	5.117087056	49.88620279	75.59216757
Triphasia trifolia	introduced	16.64144688	2.881028879	-	-	-	-
Turnera angustifolia	introduced	-1.157200496	-2.0415116	-2.712578844	-2.012680038	-2.271519553	-6.168993593
Turraea thouarsiana	native	0.855342929	0.648317896	0.835934079	-	0.852392375	6.19252943
Tylophora coriacea	native	-0.156098333	1.25332361	1.414600559	-	-1.346219087	0.973251366
Wikstroemia indica	introduced	-	-3.703714041	-	-3.648411919	-8.949351601	-10.1629836

*Species is cryptogenic but for the purpose of analyses it is classified as native

5.5 Discussion

In this study DNA metabarcoding was used in conjunction with ecological data to assess how grazing by Aldabra giant tortoises, introduced as ecological analogues, are affecting the plant community and the knock-on effects that this has across a major component of the food web. This is the first time that DNA metabarcoding has been used to assess the impact of an analogue species.

5.5.1 The diet of the Aldabra giant tortoise on Ile aux Aigrettes

Aldabra giant tortoises, introduced as ecological analogues, consume and prefer both native and introduced plants across the seasons. The hypothesis that the tortoises primarily eat alien plants is clearly rejected. Indeed, species richness of native plants in tortoise diet was higher than that of introduced plants. This is in contrast to earlier work where feeding observations and identification of plant dietary items from tortoise faeces on Round Island indicated that 81% of the diet was composed of non-native vegetation (Griffiths *et al.* 2013). In that study, the grazing witnessed on native species was largely thought to be tortoises testing novel food items in a new environment. However, this argument cannot apply to the present study since the tortoises have been free-roaming on Ile aux Aigrettes for several years. Moreover, the differences between the two studies are more likely to be due to the different techniques used to gather dietary information in addition to the differing plant community compositions on the two islands. DNA metabarcoding is thought to provide more detailed dietary information than more traditional approaches (e.g. Soininen *et al.* 2009; Ando *et al.* 2013), which may explain why more native plants were identified in this study. In addition, although both islands have a similar proportion of native plants (Ile aux Aigrettes 57%, Round Island: 69%, Chapter 2 Table 2.1), the availability of native versus introduced plant species on the two islands may be very different and this may affect grazing patterns. If true, it is an indication that the impacts of Aldabra giant tortoises as an analogue species may be site specific.

There were 31 plant species detected on Ile aux Aigrettes but never detected in the faecal samples of giant tortoises. Of particular note is *Vetiveria arguta,* an endemic grass that became rare on Round Island after introduced mammalian herbivores were eradicated. It is hypothesized that *V. arguta* is a poor competitor in comparison to the exotic vegetation present but flourishes in grazed habitats (North & Bullock 1986; North

et al. 1994). Griffiths *et al.* (2013) morphologically identified *V. arguta* in tortoise faecal samples from Round Island, but suggested that this species was probably not intentionally eaten and it had recolonized tortoise grazed areas. The molecular data support the notion that introduced tortoises are unlikely to be a threat to this species, and indeed their grazing pressure is likely to provide competitor release. *V. arguta* is a tussock-forming grass, which are known to be important habitats for invertebrates, seabirds, reptiles and invertebrates (Cole, N. pers.comm, Jones, C. pers.comm) in Mauritius, so this may be a mechanism by which tortoise grazing is engineering landscapes to benefit biodiversity.

5.5.2 The impacts of tortoise grazing on the plant community

Tortoise grazing affects plant community structure by reducing plant height, which has also been shown on Round Island (Griffiths *et al.* 2013). The cropping of native vegetation is restricted to open areas whereas introduced plants are also affected in forested areas. This hints at the creation of a vegetation mosaic where all vegetation is impacted in open areas but native vegetation remains unaffected in forested areas. In open areas tortoises are creating and maintaining 'tortoise lawns' akin to those seen on Aldabra atoll (Grubb 1971), and this is readily observed on Ile aux Aigrettes. This engineering and maintenance of a grazed climax community is performed elsewhere by megaherbivores, for example white rhinoceros (Krook *et al.* 2007; Waldram *et al.* 2008) and hippopotamus (*Hippopotamus amphibius*) (Owen-Smith 1987). Such grazing reduces the size and frequency of fires, benefits other grazing fauna (Waldram *et al.* 2008), and adds to ecosystem biodiversity (Krook *et al.* 2007). The potential benefits and costs of a vegetation mosaic composed of forests and grazed lawns on Ile aux Aigrettes remains unknown. After one year of tortoise exclusion, there is no significant change in the abundance, richness or diversity of plants. However, structural changes alone may have knock-on effects on the native and introduced fauna. This was not measured directly and is a priority for future research.

Tortoises reduce the biomass of introduced species on Ile aux Aigrettes, including at least two species that are targeted by an annual weeding effort on the island, *P. suberosa* and *L. leucocephala* (Newfield *et al.* 2003). This suggests that tortoises may contribute to the restoration of the island by removing exotic plant material. This is in agreement with an earlier study on Round Island (Griffiths *et al.* 2013). However, the extent to which the tortoise population can control introduced plants is likely to be density dependent and control will be limited to areas of the island that are accessible to tortoises. If left

unweeded, tortoise free areas will act as a source for recolonizing grazed areas. Thus, although tortoises play a role in weed control, it is unlikely that they can replace manual weeding at the present time.

Research into tortoise movement ecology is currently underway on both Aldabra and Round Island. Understanding the barriers to tortoise range expansion is essential for predicting future tortoise ranges. These barriers may be different for tortoises of different ages and sexes. For example, hatchling tortoises are often located in areas of Ile aux Aigrettes where the terrain is particularly uneven and so more challenging for tortoises to utilise (personal observation). Incidentally, two common weeds, *L. leucocephala* and *P. suberosa,* were particularly prevalent in the diet of hatchlings. Thus, an increased density of young tortoises may be beneficial for weed control in areas where large tortoises cannot access.

5.5.3 The knock-on effects of tortoise grazing on Pink Pigeons and Telfair's skinks

There is considerable dietary overlap between tortoises and the endemic fauna. However, the impact of tortoise grazing on a given species relies heavily on the plant tissue type consumed. This cannot be determined by DNA metabarcoding in isolation from other methods. For example, tortoises eating the fruits of native species may indeed be competing for resources with the native fauna in the short-term but in the long-term seed dispersal and increased seedling success (Griffiths *et al.* 2011) will benefit island restoration and increase food resources. Conversely, any native seedlings that lack herbivore defenses may be consumed and those species may suffer from reduced recruitment in the presence of tortoise grazing, which may have cascading effects on the native fauna. However, knowing which species tortoises eat allows for their targeted monitoring.

Excluding tortoises from small plots on Ile aux Aigrettes does not allow for the impact of tortoise grazing on all plant species to be fully determined. Rare species, in particular, will likely be missed. However, impacts on common species can be determined and thus the knock-on effects on the native fauna can be revealed. Tortoise grazing controls the biomass of *L. leucocephala* and *P. suberosa,* both common weeds on Ile aux Aigrettes. However, Pink Pigeons have preferences for both species in both seasons and Telfair's skinks exhibit preferences for *P. suberosa*. Thus, tortoise grazing in addition to manual weeding, reduces the availability of important food resources for the endemic fauna. Although the direct impacts of this on the fitness and fecundity of the endemic fauna is

unknown, there is evidence to suggest that the presence of weeding on the island contributes to reduced survival of adult Pink Pigeons (Concannon 2014). The mechanism by which this occurs may be, at least in part, through the loss of key dietary resources. However, Pink Pigeons have a broad dietary niche so the loss of exotic dietary items may be buffered if preferred native plants are readily available. This highlights the importance of considering cascading effects on the native fauna when assessing the impacts of ecological analogues.

5.5.4 Implications for management and future research

Understanding, at the species level, what introduced tortoises eat allows for the targeted monitoring of those species to determine the impacts of tortoise grazing. Furthermore, those species that are important dietary items for the native fauna must be priorities for monitoring given the substantial dietary overlap with the tortoises.

Knowing which native plants are important for the native fauna allows for managers to work towards increasing their abundance. This is particularly important considering that tortoise grazing reduces the availability of at least two important dietary items and their losses must be buffered to prevent negative cascading effects. However, both Telfair's skinks and Pink Pigeons have broad dietary niches, meaning that the removal of preferred food items may simply result in dietary switching so negative affects may already be buffered by the presence of alternative food sources. Alternatively, the considerable dietary overlap between the tortoises and the endemic fauna may reduce the scope for such dietary flexibility.

The five most frequently occurring dietary items across tortoise faecal samples were *I. violacea, P. suberosa, H. tiliaceus, H. petiolaris* and *L. leucocephala*. All of these species are also eaten more than expected given their availability, except *L. leucocephala.* Despite *L. leucocephala* not being a preferred dietary item, it is important to note that tortoise grazing still reduces its biomass. This illustrates the disproportionate affect keystone grazers have on an ecosystem. The remaining four plants are also selected for by Telfair's skinks and Pink Pigeons but, with the exception of *P. suberosa,* the impacts of tortoise grazing on these species is unknown. As a starting point, the plant tissue types the tortoises are consuming (fresh leaf material, seedlings, fruits, flowers) should be determined, followed by the monitoring of these species to establish whether tortoises are influencing food availability for the endemic fauna.

Ile aux Aigrettes may be missing native plant species that are important dietary items for the native fauna. The dietary analysis of other subpopulations of Pink Pigeons and Telfair's skinks, for example, by metabarcoding would shed light on this. Subsequently, those missing species could be (re)established on the island. Interventions such as these may help to boost population numbers on Ile aux Aigrettes and reduce the need for supplementary feeding in the case of the Pink Pigeon. Furthermore, increasing numbers of Telfair's Skinks is thought to be a pathway by which the invasive Asian musk shrew (*Suncus murinus*) population on Ile aux Aigrettes can be eradicated (Cole *et al.* 2009; Cole *et al.* 2013).

There were 27 plant species recorded on Ile aux Aigrettes during vegetation surveys but never detected in the diets of tortoises, Telfair's skinks or Pink pigeons. This may be because they were never consumed due to having plant defenses to deter herbivory (unpalatable or heterophyllic for example) or because better preferred plant food items were available, because the sample size may not have been high enough to capture the whole diet or because there was a mismatch between fruiting/flowering periods for particular plant species and sample collection, or because there were biases present in the molecular analyses that may preclude these species from being detected. It is likely that a combination of these factors explain the absence of the 27 plant species in the molecular analyses. For example, the absence of *V. arguta* is likely to be a true reflection of absence in the diet due to this species being well defended against herbivory whereas the absence of *Angraecum eburneum* and *Oeoniella polystachys*, both orchids, may be because it was never consumed or because there is a known PCR bias against this plant family (see Chapter 3). Current methods for the *In silico* testing of PCR primers are unable to predict how amplification is influenced by the presence of multiple DNA targets in a pooled sample. Therefore, even if *in silico* testing suggests that PCR primers are a good fit for a particular plant species, the presence of another plant species that is a better fit may influence which species are detected by metabarcoding. Primer behaviour in pooled samples in addition to interacting factors such as amplicon length biases and the susceptibility of different plant species and tissues to digestion may all influence which species are detected by metabarcoding. The analysis of mock samples alongside captive feeding trials can clarify where such biases exist and this is recommended for future work and the list of plant species never detected in Appendix 3.3 is available for use as a starting point for future experiments.

Tortoises reduce the biomass of introduced plants on Ile aux Aigrettes. However, the point at which the density of tortoises is high enough for their grazing to replace manual weeding is unknown. It must also be considered that despite reducing biomass, tortoises may not reduce plant numbers overall. This is fundamentally different to weeding, where plants are removed entirely. Furthermore, the barriers to tortoise range expansion are not fully understood. These are likely to be fruitful areas of research and will be particularly informative for future introductions.

Tortoises also crop vegetation in open areas, forming a mosaic habitat. Elsewhere, such mosaics have been shown to benefit ecosystem functioning and biodiversity. The effect of this on Ile aux Aigrettes is unknown and should be explored. Additionally, the exclosure study results are based on just one year of tortoise exclusion. Often, the short-term effects can be profoundly different to long-term effects (Suttle *et al.* 2007). Thus, it is important to continue monitoring the exclusion experiment to determine the long-term effects of tortoise grazing.

This study begins to piece together the trophic interactions that occur on Ile aux Aigrettes. By focusing on a keystone grazer one major driver of food web dynamics has been investigated. However, the study focuses on a small portion of the food web. The impacts of tortoises on the remaining invertebrates, birds and reptiles, are unknown. To begin to fully understand the consequences of introducing an analogue species, a detailed understanding of trophic interactions is required. With the comprehensive DNA barcode library (Chapter 2) and novel PCR primers to study herbivory by DNA metabarcoding (Chapter 3), such analysis of trophic interactions can be extended to the remaining herbivores, omnivores and pollinators that are members of the Ile aux Aigrettes ecological network.

5.6 Acknowledgements

Thank you to Ian Vaughan for help with applying econullnetr and to Helen Hipperson and the NBAF team at the University of Sheffield for carrying out the Illumina library preparation, sequencing and for training in bioinformatics. Thank you to the Mauritian Wildlife Foundation Pink Pigeon field staff and the Islands Restoration Team for assistance collecting faecal samples.

Chapter Six – General discussion

6.1 Aims

This PhD research aimed to analyse the effects of ecological replacement, where analogue species were introduced as proxies for species that were believed to be keystone grazers. This project aimed to explore the effects of selective grazing by an analogue species on the plant community and the knock on effects this may have on other herbivorous or omnivorous species.

The primary aim of this PhD project was to disentangle the impacts and trophic interactions of introduced Aldabra giant tortoises (*Aldabrachelys gigantea*) on Ile aux Aigrettes using DNA metabarcoding. The focus was primarily on the direct effects of giant tortoise grazing on the plant community and the indirect effects on two endemic species; the Pink Pigeon (*Nesoenas mayeri*) and the Telfair's skink (*Leiolopisma telfairii*).

Specific aims were to (i) compile a comprehensive DNA barcode library to lay the foundations to study trophic interactions on both Ile aux Aigrettes and Round Island by DNA metabarcoding; (ii) design novel PCR primers for metabarcoding at the ITS2 region, appropriate for use in the Mauritian study system and elsewhere; (iii) understand the indirect interactions between introduced giant tortoises and the endemic Pink Pigeon and Telfair's skink, on Ile aux Aigrettes, by analysing their dietary preferences via metabarcoding; and finally (iv) assess the direct affect of giant tortoise grazing on the plant community by carrying out a giant tortoise exclusion experiment. The exclusion data were combined with the dietary data to elucidate the impact of introduced giant tortoises.

6.2 Completion of aims

The plant communities on both Ile aux Aigrettes and Round Island were comprehensively DNA barcoded (Chapter 2). Included in the barcode libraries were 99% of the angiosperms (163 of 164 species) and 25% of the ferns (1 of 4 species) known to be present on the islands. The only missing angiosperm was the Critically Endangered Hurricane Palm, *Dictyosperma album* var. *conjugatum.* Failure to amplify and sequence the hurricane palm was unexpected and potential reasons for this include primer mismatch, intraspecific variation at the ITS2 region, or the presence of a symbiont, such as a fungus, that is also amplified by the primers and prevents a readable sequence being obtained through Sanger sequencing. The difficulties amplifying ferns

were expected since the PCR primers used were not designed for ferns (Chen *et al.* 2010; Cheng *et al.* 2016). Fortunately, *Phymatodes scolopendria,* the fern included in the library*,* is the only very common fern on Ile aux Aigrettes. Of the plants tested, 98.6% could be correctly assigned to their species using a BLASTn search. This equates to just two species that cannot be differentiated, both in the genus *Fimbristylis*, and their identification as two separate species is in debate (see Chapter 2).

A suite of universal plant DNA metabarcoding PCR primers were designed for the ITS2 region and initial testing on a subset of plant species revealed that one primer pair were superior (Chapter 3). This primer pair, UniPlantF and UniPlantR, were tested *in silico* and *in vitro* against all of the plant species present in the comprehensive DNA barcode library (see Chapter 2) and a subset of the UK flora to explore whether these molecular tools could be applied to other systems. *In silico* PCR using ecoPCR within the Obitools software package (Boyer *et al.* 2016) revealed that 88% (n = 1111 species) of the tested species had negligible primer mismatches and should amplify in practice. Of the Mauritian species tested, the primer match was also 88% (n = 131 species). These *in silico* analyses predicted that there may be problems amplifying species in the Orchidaceae and Cyperaceae families. Testing PCR amplification in the lab gave much more promising results with 99% of the Mauritian samples (n=169) amplifying successfully. In addition, Cyperaceae were detected in the dietary case studies (Chapter 4, Chapter 5). No Orchidaceae were detected in the diet, which could indicate no consumption or amplification failure. Taxonomic discrimination was high: 86.1%, 99.4% and 99.9% at the species, genus and family levels respectively (n=1577 species). Taxonomic discrimination within the Mauritian samples only, matched the discrimination capacity of the longer ITS2 amplicon used to build the comprehensive DNA barcode library in Chapter Two. The clustering analyses indicated that during the metabarcoding bioinformatics pipeline, it was not advisable to cluster the DNA sequences into molecular taxonomic units since the extent of differentiation varied considerably across the species tested. Instead, assigning all unique sequences to taxa before merging the data for different haplotypes is recommended. For Mauritian samples in particular the prior comprehensive barcoding greatly facilitated this process.

In Chapter Four the novel DNA metabarcoding primers were used in conjunction with the comprehensive DNA barcode library to assess the diet of Pink Pigeons and Telfair's skinks on Ile aux Aigrettes. Using metabarcoding to delineate diet from faecal samples revealed that both species were generalists with broad dietary niches. Food available

was compared to the food consumed, which indicated that both species have dietary preferences (positive or negative) for the different species of both introduced and native plants. Exotic plants are an important component of Pink Pigeon diet, which has implications for the restoration activities that are carried out on Ile aux Aigrettes. Pink Pigeons also rarely consume supplementary feed, and when eaten, is used more frequently by males in comparison to females. This suggests that supplementary feed may not be as important for the Ile aux Aigrettes' Pink Pigeon subpopulation as previously thought. A modification of the current supplementary feeding regime would reduce project costs and may also benefit Pink Pigeon fitness by reducing the risk of disease transmission at supplementary feeding sites. The metabarcoding data was compared with dietary data for Telfair's skink as determined by the morphological examination of faecal samples. Using morphology alone, it would have been concluded that the Telfair's skinks specialize on fewer plants in comparison to the metabarcoding technique. Thus the breadth of the diet cannot be captured by morphology alone. However, using morphological analyses the type of plant tissue consumed (fruit, leaf, flower etc.) can be determined. Using DNA metabarcoding in conjunction with morphological and nutritional analyses is advised to more fully understand the importance of trophic interactions in threatened species recovery programmes and ecosystems undergoing restoration.

The impacts and interactions of introduced giant tortoises on Ile aux Aigrettes begun to be disentangled in Chapter Five. Giant tortoise, Telfair's skinks and Pink Pigeon dietary data were coalesced with data from a giant tortoise exclusion experiment. This study found that giant tortoises ate and had preferences for a wide variety of plant species, both native and introduced. There was a great degree of overlap between the diet of the giant tortoises, Telfair's skinks and Pink Pigeons. This is an indication of competition for resources (if resources are limited), however detecting the direct effect of giant tortoise grazing on each individual plant species (promotion or suppression) was beyond the limits of the experimental design. However, the experiment showed that giant tortoises reduced the biomass of two exotic weeds that are also important components of Pink Pigeon diet. Since Pink Pigeons occupy a broad dietary niche, a reduction in the availability of some food resources may not negatively affect their population viability. Overall, giant tortoise grazing reduces vegetation height, particularly in open areas, leading to a mosaic landscape, however the knock-on effects of this on the native fauna and ecosystem functioning overall are unknown.

6.3 Management recommendations

It is best practice to monitor trophic interactions subsequent to (re)introductions (IUCN/SSC 2013). By understanding trophic interactions dietary overlap, potential competition and species at risk due to inflexible niches can be detected, supplementary feed requirements can be monitored, the vulnerability of interaction networks can be assessed, and human-wildlife conflict can be preempted (Edmunds *et al.* 2008; Kowalczyk *et al.* 2011; Brown *et al.* 2014; Clare 2014; Jung *et al.* 2015). This section outlines conservation management recommendations based on the findings from this PhD.

6.3.1 Targeted monitoring of plant species consumed by introduced tortoises

Introduced Aldabra giant tortoises show both preference and avoidance for different species of native and introduced plant species. This refutes the hypothesis that giant tortoises primarily prefer exotic plants. Indeed, the species richness of native plants in the diet was significantly higher than that of introduced plants. The giant tortoise exclusion experiment does not allow for the effect of giant tortoise grazing on each individual plant species to be assessed. However, the data show that giant tortoise grazing reduces the biomass of both introduced and exotic plants overall. Therefore, targeted monitoring of those native plant species that occur frequently in the diet of giant tortoises is recommended in order to detect the impact of giant tortoise grazing on plant survival and recruitment. The five most frequently occurring native plant species across giant tortoise samples were *Ipomoea violacea, Hibiscus tiliaceus, Hilsenbergia petiolaris, Ficus reflexa,* and *Stenotaphrum dimidatum.* Targeted monitoring should begin with these species. Frequency of occurrence has been chosen over dietary preferences for these recommendations because giant tortoise do not have to prefer a species to impact upon its biomass (Chapter 5). The monitoring of *I. violacea, H. petiolaris,* and *F. reflexa* are particularly important since they are preferred dietary items for endemic Pink Pigeons and Telfair's skinks (Chapter 4).

6.3.2 Recommendations for weeding practice on Ile aux Aigrettes

Giant tortoise grazing reduces the biomass of exotic species overall, including two particularly invasive weeds that are targeted by an annual weeding effort on the island (Newfield *et al.* 2003): *Passiflora suberosa* and *Leucaena leucocephala* (Chapter 5). However, giant tortoises are unlikely to completely replace manual weeding on

Mauritian islands at this time; a conclusion also reached by Griffiths *et al.* (2013) after studying the plant communities on Round Island. As shown in Chapter Five, introduced adult giant tortoises do not inhabit the entirety of Ile aux Aigrettes. If left unweeded, these areas could act as sources for weed regeneration across the island. Furthermore, giant tortoises reduce plant biomass but may not reduce weed numbers from an area, which is fundamentally different to weeding. It is recommended that areas where giant tortoises do not inhabit become priorities for weeding whilst the remainder of the island is reassessed periodically for weeding requirements.

It has been shown that Aldabra giant tortoises have the capacity to disperse viable seeds of introduced plant species found in Mauritius (Waibel *et al.* 2013). Furthermore, Galápagos tortoises, *Chelonoidis nigra*, disperse the seeds of both native and exotic plant species (Blake *et al.* 2015). Thus, it is likely that Aldabra giant tortoises play a role in dispersing exotic seeds in their introduced range. It is recommended that the list of introduced plant species consumed by giant tortoises be used as baseline information to determine which plant tissue types are eaten. If only leaf material, for example, is found in faecal samples, this is an indication that giant tortoises may reduce the biomass of that species. Conversely, if seeds are found, giant tortoises may increase the biomass of those species through seed dispersal. This is of particular importance for those introduced species frequently consumed by giant tortoises: *P. suberosa*, *L. leucocephala* and *Euphorbia hirta.*

Leucaena leucocephala seeds are frequently identified in the faecal samples of Aldabra giant tortoises (Moorhouse-Gann, unpublished data). During the annual weeding activities on Ile aux Aigrettes, it is common practice for the weeded plant material to be left in piles on the island. Giant tortoises are seen feeding on these piles of vegetation, which often contain *L. leucocephala* seed pods (Fig. 6.1). Thus the interaction between this weeding practice and an ecological analogue is likely to contribute to dispersing the seeds of the highly invasive *L. leucocephala.* Understandably there are logistical difficulties removing large volumes of vegetation from the island and burning is not an option since reptiles utilise the vegetation piles. Therefore, it is recommend that the seedpods be removed from the weeded *L. leucocephala* and removed from the island. Scattering, rather than piling, the remainder of the vegetation may also benefit the invertebrate community by increasing resource availability (Cole, pers.comm).

Figure 6.1. A pile of weeded vegetation on Ile aux Aigrettes. Many *Leucaena leucocephala* seed pods can be seen on the weeded vegetation.

Pink Pigeons and Telfair's skinks have dietary preferences for introduced weeds, some of which are targeted for sustained control on Ile aux Aigrettes (Chapter 4, Newfield *et al.* 2003). Concannon (2014) found that the presence of weeding on Ile aux Aigrettes was associated with the reduced survival of adult Pink Pigeons. The mechanism by which this occurs is unknown, but given that the Pink Pigeons exhibit preferences for the weeds it is likely that the loss of key dietary resources plays a role. Conservation managers must be mindful of this when rapidly removing exotic plant species from the island. Indeed a mechanism by which the reduction in exotic food availability can be mitigated is by increasing the availability of natural native species. The diet of the Critically Endangered red-headed wood pigeon, endemic to the Ogasawara Islands, Japan, frequently contained introduced species (Ando *et al.* 2013). Here, the authors warned that the eradication of introduced plants must be balanced with the restoration of native plant species to mitigate the loss of introduced dietary items.

6.3.3 Increase natural food availability for endemic vertebrates

This study represents the first time that herbivory by the endemic Pink Pigeon and Telfair's skink has been analysed by metabarcoding. This dietary knowledge can be used to inform ongoing island restoration. A long-term plan to increase the availability

of those species both frequently consumed and preferred by Pink Pigeons and Telfair's skinks may benefit threatened species recovery programmes. Thus, it is recommended that conservation managers consider increasing the availability of *H. petiolaris, Premna serratifolia, I. violacea, F. reflexa, F. rubra,* and *Margaritaria anomala* on Ile aux Aigrettes. This is particularly important to balance the impact of losing alien plant dietary items through weeding.

6.3.4 Pink Pigeon supplementary feeding regime

Despite the *ad libitum* provision of supplementary feed throughout the year, Pink Pigeons infrequently use this food resource. It has previously been suggested that supplementary food is an important resource for Pink Pigeons when there is a shortfall in naturally available food resources (Edmunds *et al.* 2008). Combining the Pink Pigeon dietary data presented in Chapter Four with phenology data for Ile aux Aigrettes to identify any periods when there is a deficit of natural food is recommended. Subsequently, managers can work towards only supplying supplementary feed during these periods. This strategy, in combination with increasing the availability of natural food items, may reduce the need for supplementary feed and provide economic benefits to the restoration project in addition to potentially increasing the health of the subpopulation by reducing disease transmission. A potential drawback of reducing supplementary feed is that it may become more difficult to monitor individuals within the population if birds stop visiting the feeding hoppers.

6.3.5 Long-term monitoring of introduced giant tortoise impacts and future introductions

In this PhD the results of a giant tortoise exclusion experiment on Ile aux Aigrettes were presented after excluding giant tortoises from plots for one year. However, the long-term effects of giant tortoise exclusion are unknown and may differ markedly from the short-term effects. Henceforth the annual monitoring of the on-going giant tortoise exclusion experiment each May is recommended.

Furthermore, giant tortoise exclusion experiments are not the optimal method for determining the impacts of ecological analogues on plant communities and ecosystem functioning since it is difficult to capture the species-level responses in the plant community (Chapter 5). An alternative is to conduct a comprehensive plant community survey before giant tortoises are introduced, alongside gathering information on the resident vertebrate fauna to detect the cascading effects of giant tortoise introductions.

This should include collecting faecal samples in order to analyse animal diets functioning in a system before ecological replacement is implemented. It is recommended to consider these suggestions prior to any future introductions.

6.4 Future research directions

6.4.1 Combining methodologies to better understand the importance of trophic interactions

DNA metabarcoding has the capacity to provide information on more dietary items at a finer taxonomic resolution than traditional methods of diet assessment such as morphology and observation (Chapter 4; Soininen *et al.* 2009; Ando *et al.* 2013; Alonso *et al.* 2014). However, metabarcoding cannot identify the tissue type consumed and it provides no nutritional information. Combining the metabarcoding data with the morphological examination of faecal samples and feeding observations to determine which tissue types are being consumed is recommended. This can then be followed by the nutritional analysis of those tissue types for each species. A combined approach will give a clear indication of the importance of each dietary item for the fitness of the consumer. Combining techniques will also shed light on seed dispersal and pollination mechanisms within the system and elucidate the impacts of tortoise grazing on each plant species.

6.4.2 Use dietary data from other subpopulations to inform restoration activities on Ile aux Aigrettes

Ile aux Aigrettes has suffered a history of deforestation, but thanks to intensive restoration activities by the Mauritian Wildlife Foundation it now supports a native forest once again. However, some native plant species that historically provided food for the endemic fauna may be missing. Using DNA metabarcoding to analyse the diet of the Pink Pigeon subpopulations on the mainland and the Telfair's skink in their native Round Island range may reveal important native plant species that are missing from Ile aux Aigrettes. This data can then be used to inform restoration activities on Ile aux Aigrettes. Alongside the Ile aux Aigrettes data presented in this thesis, herbivory by Telfair's skinks on Round Island was also assessed by metabarcoding (to be published elsewhere), but analysing the diets of the Pink Pigeon mainland subpopulations is recommended. In addition, the metabarcoding dietary data presented in this thesis can

be used to identify important dietary items missing from other islands and the mainland. Restoring these plant species may contribute to restoring functionality.

6.4.3 What density of giant tortoises is required to control invasive weeds?

In Chapter Five it was illustrated that giant tortoises can reduce the biomass of introduced weeds so they have the potential to contribute to the weeding effort on Ile aux Aigrettes. However, the density of giant tortoises required to control invasive weeds is unknown. This is an important area of research, especially if giant tortoises are to be introduced to other Mauritian islands, or elsewhere, to play a role in weed control. Furthermore, there is an indication (Chapter 5) that giant tortoises of different age-classes may differ in how and where they control weeds by their capacity to access to different terrain.

6.4.4 How does a vegetation mosaic on Ile aux Aigrettes affect ecosystem functioning?

In Chapter Five it was shown that giant tortoises crop native and exotic plants in open areas and exotic plants only in forested areas. In open areas, it is clear that giant tortoises are creating and maintaining tortoise lawns, as is seen in their native range of the Aldabra atoll (Gibson & Hamilton 1983). This suggests that giant tortoises are engineering a mosaic habitat. The effect of this on the native fauna and ecosystem functioning on Ile aux Aigrettes is unknown. Elsewhere, grazing by white rhinoceros (*Ceratotherium simum*) maintains short grass, which benefits smaller herbivores that are short grass specialists. In that same study, it was shown that white rhinoceros grazing engineers a short grass mosaic that halts the spread of fire (Waldram *et al.* 2008). A mosaic of grazing lawns and tussock-like grassland has been shown to benefit bird biodiversity (Krook *et al.* 2007). Determining the diversity of species (animal and plant) that use each habitat type alongside assessing their trophic interactions should be considered. This would determine the effect of an Ile aux Aigrettes mosaic landscape on ecosystem functioning.

6.4.5 A complete food web approach to understand ecological replacement

The comprehensive DNA barcode library and novel plant DNA metabarcoding primers are the basic tools necessary to begin studying herbivory and piecing together the trophic interactions on Ile aux Aigrettes and Round Island. This research has focused on

assessing the interactions and impacts of an ecological analogue, and the construction of the Ile aux Aigrettes food web has begun (Chapter 4, Chapter 5). However, the impact of introduced giant tortoises on the invertebrates and other birds and reptiles in the system are unknown. Using the molecular tools and methodologies developed here, the analyses of trophic interactions can be extended to the remaining herbivores, omnivores, predators and pollinators that are present in the ecological network. DNA barcoding the Ile aux Aigrettes invertebrate communities would also allow for the study of predation within the system. The absence of a comprehensive invertebrate DNA barcode library for the Ile aux Aigrettes has resulted in low taxonomic discrimination in an earlier DNA metabaracoding study (Brown *et al.* 2014). An invertebrate library would allow for the data within that study to be reanalysed, in addition for new predation studies to be developed. DNA barcoding the entire island system would allow for the comprehensive analysis of the trophic interactions in the ecological network. Not only would this allow for the impacts of ecological replacement to be better understood, but it would also allow for the resilience of the system to be tested. This is particularly important in the context of global change.

6.5 Advances in assessing the impact of ecological replacement

In the age of the Anthropocene where biodiversity continues to decline, ecosystems are increasingly more likely to lose their structural and functional complexity (Chapin *et al.* 2000; Crutzen 2002; Sanderson *et al.* 2002; Wake & Vredenburg 2008; Dirzo *et al.* 2014; Birnie-Gauvin *et al.* 2017; Fernandez *et al.* 2017). Recently there has been a call to include rewilding approaches, such as ecological replacement, in long-term strategies for the conservation of biodiversity in order to restore the complexity of ecosystems (Fernandez *et al.* 2017). Currently however, there is a deficit of empirical studies that assess the impact of rewilding interventions (Donlan *et al.* 2006; Svenning *et al.* 2016; Fernandez *et al.* 2017). Until this changes, such ambitious conservation interventions are likely to remain controversial (Smith 2005; Rubenstein *et al.* 2006; Caro 2007; Ricciardi & Simberloff 2009a, b; Fernandez *et al.* 2017).

This PhD research represents the first time that DNA metabarcoding has been used to assess the impact and interactions of an analogue species introduced by ecological replacement. Analysing trophic links and combining these data with field data has furthered current understanding of ecological replacement. This study has also explored the indirect effects that ecological replacement may have on other species in the

ecological network, which is an area of concern in rewilding projects (Rubenstein *et al.* 2006; Ricciardi & Simberloff 2009a, b).

Although a myriad of questions regarding the impact of introduced giant tortoises remain unanswered (see sections 6.3 and 6.4 earlier in this chapter), DNA metabarcoding has the capacity to enhance current understanding of ecological replacement and can also benefit threatened species recovery programmes. Based on the findings from this PhD research, using DNA metabarcoding as a tool to assess the trophic interactions and impacts of analogue species is encouraged.

Appendix One – Supplementary information relating to Chapter Three

Appendix 1.1 – Mauritian and UK plant species used for primer design

Table A1.1.1 Mauritian species (native and exotic) used for primer design, alongside Order, Family and local name (where present). All accession numbers are from sequences described in Chapter 2.

Order	Family	Species	Local name	GenBank Accession number
Apiales	Araliaceae	*Polyscias maraisiana*	Bois de Boeuf, Bois d'éponge	KY700450
Arecales	Arecaceae	*Hyophorbe lagenicaulis*	Palmiste Bouteille, Palmiste gargoulett	KY700379
Asparagales	Asparagaceae	*Asparagus setaceus*	Liane asperge	KY700230
Asparagales	Asparagaceae	*Asparagus umbellatus*	Asperge sauvage	KY700233
Asparagales	Orchidaceae	*Oeoniella polystachys*	-	KY700424
Asparagales	Xanthorrhoeaceae	*Aloe tormentorii*	Mazambron	KX689270
Asterales	Asteraceae	*Chromolaena odorata*	-	KY700271
Asterales	Asteraceae	*Psiadia arguta*	Baume de l'île Plate	KY700461
Asterales	Goodeniaceae	*Scaevola taccada*	Veloutier vert	KY700472
Boraginales	Boraginaceae	*Cordia curassavica*	Herbe Condé	KY700286
Boraginales	Boraginaceae	*Hilsenbergia petiolaris*	Bois pipe	KY700373
Boraginales	Boraginaceae	*Tournefortia argentea*	Veloutier blanc	KY700514
Caryophyllales	Amaranthaceae	*Aerva congesta*	-	KY700209
Caryophyllales	Amaranthaceae	*Amaranthus dubius*	Brède malabar	KY700217
Caryophyllales	Petiveriaceae	*Rivina humilis*	Petite groseille	KY700467
Caryophyllales	Portulacaceae	*Portulaca oleracea*	Pourpier rouge	KY700453
Celastrales	Celastraceae	*Cassine orientalis*	Bois d'olive	KY700255
Celastrales	Celastraceae	*Maytenus pyria*	Bois à poudre	KY700412
Ericales	Ebenaceae	*Diospyros tesselaria*	Bois d'ébène noir	KY700307

Table A1.1.1 Mauritian species (native and exotic) used for primer design, alongside Order, Family and local name (where present). All accession numbers are from sequences described in Chapter 2.

Order	Family	Species	Local name	GenBank Accession number
Ericales	Lecythidaceae	*Foetidia mauritiana*	Bois puant	KY700361
Ericales	Sapotaceae	*Sideroxylon boutonianum*	Bois de fer	KX689341
Fabales	Fabaceae	*Caesalpinia bonduc*	Cadoque	KY700251
Fabales	Fabaceae	*Dendrolobium umbellatum*	Bois malgache	KX689290
Fabales	Fabaceae	*Desmanthus virgatus*	Petit acacia	KY700299
Fabales	Fabaceae	*Gagnebina pterocarpa*	Acacia indigene	KY700363
Fabales	Fabaceae	*Leucaena leucocephala*	Acacia indigène	KY700392
Fabales	Fabaceae	*Millettia pinnata*	Pongame	KY700415
Fabales	Fabaceae	*Pithecellobium dulce*	Cassie de Manille	KY700366
Fabales	Fabaceae	*Sophora tomentosa*	Bois chapelet	KY700495
Gentianales	Apocynaceae	*Catharanthus roseus*	Pervenche de Madagascar	KY700261
Gentianales	Apocynaceae	*Cynanchum staubii*	Liane calle	KX689283
Gentianales	Apocynaceae	*Ochrosia borbonica*	Bois jaune	KX689310
Gentianales	Apocynaceae	*Secamone dilapidens*	Liane bois d'olive, liane a ouate	KX689337
Gentianales	Apocynaceae	*Tylophora coriacea*	Ipéca du Pays	KY700526
Gentianales	Rubiaceae	*Coffea myrtifolia*	-	KY700288
Gentianales	Rubiaceae	*Coptosperma borbonica*	Bois de rat	KY700282
Gentianales	Rubiaceae	*Fernelia buxifolia*	Bois buis	KY700341
Gentianales	Rubiaceae	*Morinda citrifolia*	Bois tortue	KY700418
Gentianales	Rubiaceae	*Oldenlandia sieberi*	-	KX689313
Lamiales	Acanthaceae	*Asystasia gangetica*	Herbe pistache	KY700228
Lamiales	Acanthaceae	*Barleria observatrix*	-	KX689273
Lamiales	Bignoniaceae	*Tabebuia pallida*	Técoma	KX689347
Lamiales	Lamiaceae	*Premna serratifolia*	Bois sureau	KY700459

Table A1.1.1 Mauritian species (native and exotic) used for primer design, alongside Order, Family and local name (where present). All accession numbers are from sequences described in Chapter 2.

Order	Family	Species	Local name	GenBank Accession number
Lamiales	Lantaneae	*Lantana camara*	Vieille fille	KY700389
Lamiales	Lauraceae	*Clerodendrum heterophyllum*	Bois cabris	KY700274
Lamiales	Oleaceae	*Chionanthus ayresii*	Bois blanc	KX689274
Lamiales	Oleaceae	*Olea europaea var. africana*	Olivier de bourbon	KY700425
Malpighiales	Erythroxylaceae	*Erythroxylum sideroxyloides*	Bois de ronde	KY700318
Malpighiales	Euphorbiaceae	*Acalypha indica*	Herbe chatte	KY700205
Malpighiales	Euphorbiaceae	*Euphorbia hirta*	Jean Robert	KY700326
Malpighiales	Euphorbiaceae	*Euphorbia prostrata*	Rougette	KY700340
Malpighiales	Euphorbiaceae	*Stillingia lineata*	Fangame	KY700505
Malpighiales	Passifloraceae	*Passiflora suberosa*	Liane poc poc	KY700430
Malpighiales	Passifloraceae	*Turnera angustifolia*	-	KX689353
Malpighiales	Phyllanthaceae	*Margaritaria anomala*	Bois chenille	KY700409
Malpighiales	Phyllanthaceae	*Phyllanthus casticum*	Bois castique	KY700442
Malpighiales	Phyllanthaceae	*Phyllanthus mauritianus*	-	KX689319
Malpighiales	Phyllanthaceae	*Phyllanthus revaughanii*	-	KX689324
Malpighiales	Phyllanthaceae	*Phyllanthus tenellus*	-	KY700446
Malpighiales	Salicaceae	*Flacourtia indica*	Prune malgache	KY700356
Malpighiales	Salicaceae	*Ludia mauritiana*	Bois mozambique	KY700403
Malvales	Malvaceae	*Dombeya mauritiana*	-	KY700311
Malvales	Malvaceae	*Hibiscus tiliaceus*	Var	KY700376
Malvales	Malvaceae	*Trochetia boutoniana*	-	KY700517
Malvales	Malvaceae	*Urena lobata*	Herbe panier	KY700528
Malvales	Thymelaeaceae	*Wikstroemia indica*	Herbe tourterelle	KY700531
Myrtales	Combretaceae	*Terminalia bentzoe*	Bois benjoin	KX689350

Table A1.1.1 Mauritian species (native and exotic) used for primer design, alongside Order, Family and local name (where present). All accession numbers are from sequences described in Chapter 2.

Order	Family	Species	Local name	GenBank Accession number
Myrtales	Lythraceae	*Pemphis acidula*	Bois matelot	KY700436
Myrtales	Myrtaceae	*Eugenia lucida*	Bois clou	KY700332
Oxalidales	Oxalidaceae	*Oxalis corniculata*	Petite oseille	KY700428
Poales	Cyperaceae	*Cyperus dubius*	-	KY700386
Poales	Cyperaceae	*Fimbristylis cymosa*	-	KY700346
Poales	Poaceae	*Vetiveria arguta*	-	KX689356
Rosales	Moraceae	*Ficus reflexa*	Lafouche bâtard	KY700354
Rosales	Moraceae	*Ficus rubra*	Affouche rouge	KX689294
Rosales	Rhamnaceae	*Scutia myrtina*	Liane bambara	KY700477
Santalales	Santalaceae	*Santalum album*	Bois de santal	KY700470
Sapindales	Anacardiaceae	*Poupartia borbonica*	Bois poupart	KX689316
Sapindales	Burseraceae	*Protium obtusifolium*	Colophane bâtard	KY700457
Sapindales	Meliaceae	*Turraea thouarsiana*	Bois quivi	KY700524
Sapindales	Sapindaceae	*Dodonaea viscosa*	Bois de reinette	KY700314
Solanales	Convolvulaceae	*Ipomoea violacea*	-	KX689306
Solanales	Convolvulaceae	*Ipomoea obscura*	-	KY700384
Vitales	Vitaceae	*Cyphostemma mappia*	Mapou	KY700293

Table A1.1.2 UK species used for primer design, along with Order, Family and common name. Accession numbers beginning KT9486 are those uploaded from this study, the rest were downloaded from GenBank. All species were either known from previous studies of turtle dove diet (Murton *et al.* 1964; Browne *et al*. 2003), or common at our field sites or in supplementary or planted seed mixes (Dunn *et al.* 2015). Species with (spp.) after the common name are those which were not identified to the species level in previous dietary studies and for which we selected a representative species for primer design. Where multiple accession numbers are provided, these sequences were stitched together in order to cover the entire ITS2 and primer binding regions.

Order	Family	Species	Common Name	Genbank accession number (s)
Apiales	Apiaceae	*Anthriscus sylvestris*+	Cow parsley	AY548228 and KT948614
Asterales	Asteraceae	*Anthemis cotula*	Stinking chamomile	EU179216
Asterales	Asteraceae	*Carthamus tinctorius*+	Safflower	JQ230977 and KT948630
Asterales	Asteraceae	*Cirsium vulgare*	Spear thistle (spp.)	JX867638
Asterales	Asteraceae	*Guizotia abyssinica*+^	Niger seed	KT948615
Asterales	Asteraceae	*Helianthus annuus*+	Sunflower	JN115024
Asterales	Asteraceae	*Helminthotheca echoides*	Bristly ox-tongue	AF528491
Asterales	Asteraceae	*Senecio vulgaris*+	Groundsel	EF538396 and KT948631
Brassicales	Brassicaceae	*Brassica napus*+	Oil seed rape	JQ085860 and KT948616
Brassicales	Brassicaceae	*Capsella bursa-pastoris*+	Shepherd's purse	DQ310531 and KT948632
Brassicales	Brassicaceae	*Sinapsis alba*	Field mustard	FJ609733
Brassicales	Resedaceae	*Reseda lutea*^	Wild mignonette	DQ987096*
Caryophyllales	Amaranthaceae	*Atriplex patula*	Orache	HM005859*
Caryophyllales	Caryophyllaceae	*Cerastium fontanum*	Common mouse-ear	GU444015
Caryophyllales	Caryophyllaceae	*Silene latifolia subsp. alba*	White campion	AY594308
Caryophyllales	Caryophyllaceae	*Silene vulgaris*	Bladder campion	FN821149
Caryophyllales	Caryophyllaceae	*Spergula arvensis*	Corn spurrey	JX274532

Table A1.1.2 UK species used for primer design, along with Order, Family and common name. Accession numbers beginning KT9486 are those uploaded from this study, the rest were downloaded from GenBank. All species were either known from previous studies of turtle dove diet (Murton *et al.* 1964; Browne *et al*. 2003), or common at our field sites or in supplementary or planted seed mixes (Dunn *et al.* 2015). Species with (spp.) after the common name are those which were not identified to the species level in previous dietary studies and for which we selected a representative species for primer design. Where multiple accession numbers are provided, these sequences were stitched together in order to cover the entire ITS2 and primer binding regions.

Order	Family	Species	Common Name	Genbank accession number (s)
Caryophyllales	Caryophyllaceae	*Stellaria graminea*	Lesser stitchwort (spp.)	AY594304
Caryophyllales	Caryophyllaceae	*Stellaria media*+	Chickweed	JN589063 and KT948633
Caryophyllales	Chenopodiaceae	*Chenopodium album*+	Fat hen	FN561552 and KT948617
Caryophyllales	Polygonaceae	*Persicaria maculosa*+	Redshank	HQ843137 and KT948635
Caryophyllales	Polygonaceae	*Polygonum aviculare*+	Knotgrass	KJ025070
Caryophyllales	Polygonaceae	*Rumex obtusifolius*+	Broad-leaved dock	GQ340059*
Ericales	Primulaceae	*Anagallis arvensis*+	Scarlet pimpernel	AY855135 and KT948628
Fabales	Fabaceae	*Lotus corniculatus*+	Birds-foot trefoil	DQ312207 and KT948621
Fabales	Fabaceae	*Medicago lupulina*+	Black medick	DQ311980
Fabales	Fabaceae	*Trifolium pratense*+	Red clover	AF053171 and KT948619
Fabales	Fabaceae	*Trifolium repens*+	White clover	DQ311962 and KT948620
Fabales	Fabaceae	*Vicia sativa*+	Common vetch	KJ787165
Gentianales	Rubiaceae	*Galium aparine*+	Goosegrass	DQ006036
Geraniales	Geraniaceae	*Geranium dissectum*+	Cut-leaved cranesbill	AY944413 and KT948622
Lamiales	Plantaginaceae	*Veronica persica*+	Common field speedwell	AF313001 and KT948624
Lamiales	Scrophulariaceae	*Kickxia spuria*	Round-leaf fluellen	AF513880

Table A1.1.2 UK species used for primer design, along with Order, Family and common name. Accession numbers beginning KT9486 are those uploaded from this study, the rest were downloaded from GenBank. All species were either known from previous studies of turtle dove diet (Murton *et al.* 1964; Browne *et al*. 2003), or common at our field sites or in supplementary or planted seed mixes (Dunn *et al.* 2015). Species with (spp.) after the common name are those which were not identified to the species level in previous dietary studies and for which we selected a representative species for primer design. Where multiple accession numbers are provided, these sequences were stitched together in order to cover the entire ITS2 and primer binding regions.

Order	Family	Species	Common Name	Genbank accession number (s)
Malpighiales	Euphorbiaceae	*Euphorbia esula*	Green spurge (spp.)	JN010042
Malpighiales	Violaceae	*Viola arvensis*[+]	Field pansy	DQ005347 and KT948636
Malpighiales	Violaceae	*Viola tricolor*	Heartsease	DQ055406
Poales	Poaceae	*Alopecurus myosuroides*[+^]	Black grass	KT948627
Poales	Poaceae	*Festuca pratensis*	Meadow fescue (spp.)	KJ598995
Poales	Poaceae	*Hordeum vulgare*[+]	Barley	KM217265 and KT948626
Poales	Poaceae	*Panicum miliaceum*[+]	Millet	KT948629 and JX576677
Poales	Poaceae	*Poa annua*[+]	Meadow grass	KJ599003 and KT948634
Poales	Poaceae	*Poa trivialis*	Rough meadow-grass (spp.)	KJ598983
Poales	Poaceae	*Sorghum bicolor*[+]	White sorghum	GQ856358
Poales	Poaceae	*Triticum aestivum*[+]	Wheat	KF482086 and KT948625
Poales	Poaceae	*Zea mays*[+]	Maize	DQ683016*
Ranunculales	Papaveraceae	*Fumaria officinalis*[+]	Common fumitory	HE603306 and KT948623
Ranunculales	Papaveraceae	*Papaver rhoeas*	Poppy	DQ912886
Ranunculales	Ranunculaceae	*Ranunculus repens*	Creeping buttercup	JN115047*
Rosales	Urticaceae	*Urtica dioica*	Common nettle	KF454275 and KF137936

Table A1.1.2 UK species used for primer design, along with Order, Family and common name. Accession numbers beginning KT9486 are those uploaded from this study, the rest were downloaded from GenBank. All species were either known from previous studies of turtle dove diet (Murton *et al.* 1964; Browne *et al*. 2003), or common at our field sites or in supplementary or planted seed mixes (Dunn *et al.* 2015). Species with (spp.) after the common name are those which were not identified to the species level in previous dietary studies and for which we selected a representative species for primer design. Where multiple accession numbers are provided, these sequences were stitched together in order to cover the entire ITS2 and primer binding regions.

Order	Family	Species	Common Name	Genbank accession number (s)
Solanales	Convolvulaceae	*Convolvulus arvensis*+	Field bindweed	AY558826

^Sequence does not or only partially overlaps forward primer region

* Sequence does not or only partially overlaps reverse primer region

+ Primers successfully tested *in vitro* using these species

Appendix 1.2 – Primers designed in this study

Table A1.2.1 Primers designed in this study with *in vitro* testing results

Forward primer	Primer sequences 5'-3'	Reverse primer	Primer sequences 5'-3'	No. species tested *in vitro*	Amplification success (%)
TAS1 forward	GCRAGTTGCGCCYVRVK	TAS1 reverse	TGCTTAARCTCRGYGGGTRDY	10	0
		TAS2 reverse	ATATGCTTAARCTCRGYGGGT	7	0
TAS2 forward	TTKRAWYGCRAGTTGCG	TAS3 reverse	CCGCTTAKTKATATGC	4	75
		TAS4 reverse	TATGCTTAARCTCRGCGGG	4	75
		TAS5 reverse	CTCCGCTTAKTKATATGC	117	44
TAS3 forward	TTKRAWYGCRAGTTGCGCC	TAS3 reverse	CCGCTTAKTKATATGC	4	75
		TAS4 reverse	TATGCTTAARCTCRGCGGG	4	75
		TAS5 reverse	CTCCGCTTAKTKATATGC	4	75
UniPlantF	TGTGAATTGCARRATYCMG	UniPlantR	CCCGHYTGAYYTGRGGTCDC	195	99
		Seed2 reverse	ATATGCTTAAAYTCAGCGGGYV	3	0
Seed2 forward	TTTGAACGCAMRTTGCGCC	Seed2 reverse	ATATGCTTAAAYTCAGCGGGYV	3	67
		UniPlantR	CCCGHYTGAYYTGRGGTCDC	3	67

Appendix 1.3 – *In silico* analyses for short DNA sequence lengths

Table A1.3.1. Results of *in silico* analysis of primer fit for UniPlantR for Mauritian plant families where forward primer fit could not be tested due to short sequence lengths. Matches are where primers fit with a maximum of 3bp mismatches and no mismatches in the last two bp at the 3 prime end. For all Mauritian sequences, if the UniPlantF priming site was present, the UniplantR priming site was also present (Table 3.1).

Order	Family	No. species where reverse primer only tested *in silico*	No. species where reverse primer matches *in silico*	% matches reverse primer only
Apiales	Araliaceae	1	1	100
Arecales	Arecaceae	2	2	100
Asparagales	Amaryllidaceae	1	1	100
Asparagales	Orchidaceae	2	0	0
Asparagales	Xanthorrhoeaceae	1	1	100
Asterales	Asteraceae	6	6	100
Asterales	Goodeniaceae	1	1	100
Boraginales	Boraginaceae	3	3	100
Brassicales	Caricaceae	1	1	100
Caryophyllales	Aizoaceae	1	1	100
Caryophyllales	Amaranthaceae	1	1	100
Caryophyllales	Nyctaginaceae	1	1	100
Caryophyllales	Petiveriaceae	1	1	100

Table A1.3.1. Results of *in silico* analysis of primer fit for UniPlantR for Mauritian plant families where forward primer fit could not be tested due to short sequence lengths. Matches are where primers fit with a maximum of 3bp mismatches and no mismatches in the last two bp at the 3 prime end. For all Mauritian sequences, if the UniPlantF priming site was present, the UniplantR priming site was also present (Table 3.1).

Order	Family	No. species where reverse primer only tested *in silico*	No. species where reverse primer matches *in silico*	% matches reverse primer only
Caryophyllales	Portulacaceae	1	1	100
Celastrales	Celastraceae	2	2	100
Commelinales	Commelinaceae	1	1	100
Ericales	Ebenaceae	2	2	100
Ericales	Lecythidaceae	1	1	100
Ericales	Sapotaceae	1	1	100
Fabales	Fabaceae	10	7	70
Gentianales	Apocynaceae	6	4	67
Gentianales	Rubiaceae	4	4	100
Lamiales	Acanthaceae	1	1	100
Lamiales	Bignoniaceae	1	1	100
Lamiales	Lamiaceae	1	1	100
Lamiales	Lauraceae	1	1	100
Lamiales	Oleaceae	2	1	50
Lamiales	Scrophulariaceae	1	1	100
Lamiales	Verbenaceae	2	2	100
Malpighiales	Erythroxylaceae	1	1	100
Malpighiales	Euphorbiaceae	6	6	100

Table A1.3.1. Results of *in silico* analysis of primer fit for UniPlantR for Mauritian plant families where forward primer fit could not be tested due to short sequence lengths. Matches are where primers fit with a maximum of 3bp mismatches and no mismatches in the last two bp at the 3 prime end. For all Mauritian sequences, if the UniPlantF priming site was present, the UniplantR priming site was also present (Table 3.1).

Order	Family	No. species where reverse primer only tested *in silico*	No. species where reverse primer matches *in silico*	% matches reverse primer only
Malpighiales	Passifloraceae	2	1	50
Malpighiales	Phyllanthaceae	8	5	63
Malpighiales	Salicaceae	3	3	100
Malvales	Malvaceae	8	5	63
Malvales	Thymelaeaceae	1	1	100
Myrtales	Combretaceae	1	1	100
Myrtales	Lythraceae	1	1	100
Myrtales	Myrtaceae	1	1	100
Poales	Cyperaceae	3	0	0
Poales	Poaceae	17	12	71
Polypodiales	Pteridaceae	1	0	0
Ranunculales	Papaveraceae	1	1	100
Rosales	Moraceae	3	2	67
Rosales	Rhamnaceae	3	2	67
Rosales	Urticaceae	1	1	100
Santalales	Santalaceae	1	1	100
Sapindales	Anacardiaceae	1	1	100
Sapindales	Burseraceae	1	1	100

Table A1.3.1. Results of *in silico* analysis of primer fit for UniPlantR for Mauritian plant families where forward primer fit could not be tested due to short sequence lengths. Matches are where primers fit with a maximum of 3bp mismatches and no mismatches in the last two bp at the 3 prime end. For all Mauritian sequences, if the UniPlantF priming site was present, the UniplantR priming site was also present (Table 3.1).

Order	Family	No. species where reverse primer only tested *in silico*	No. species where reverse primer matches *in silico*	% matches reverse primer only
Sapindales	Rutaceae	1	0	0
Sapindales	Sapindaceae	2	1	50
Saxifragales	Crassulaceae	1	1	100
Solanales	Convolvulaceae	2	2	100
Solanales	Solanaceae	3	3	100
Vitales	Vitaceae	1	1	100
Totals		**132**	**104**	**79**

Table A1.3.2a. Results of *in silico* analysis of primer fit for UniPlantF for plant families across both UK databases, where UniPlantR primer fit could not be tested due to short sequence lengths.

		UK database		Turtle Dove diet database		Overall		
Family	Order	F tested	F matches	F tested	F matches	F tested	F matches	% match
Acanthaceae	Lamiales	1	1			1	1	100
Aceraceae	Sapindales	1	1			1	1	100
Acoraceae	Acorales	1	1			1	1	100
Adoxaceae	Dipsacales	4	4			4	4	100
Aizoaceae	Caryophyllales	3	3			3	3	100
Alismataceae	Alismatales	7	5			7	5	71
Alstroemeriaceae	Liliales	1	1			1	1	100
Amaranthaceae	Caryophyllales	1	1			1	1	100
Amaryllidaceae	Asparagales	10	9			10	9	90
Anacardiaceae	Sapindales	1	1			1	1	100
Apiaceae	Apiales	52	49	1	1	52	49	94
Aponogetonaceae	Alismatales	1	1			1	1	100
Aquifoliaceae	Aquifoliales	1	1			1	1	100
Araceae	Alismatales	4	4			4	4	100
Araliaceae	Apiales	4	4			4	4	100
Araucariaceae	Pinales	1	1			1	1	100
Aristolochiaceae	Piperales	2	1			2	1	50
Asparagaceae	Asparagales	5	4			5	4	80

Table A1.3.2a. Results of *in silico* analysis of primer fit for UniPlantF for plant families across both UK databases, where UniPlantR primer fit could not be tested due to short sequence lengths.

		UK database		Turtle Dove diet database		Overall		
Family	Order	F tested	F matches	F tested	F matches	F tested	F matches	% match
Aspleniaceae	Polypodiales	1	0			1	0	0
Asteraceae	Asterales	122	115	6	6	122	115	94
Azollaceae	Salviniales	1	1			1	1	100
Balsaminaceae	Ericales	1	1			1	1	100
Berberidaceae	Ranunculales	1	1			1	1	100
Betulaceae	Fagales	6	6			6	6	100
Boraginaceae	Boraginales	22	22			22	22	100
Brassicaceae	Brassicales	75	70	3	3	76	71	93
Butomaceae	Alismatales	1	1			1	1	100
Buxaceae	Buxales	2	2			2	2	100
Cabombaceae	Nymphaeales	1	1			1	1	100
Calceolariaceae	Lamiales	1	1			1	1	100
Campanulaceae	Asterales	13	13			13	13	100
Cannabaceae	Rosales	2	2			2	2	100
Caprifoliaceae	Dipsacales	12	12			12	12	100
Caryophyllaceae	Caryophyllales	53	51	6	6	54	52	96
Celastraceae	Celastrales	3	3			3	3	100
Ceratophyllaceae	Ceratophyllales	2	2			2	2	100
Chenopodiaceae	Caryophyllales	24	21	2	2	24	21	88
Cistaceae	Malvales	2	2			2	2	100
Colchicaceae	Liliales	1	1			1	1	100
Convolvulaceae	Solanales	5	5	1	1	5	5	100

Table A1.3.2a. Results of *in silico* analysis of primer fit for UniPlantF for plant families across both UK databases, where UniPlantR primer fit could not be tested due to short sequence lengths.

		UK database		Turtle Dove diet database		Overall		
Family	Order	F tested	F matches	F tested	F matches	F tested	F matches	% match
Cornaceae	Cornales	1	1			1	1	100
Crassulaceae	Saxifragales	6	6			6	5	83
Cucurbitaceae	Cucurbitales	5	5			5	5	100
Cupressaceae	Pinales	8	8			8	8	100
Cymodoceaceae	Alismatales	1	1			1	1	100
Cyperaceae	Poales	50	44			50	44	88
Dennstaedtiaceae	Polypodiales	1	1			1	1	100
Diapensiaceae	Ericales	1	1			1	1	100
Droseraceae	Caryophyllales	2	2			2	2	100
Elaeagnaceae	Rosales	2	2			2	2	100
Equisetaceae	Equisetales	1	1			1	1	100
Ericaceae	Ericales	21	20			21	20	95
Euphorbiaceae	Malpighiales	6	6	1	1	7	7	100
Fabaceae	Fabales	68	64	5	5	71	67	94
Fagaceae	Fagales	3	3			3	3	100
Garryaceae	Garryales	1	1			1	1	100
Gentianaceae	Gentianales	8	8			8	8	100
Geraniaceae	Geraniales	15	15	1	1	15	15	100
Gesneriaceae	Lamiales	1	1			1	1	100
Griseliniaceae	Apiales	1	1			1	1	100
Grossulariaceae	Saxifragales	1	1			1	1	100
Gunneraceae	Gunnerales	1	1			1	1	100

Table A1.3.2a. Results of *in silico* analysis of primer fit for UniPlantF for plant families across both UK databases, where UniPlantR primer fit could not be tested due to short sequence lengths.

		UK database		Turtle Dove diet database		Overall		
Family	Order	F tested	F matches	F tested	F matches	F tested	F matches	% match
Haloragaceae	Saxifragales	1	1			1	1	100
Hippocastanaceae	Sapindales	1	1			1	1	100
Hyacinthaceae	Asparagales	3	3			3	3	100
Hydrangeaceae	Cornales	3	2			3	2	67
Hydrocharitaceae	Alismatales	8	8			8	8	100
Hydrophyllaceae	Boraginales	1	1			1	1	100
Hypericaceae	Malpighiales	7	7			7	7	100
Iridaceae	Asparagales	3	3			3	3	100
Isoetaceae	Isoetales	1	1			1	1	100
Juglandaceae	Fagales	1	1			1	1	100
Juncaceae	Poales	23	17			23	17	74
Juncaginaceae	Alismatales	1	1			1	1	100
Lamiaceae	Lamiales	28	27			28	27	96
Lauraceae	Laurales	1	1			1	1	100
Lentibulariaceae	Lamiales	4	3			4	3	75
Liliaceae	Liliales	7	6			7	6	86
Limnanthaceae	Brassicales	1	1			1	1	100
Linaceae	Malpighiales	3	3			3	3	100
Lycopodiaceae	Lycopodiales	1	1			1	1	100
Lythraceae	Myrtales	1	1			1	1	100
Malvaceae	Malvales	15	15			15	15	100
Melanthiaceae	Liliales	1	1			1	1	100

Table A1.3.2a. Results of *in silico* analysis of primer fit for UniPlantF for plant families across both UK databases, where UniPlantR primer fit could not be tested due to short sequence lengths.

		UK database		Turtle Dove diet database		Overall		
Family	Order	F tested	F matches	F tested	F matches	F tested	F matches	% match
Menyanthaceae	Asterales	2	2			2	2	100
Montiaceae	Caryophyllales	2	2			2	2	100
Moraceae	Rosales	2	2			2	2	100
Myricaceae	Fagales	1	1			1	1	100
Myrtaceae	Myrtales	3	3			3	3	100
Nothofagaceae	Fagales	1	1			1	1	100
Nymphaeaceae	Nymphaeales	2	2			2	2	100
Oleaceae	Lamiales	5	5			5	5	100
Onagraceae	Myrtales	15	14			15	14	93
Orchidaceae	Asparagales	26	23			26	23	88
Orobanchaceae	Lamiales	25	25			25	25	100
Osmundaceae	Osmundales	1	1			1	1	100
Oxalidaceae	Oxalidales	2	1			2	1	50
Paeoniaceae	Saxifragales	1	1			1	1	100
Papaveraceae	Ranunculales	13	13	2	2	15	15	100
Paulowniaceae	Lamiales	1	1			1	1	100
Phrymaceae	Lamiales	1	1			1	1	100
Phytolaccaceae	Caryophyllales	1	1			1	1	100
Pinaceae	Pinales	8	8			8	8	100
Pittosporaceae	Apiales	1	1			1	1	100
Plantaginaceae	Lamiales	29	25	2	2	31	27	87
Platanaceae	Proteales	1	1			1	1	100

Table A1.3.2a. Results of *in silico* analysis of primer fit for UniPlantF for plant families across both UK databases, where UniPlantR primer fit could not be tested due to short sequence lengths.

		UK database		Turtle Dove diet database		Overall		
Family	Order	F tested	F matches	F tested	F matches	F tested	F matches	% match
Plumbaginaceae	Caryophyllales	3	3			3	3	100
Poaceae	Poales	127	121	8	8	127	121	95
Polemoniaceae	Ericales	2	2			2	2	100
Polygalaceae	Fabales	3	3			3	3	100
Polygonaceae	Caryophyllales	14	12	3	3	15	13	87
Portulacaceae	Caryophyllales	1	1			1	1	100
Potamogetonaceae	Alismatales	6	6			6	6	100
Primulaceae	Ericales	10	10	1	1	10	10	100
Pteridaceae	Polypodiales	1	1			1	1	100
Ranunculaceae	Ranunculales	27	27	1	1	27	27	100
Resedaceae	Brassicales	1	1			1	1	100
Rhamnaceae	Rosales	2	2			2	2	100
Rosaceae	Rosales	86	82			86	82	95
Rubiaceae	Gentianales	5	5	1	1	5	5	100
Salicaceae	Malpighiales	14	14			14	14	100
Sapindaceae	Sapindales	1	1			1	1	100
Sarraceniaceae	Ericales	1	1			1	1	100
Saxifragaceae	Saxifragales	18	18			18	18	100
Scheuchzeriaceae	Alismatales	1	0			1	0	0
Scrophulariaceae	Lamiales	6	6			6	6	100
Selaginellaceae	Selaginellales	1	1			1	1	100
Simaroubaceae	Sapindales	1	1			1	1	100

Table A1.3.2a. Results of *in silico* analysis of primer fit for UniPlantF for plant families across both UK databases, where UniPlantR primer fit could not be tested due to short sequence lengths.

		UK database		Turtle Dove diet database		Overall		
Family	Order	F tested	F matches	F tested	F matches	F tested	F matches	% match
Solanaceae	Solanales	12	12			12	12	100
Tamaricaceae	Caryophyllales	1	1			1	1	100
Taxaceae	Pinales	1	1			1	1	100
Thesiaceae	Santalales	1	1			1	1	100
Thymelaeaceae	Malvales	2	1			2	1	50
Tofieldiaceae	Alismatales	1	1			1	1	100
Tropaeolaceae	Brassicales	1	1			1	1	100
Typhaceae	Poales	4	4			4	4	100
Ulmaceae	Rosales	3	3			3	3	100
Urticaceae	Rosales	3	3	1	1	3	3	100
Verbenaceae	Lamiales	1	1			1	1	100
Violaceae	Malpighiales	7	6	2	2	9	8	89
Viscaceae	Santalales	1	0			1	0	0
Vitaceae	Vitales	1	1			1	1	100
Xanthorrhoeaceae	Asparagales	2	1			2	1	50
Zosteraceae	Alismatales	1	1			1	1	100
Total species		1286	1213	47	47	1299	1225	94
Total genera		824	806	42	42	824	806	98
Total families		144	141	18	18	144	141	97

Table A1.3.2b. Results of *in silico* analysis of primer fit for UniPlantR for plant families across all three UK databases, where UniPlantF primer fit could not be tested due to short sequence lengths.

		UK Genus level		Turtle Dove database		Overall		
Family	Order	R tested	R matches	R tested	R matches	R tested	R matches	% match
Acanthaceae	Lamiales	1	1			1	1	100
Aceraceae	Sapindales	1	1			1	1	100
Acoraceae	Acorales	1	1			1	1	100
Adoxaceae	Dipsacales	4	4			4	4	100
Aizoaceae	Caryophyllales	1	1			1	1	100
Alismataceae	Alismatales	6	6			6	6	100
Amaranthaceae	Caryophyllales	5	5			5	5	100
Amaryllidaceae	Asparagales	10	9			10	9	90
Anacardiaceae	Sapindales	1	1			1	1	100
Apiaceae	Apiales	58	55	1	1	58	55	95
Aponogetonaceae	Alismatales	1	1			1	1	100
Aquifoliaceae	Aquifoliales	1	1			1	1	100
Araceae	Alismatales	4	4			4	4	100
Araliaceae	Apiales	3	3			3	3	100
Araucariaceae	Pinales	1	1			1	1	100
Aristolochiaceae	Piperales	1	0			1	0	0
Asparagaceae	Asparagales	4	3			4	3	75
Aspleniaceae	Polypodiales	1	0			1	0	0
Asteraceae	Asterales	122	121	7	7	123	122	99
Azollaceae	Salviniales	1	0			1	0	0
Balsaminaceae	Ericales	2	2			2	2	100

Table A1.3.2b. Results of *in silico* analysis of primer fit for UniPlantR for plant families across all three UK databases, where UniPlantF primer fit could not be tested due to short sequence lengths.

		UK Genus level		Turtle Dove database		Overall		
Family	Order	R tested	R matches	R tested	R matches	R tested	R matches	% match
Berberidaceae	Ranunculales	1	1			1	1	100
Betulaceae	Fagales	7	7			7	7	100
Boraginaceae	Boraginales	23	23			23	23	100
Brassicaceae	Brassicales	76	73	3	3	77	74	96
Butomaceae	Alismatales	1	1			1	1	100
Buxaceae	Buxales	1	1			1	1	100
Cabombaceae	Nymphaeales	1	1			1	1	100
Calceolariaceae	Lamiales	1	1			1	1	100
Campanulaceae	Asterales	15	14			15	14	93
Cannabaceae	Rosales	2	2			2	2	100
Caprifoliaceae	Dipsacales	7	6			7	6	86
Caryophyllaceae	Caryophyllales	69	68	6	6	69	68	99
Celastraceae	Celastrales	2	2			2	2	100
Ceratophyllaceae	Ceratophyllales	2	2			2	2	100
Chenopodiaceae	Caryophyllales	24	24	1	1	24	24	100
Cistaceae	Malvales	2	2			2	2	100
Convolvulaceae	Solanales	7	7	1	1	7	7	100
Cornaceae	Cornales	1	1			1	1	100
Crassulaceae	Saxifragales	8	6			8	6	75
Cucurbitaceae	Cucurbitales	3	3			3	3	100
Cupressaceae	Pinales	4	4			4	4	100
Cymodoceaceae	Alismatales	1	0			1	0	0
Cyperaceae	Poales	79	0			79	0	0

Table A1.3.2b. Results of *in silico* analysis of primer fit for UniPlantR for plant families across all three UK databases, where UniPlantF primer fit could not be tested due to short sequence lengths.

		UK Genus level		Turtle Dove database		Overall		
Family	Order	R tested	R matches	R tested	R matches	R tested	R matches	% match
Diapensiaceae	Ericales	1	1			1	1	100
Droseraceae	Caryophyllales	3	3			3	3	100
Dryopteridaceae	Polypodiales	1	0			1	0	0
Elatinaceae	Malpighiales	1	1			1	1	100
Ericaceae	Ericales	23	22			23	22	96
Euphorbiaceae	Malpighiales	9	9	1	1	10	10	100
Fabaceae	Fabales	76	68	5	5	76	68	89
Fagaceae	Fagales	3	3			3	3	100
Gentianaceae	Gentianales	12	12			12	12	100
Geraniaceae	Geraniales	15	15	1	1	15	15	100
Gesneriaceae	Lamiales	1	0			1	0	0
Griseliniaceae	Apiales	1	1			1	1	100
Grossulariaceae	Saxifragales	2	2			2	2	100
Gunneraceae	Gunnerales	1	1			1	1	100
Haloragaceae	Saxifragales	4	4			4	4	100
Hyacinthaceae	Asparagales	2	2			2	2	100
Hydrangeaceae	Cornales	1	1			1	1	100
Hydrocharitaceae	Alismatales	6	3			6	3	50
Hydrophyllaceae	Boraginales	1	1			1	1	100
Hypericaceae	Malpighiales	10	10			10	10	100
Iridaceae	Asparagales	2	2			2	2	100
Juglandaceae	Fagales	1	1			1	1	100
Juncaceae	Poales	31	31			31	31	100

Table A1.3.2b. Results of *in silico* analysis of primer fit for UniPlantR for plant families across all three UK databases, where UniPlantF primer fit could not be tested due to short sequence lengths.

		UK Genus level		Turtle Dove database		Overall		
Family	Order	R tested	R matches	R tested	R matches	R tested	R matches	% match
Juncaginaceae	Alismatales	2	2			2	2	100
Lamiaceae	Lamiales	29	27			29	27	93
Lentibulariaceae	Lamiales	5	5			5	5	100
Liliaceae	Liliales	6	6			6	6	100
Linaceae	Malpighiales	3	3			3	3	100
Lythraceae	Myrtales	2	2			2	2	100
Malvaceae	Malvales	13	11			13	11	85
Melanthiaceae	Liliales	1	1			1	1	100
Menyanthaceae	Asterales	2	2			2	2	100
Montiaceae	Caryophyllales	2	2			2	2	100
Moraceae	Rosales	1	1			1	1	100
Myricaceae	Fagales	1	1			1	1	100
Myrtaceae	Myrtales	3	2			3	2	67
Nymphaeaceae	Nymphaeales	3	2			3	2	67
Oleaceae	Lamiales	3	3			3	3	100
Onagraceae	Myrtales	15	14			15	14	93
Orchidaceae	Asparagales	28	25			28	25	89
Orobanchaceae	Lamiales	34	34			34	34	100
Oxalidaceae	Oxalidales	2	2			2	2	100
Papaveraceae	Ranunculales	17	17	2	2	17	17	100
Phytolaccaceae	Caryophyllales	1	1			1	1	100
Pinaceae	Pinales	3	3			3	3	100
Pittosporaceae	Apiales	1	1			1	1	100

Table A1.3.2b. Results of *in silico* analysis of primer fit for UniPlantR for plant families across all three UK databases, where UniPlantF primer fit could not be tested due to short sequence lengths.

		UK Genus level		Turtle Dove database		Overall		
Family	Order	R tested	R matches	R tested	R matches	R tested	R matches	% match
Plantaginaceae	Lamiales	38	37	2	2	39	38	97
Platanaceae	Proteales	1	1			1	1	100
Plumbaginaceae	Caryophyllales	3	3			3	3	100
Poaceae	Poales	124	122	8	8	125	123	98
Polemoniaceae	Ericales	1	1			1	1	100
Polygalaceae	Fabales	2	2			2	2	100
Polygonaceae	Caryophyllales	17	17	2	2	17	17	100
Polypodiaceae	Polypodiales	1	0			1	0	0
Portulacaceae	Caryophyllales	1	1			1	1	100
Potamogetonaceae	Alismatales	14	14			14	14	100
Primulaceae	Ericales	13	13	1	1	13	13	100
Pteridaceae	Polypodiales	1	1			1	1	100
Ranunculaceae	Ranunculales	34	33			34	33	97
Resedaceae	Brassicales	2	2			2	2	100
Rhamnaceae	Rosales	3	3			3	3	100
Rosaceae	Rosales	93	90			93	90	97
Rubiaceae	Gentianales	4	4	1	1	4	4	100
Salicaceae	Malpighiales	16	16			16	16	100
Saxifragaceae	Saxifragales	15	15			15	15	100
Scheuchzeriaceae	Alismatales	1	1			1	1	100
Scrophulariaceae	Lamiales	5	5			5	5	100
Selaginellaceae	Selaginellales	1	1			1	1	100
Simaroubaceae	Sapindales	1	1			1	1	100

Table A1.3.2b. Results of *in silico* analysis of primer fit for UniPlantR for plant families across all three UK databases, where UniPlantF primer fit could not be tested due to short sequence lengths.

		UK Genus level		Turtle Dove database		Overall		
Family	Order	R tested	R matches	R tested	R matches	R tested	R matches	% match
Solanaceae	Solanales	10	10			10	10	100
Tamaricaceae	Caryophyllales	1	1			1	1	100
Taxaceae	Pinales	1	1			1	1	100
Thesiaceae	Santalales	1	1			1	1	100
Thymelaeaceae	Malvales	2	2			2	2	100
Tofieldiaceae	Alismatales	1	1			1	1	100
Typhaceae	Poales	4	4			4	4	100
Ulmaceae	Rosales	3	3			3	3	100
Urticaceae	Rosales	3	3	1	1	3	3	100
Verbenaceae	Lamiales	2	2			2	2	100
Violaceae	Malpighiales	12	12	2	2	12	12	100
Viscaceae	Santalales	1	1			1	1	100
Vitaceae	Vitales	1	0			1	0	0
Xanthorrhoeaceae	Asparagales	1	1			1	1	100
Zosteraceae	Alismatales	1	1			1	1	100
Total species		1385	1255	45	45	1390	1260	91
Total genera		643	603	40	40	645	605	94
Total families		128	119	17	17	128	119	93

Appendix 1.4 – Clustering analyses for full UniPlant amplicon

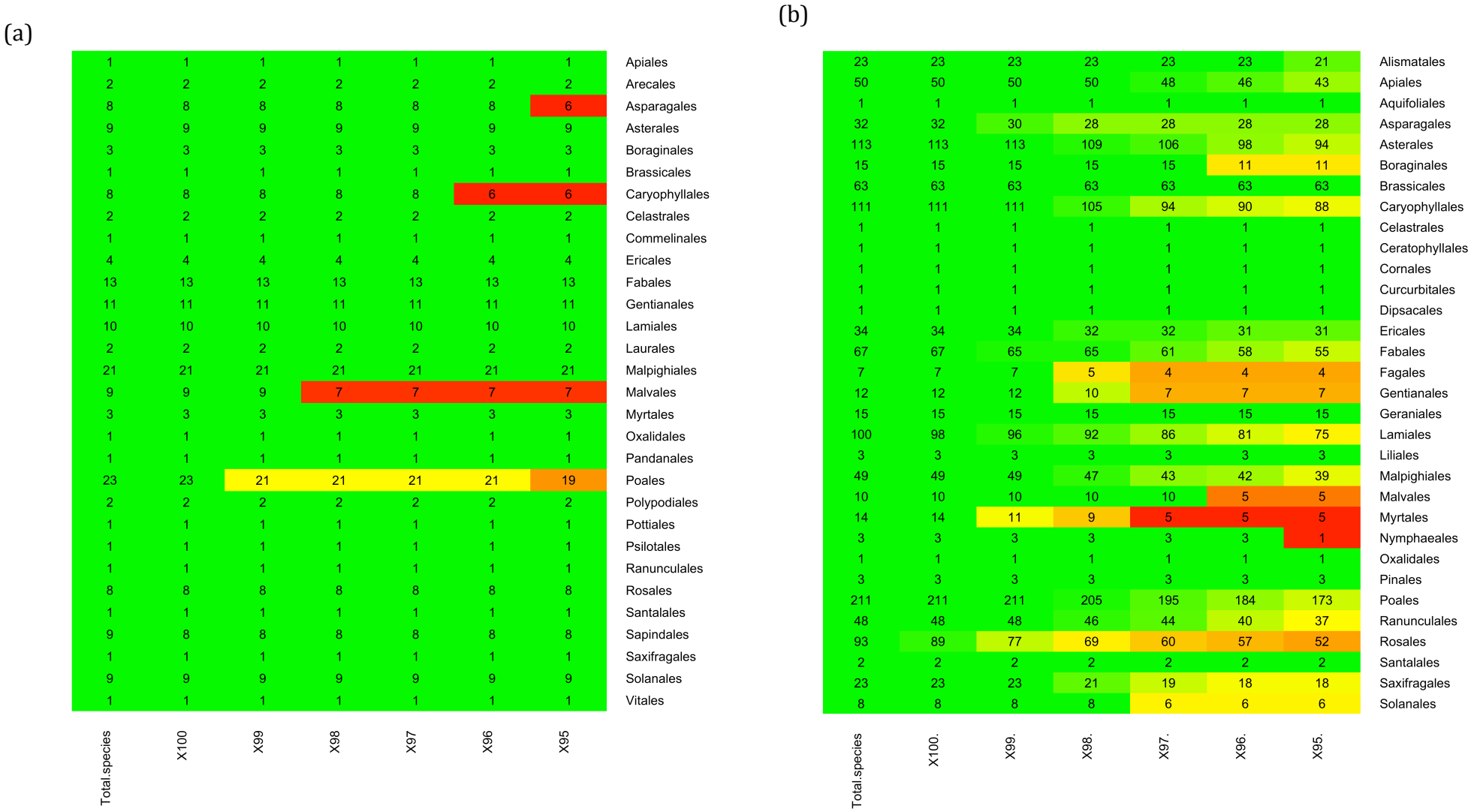


Figure. A1.4.1. Order-level summary of clustering thresholds for the full UniPlant amplicon between 95 and 100% for (a) Mauritian, n=167 species, and (b) UK databases, n=1116 species. Order names are listed on the y-axis and clustering threshold forms the x-axis. The colour of the cells represents the percentage of species within an order that can be identified to species level at a given clustering threshold. Colour gradient from green through to red signifies high species-level resolution moving towards poor species-level resolution.

Appendix 1.5 – List of Genbank accession numbers for the DNA sequences used for *in silico* analyses in this study

LC076491, LC076483, EU687533, AM920399-AM920403, KX165423-KX167996, KT948614-KT948638, KY700199-KY700571, KY700573-KY700576, KX689270-KX689363,AB000330.1, AB019948.1, AB022736.1, AB023983.1, AB032039.1, AB080562.1, AB088584.1, AB118124.1, AB120207.1, AB198348.1, AB248848.1, AB248857.1, AB261684.1, AB261687.1, AB292041.1, AB359790.1, AB359802.1, AB541095.1, AB683270.1, AB689040.1, AB851487.1, AB851493.1, AF009082.1, AF019790.1, AF019857.1, AF019873.1, AF031962.1, AF031964.1, AF037014.1, AF037624.1, AF040009.1, AF040063.1, AF040076.1, AF041343.1, AF041353.1, AF072485.1, AF077895.1, AF077900.1, AF077904.1, AF078032.1, AF088203.1, AF091952.1, AF115160.1, AF130839.1, AF136621.1, AF137539.1, AF158952.1, AF163401.1, AF163494.1, AF164001.1, AF165832.1, AF167196.1, AF169236.1, AF169757.1, AF183568.1, AF189730.1, AF209811.1, AF216544.1, AF218505.1, AF245429.1, AF245430.1, AF265281.1, AF272278.1, AF283487.1, AF301441.1, AF303026.1, AF313032.1, AF313035.1, AF318646.1, AF318715.1, AF336215.1, AF336371.1, AF351088.1, AF351121.1, AF358872.1, AF361301.1, AF367618.1, AF387520.1, AF401114.1, AF419000.1, AF422136.1, AF426378.1, AF448794.1, AF450226.1, AF450229.1, AF469682.1, AF478941.1, AF497647.1, AF497689.1, AF505631.1, AF513874.1, AF513875.1, AF513883.1, AF513888.1, AF517101.1, AF528453.1, AF528486.1, AF528490.1, AF528491.1, AF531080.1, AF540073.1, AF547727.1, AF551727.1, AJ011473.1, AJ011479.1, AJ222839.1, AJ251663.1, AJ304908.1, AJ310965.1, AJ310977.1, AJ310980.1, AJ347901.1, AJ347913.1, AJ420994.1, AJ427757.1, AJ438215.1, AJ491666.1, AJ491674.1, AJ511770.1, AJ536581.1, AJ539529.1, AJ548963.1, AJ548984.1, AJ550588.1, AJ579441.1, AJ580551.1, AJ580557.1, AJ626769.1, AJ633339.1, AJ633340.1, AJ633417.1, AJ633446.1, AJ633466.1, AJ633471.1, AJ633476.1, AJ744931.1, AJ746409.1, AJ862704.1, AJ868086.1, AM117024.1, AM267278.1, AM267287.1, AM287271.1, AM420677.1, AM503876.2, AM711744.1, AM711747.1, AM905721.1, AM905723.1, AM905724.1, AM905725.1, AM920396.1, AM943384.1, AY035750.1, AY049799.1, AY091574.1, AY092898.1, AY092900.1, AY092907.1, AY101281.1, AY146446.1, AY148270.1, AY148280.1, AY148284.1, AY176157.1, AY177603.1, AY179028.1, AY207370.1, AY236181.1, AY237921.1, AY254530.1, AY254531.1, AY254532.1, AY254541.1, AY254544.1, AY254545.1, AY263631.1, AY263679.1, AY265134.1, AY290016.1, AY290017.1, AY325280.1, AY328303.1, AY330707.1, AY331478.1, AY335961.1, AY335962.1, AY338946.1, AY341385.1, AY351379.1, AY351385.1, AY357769.1, AY357794.1, AY362767.1, AY380861.1, AY380868.1, AY438320.1, AY492098.1, AY492108.1, AY506652.1, AY508213.1, AY515398.1, AY524764.1, AY538636.1, AY548225.1, AY552528.1, AY554108.1, AY557232.1, AY558826.1, AY575439.1, AY581801.1, AY591277.1, AY594303.1, AY603257.1, AY616730.1, AY634778.1, AY635025.1, AY635034.1, AY665850.1, AY712663.1, AY722431.1, AY722459.1, AY722470.1, AY723256.1, AY731259.1,

AY740898.1, AY764040.1, AY764073.1, AY787405.1, AY839340.1, AY839344.1, AY857969.1, AY858597.1, AY870357.1, AY880236.1, AY880318.1, AY918196.1, AY926320.1, AY936278.1, AY974174.1, AY988396.1, AY996232.1, DQ005670.1, DQ005963.1, DQ005983.1, DQ005989.1, DQ005990.1, DQ006009.1, DQ006021.1, DQ006032.1, DQ006036.1, DQ006273.1, DQ022894.1, DQ059409.1, DQ066495.1, DQ074223.1, DQ092929.1, DQ180745.1, DQ184479.1, DQ217769.1, DQ224364.1, DQ249825.1, DQ249831.1, DQ249853.1, DQ250322.1, DQ276850.1, DQ277637.1, DQ304566.1, DQ304570.1, DQ310524.1, DQ310530.1, DQ310531.1, DQ311965.1, DQ311970.1, DQ312107.1, DQ314189.1, DQ336817.1, DQ336826.1, DQ336830.1, DQ336833.1, DQ340170.1, DQ340518.1, DQ354164.1, DQ354169.1, DQ357547.1, DQ385576.1, DQ435067.1, DQ444720.1, DQ451818.1, DQ467430.1, DQ467575.1, DQ468390.1, DQ518397.1, DQ539573.1, DQ539575.1, DQ539578.1, DQ539580.1, DQ539589.1, DQ539598.1, DQ539600.1, DQ642002.1, DQ667241.1, DQ667243.1, DQ667244.1, DQ667250.1, DQ667291.1, DQ667301.1, DQ683016.1, DQ888637.1, DQ912878.1, DQ912883.1, DQ975357.1, DQ987188.1, DQ996585.1, EF017402.1, EF057696.1, EF060391.1, EF065545.1, EF079387.1, EF090599.1, EF091578.1, EF127014.1, EF127040.1, EF142996.1, EF153087.1, EF185366.1, EF190030.1, EF195130.1, EF210971.1, EF368007.1, EF395535.1, EF397234.1, EF407926.1, EF416663.1, EF419452.1, EF427950.1, EF427951.1, EF436990.1, EF437224.1, EF494236.1, EF494737.1, EF517848.1, EF526367.1, EF526374.1, EF526385.1, EF538230.1, EF556350.1, EF560689.1, EF565132.1, EF577510.1, EF581935.1, EF635453.1, EF635458.1, EF635464.1, EF635471.1, EF660599.1, EU016360.1, EU070650.1, EU072550.1, EU102706.1, EU102747.1, EU179213.1, EU179215.1, EU179216.1, EU239681.1, EU239683.1, EU288566.1, EU307117.1, EU314901.1, EU331123.1, EU352241.1, EU352242.1, EU366281.1, EU391323.1, EU401308.1, EU592013.1, EU594911.1, EU606218.1, EU628253.1, EU655615.1, EU669091.1, EU687658.1, EU699446.1, EU707255.1, EU711228.1, EU720548.1, EU747245.1, EU785941.1, EU785983.1, EU792342.1, EU792364.1, EU812820.1, EU827106.1, EU850000.1, EU850027.1, EU850665.1, EU912092.1, FJ010604.2, FJ013226.1, FJ169534.1, FJ213869.1, FJ377658.1, FJ378588.1, FJ394658.1, FJ415110.1, FJ428646.1, FJ454870.1, FJ593180.1, FJ599757.1, FJ609733.1, FJ694190.1, FJ695466.1, FJ696962.1, FJ751757.1, FJ763189.1, FJ763192.1, FJ790040.1, FJ790048.1, FJ790050.1, FJ796906.1, FJ796919.1, FJ814654.1, FJ821024.1, FJ867401.1, FJ895960.1, FJ915103.2, FJ945920.1, FJ980316.1, FJ980324.1, FJ980347.1, FJ980407.1, FM243981.1, FM958516.1, FM995387.1, FM995395.1, FN263234.1, FN645827.1, FN645833.1, FN645882.1, FR715299.1, GQ285223.1, GQ285224.1, GQ285225.1, GQ285235.1, GQ373320.1, GQ379288.1, GQ395466.1, GQ464812.1, GQ464834.1, GQ464836.1, GQ464843.1, GQ470550.1, GQ470555.1, GQ478098.1, GQ478113.1, GQ856358.1, GQ862376.1, GQ888926.1, GQ901329.1, GU062347.1, GU067554.1, GU067564.1, GU176630.1, GU188570.1, GU217797.1, GU256780.1, GU323359.1, GU329698.1, GU350650.1, GU440883.1, GU444005.1, GU444008.1, GU444015.1, GU724280.1, GU724298.1, GU724302.1, GU818507.1, GU818542.1, GU818553.1, GU818558.1, GU818724.1, GU969621.1, GU977052.1, GU983033.1, HE586018.1, HE602438.1, HE602464.1, HE603307.1, HE603311.1, HE603315.1, HE603320.1, HF952973.1, HG315518.1, HG424173.1, HG518071.1, HG797486.1, HG797487.1, HG915798.1, HG934728.1, HM010981.1, HM049903.1, HM116949.1, HM176655.1, HM182015.1, HM193529.1, HM204888.1, HM235960.1, HM240422.1, HM357931.1, HM468330.1, HM468337.1, HM542614.1, HM587574.1,

HM593905.1, HQ142591.1, HQ176475.1, HQ284120.1, HQ288850.1, HQ393700.1, HQ407556.1, HQ414209.1, HQ414215.1, HQ442261.1, HQ445941.1, HQ456394.1, HQ456398.1, HQ456401.1, HQ456403.1, HQ456420.1, HQ600516.1, HQ600554.1, HQ615081.1, HQ658361.1, HQ687165.1, HQ823435.1, HQ858880.1, HQ858884.1, HQ858911.1, HQ859001.1, HQ896628.1, HQ917107.1, HQ993100.1, JF313179.1, JF331882.1, JF421553.1, JF780965.1, JF805747.1, JF900504.1, JF904803.1, JF907423.1, JF926362.1, JF976139.1, JF976297.1, JF976473.1, JF976634.1, JF977428.1, JF980331.1, JN009831.1, JN010036.1, JN113283.1, JN115012.1, JN115024.1, JN115053.1, JN234733.1, JN235076.1, JN235079.1, JN235087.1, JN235102.1, JN247411.1, JN315918.1, JN315931.1, JN375572.1, JN389222.1, JN400254.1, JN407499.1, JN407526.1, JN564914.1, JN575347.1, JN575417.1, JN578321.1, JN589051.1, JN589087.1, JN589095.1, JN589103.1, JN589107.1, JN589150.1, JN617194.1, JN680358.1, JN680360.1, JN696446.1, JN696453.1, JN900292.1, JQ011941.1, JQ033579.1, JQ041766.1, JQ062462.1, JQ062498.1, JQ218230.1, JQ230971.1, JQ230973.1, JQ230979.1, JQ283863.1, JQ388495.1, JQ388496.1, JQ388506.1, JQ388510.1, JQ392363.1, JQ392373.1, JQ392414.1, JQ392422.1, JQ392460.1, JQ392467.1, JQ405006.1, JQ424123.1, JQ669074.1, JQ669099.1, JQ669106.1, JQ669130.1, JQ669135.1, JQ669138.1, JQ776896.1, JQ792200.1, JQ895237.1, JQ898636.1, JX025227.1, JX073962.1, JX073965.1, JX078996.1, JX233505.1, JX233664.1, JX274203.1, JX274532.1, JX274538.1, JX464257.1, JX464294.1, JX467613.1, JX475146.1, JX576677.1, JX852437.1, JX867624.1, JX867643.1, KC156645.1, KC292629.1, KC455645.1, KC480352.1, KC535851.1, KC539599.1, KC575608.1, KC677958.1, KC677967.1, KC691712.1, KC691717.1, KC698935.1, KC812809.1, KC832358.1, KC861841.1, KC897915.1, KC897943.1, KC899568.1, KC922442.1, KC922448.1, KC952021.1, KC952713.1, KC958857.1, KC995016.1, KF022352.1, KF022626.1, KF022689.1, KF022704.1, KF022705.1, KF022719.1, KF137926.1, KF137936.1, KF150555.1, KF160912.1, KF163848.1, KF196320.1, KF201880.1, KF234635.1, KF265370.1, KF265388.1, KF270901.1, KF270902.1, KF301217.1, KF419387.1, KF447287.1, KF454227.1, KF454242.1, KF454259.1, KF454271.1, KF454296.1, KF454647.1, KF454681.1, KF482106.1, KF482108.1, KF493795.1, KF529544.1, KF544630.1, KF544886.1, KF547215.1, KF553460.1, KF704394.1, KF707563.1, KF713173.1, KF713204.1, KF718373.1, KF784875.1, KF806584.1, KF849103.1, KF850575.1, KF850588.1, KF850594.1, KF850608.1, KF850611.1, KF850613.1, KF861968.1, KF866381.1, KF873575.1, KF873765.1, KF897521.1, KF977441.1, KF983155.1, KF986733.1, KF993463.1, KF993513.1, KJ011903.1, KJ021874.1, KJ023678.1, KJ157634.1, KJ157635.1, KJ188998.1, KJ400904.1, KJ400936.1, KJ415356.1, KJ418133.1, KJ460085.1, KJ473882.1, KJ473895.1, KJ477049.1, KJ525046.1, KJ598893.1, KJ598933.1, KJ598938.1, KJ598940.1, KJ598941.1, KJ598943.1, KJ598945.1, KJ598947.1, KJ598985.1, KJ598998.1, KJ599003.1, KJ718098.1, KJ768883.1, KJ787175.1, KJ787202.1, KJ829446.1, KJ848154.1, KJ849660.1, KJ884130.1, KJ884178.1, KJ918495.1, KJ939168.1, KJ941156.1, KJ956490.1, KJ959254.1, KJ999384.1, KJ999385.1, KM010363.1, KM051437.1, KM051448.1, KM051450.1, KM051454.1, KM051455.1, KM051464.1, KM064889.1, KM073067.1, KM077298.1, KM113798.1, KM347931.1, KM484884.1, KM582605.1, KM875628.1, KM887371.1, KM887379.1, KM887393.1, KM887399.1, KM894999.1, KM999964.1, KP057065.1, KP098092.1, KP100268.1, KP120058.1, KP133672.1, KP159318.1, KP174260.1, KP231354.1, KP297495.1, KP297506.1,

KP334194.1, KP406141.1, KP682404.1, KP682406.1, KP682411.1, KP682414.1, KP725332.1, KP738156.1, KP751380.1, KP828810.1, KP871508.1, KP871514.1, KP873242.1, KP873338.1, KP873356.1, KP875310.1, KP972321.1, KR005087.1, KR005088.1, KR005155.1, KR011991.1, KR082780.1, KR870346.1, KT179623.1, KT249827.1, KT249843.1, KT249881.1, L11578.1, L35881.1, LC003517.1, LC027281.1, LC027919.1, LC076495.1, LC090002.1, LC090009.1, LM997566.1, LN610779.1, U24017.1, U27686.1, U30541.1, U30594.2, U56010.1, U63181.1, U63186.1, U74407.1, U78417.1, U78440.1, U88194.1, U88199.1, U90783.1, U90798.1, U90799.1, U90802.1, U96865.1, X63199.1, X98629.1, X98632.1, Z98279.1

Appendix Two – Supplementary information relating to Chapter Four

Appendix 2.1 – Modifications to the QIAGEN QIAmp® DNA stool kit protocol

The standard protocol was followed, including all recommended steps and the following modifications: (i) 36 – 44 mg of dried faecal material was used for each DNA extraction; (ii) each sample was ground dry using a single 3 mm Tungsten Carbide bead and a MP FastPrep®-24 bead beater at 5.5 m/s for 20 seconds; (iii) samples were vortexed with Buffer ASL for 30 minutes; (iv) samples were vortexed with the InhibitEX tablet for 5 minutes and then centrifuged for 6 minutes; (v) samples were incubated at 73˚C for 30 minutes with buffer AL; (vi) DNA was eluted twice with 100 µL Buffer AE after incubating at room temperature for 10 minutes each time.

Appendix 2.2 – Scripts used in the metabarcoding bioinformatics pipeline

Script 1 – Trimming

```
#Script written by Helen Hipperson

trimmomatic PE -phred33 Rose2_S2_R1_001.fastq.gz Rose2_S2_R2_001.fastq.gz
pool2_R1_trimmed_paired.fq lolpool2_R1_trimmed_unpaired.fq pool2_R2_trimmed_paired.fq
lolpool2_R2_trimmed_unpaired.fq ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10 LEADING:3
TRAILING:3 SLIDINGWINDOW:4:20 MINLEN:135

fastqc pool2_R1_trimmed_paired.fq --outdir=./
fastqc pool2_R2_trimmed_paired.fq --outdir=./
```

Script 2 – Align paired reads to obtain complete amplicon sequence

```
#Script written by Helen Hipperson

/usr/local/extras/Genomics/workshops/EOS2015/FLASH-1.2.11/flash
pool2_R1_trimmed_paired.fq pool2_R2_trimmed_paired.fq -M 250 > flash_out

# convert the fastq file to fasta format

fastq_to_fasta -i out.extendedFrags.fastq -Q 33 > pool2_aligned.fa
```

Script 3 – Allocate MID-tag combinations to samples and remove primer sequences

```
#Script written by Helen Hipperson

#Oligos_Pool2 is a text file where the first column reads #‘primer’, the second and third columns
are the forward and #reverse primer and MID-tag combinations for a particular #sample, and the
fourth column is the sample ID annotated with #an additional ‘a’ or ‘b’. ‘a’ is used when the
forward primer #is in column 2 and the reverse is in column 3. ‘b’ is used #when this order is
reversed. This means that the total number #of rows should be twice the number of samples.
```

```
mothur "#trim.seqs(fasta=pool2_aligned.fa,oligos=Oligos_Pool2,pdiffs=1,checkorient=T"
```

Script 4 – Demultiplex part 1

```
#Script written by Helen Hipperson

#SampleList_Pool2_deplex1.txt is a text that is identical to #the fourth column of the
Oligos_Pool2 file described in #Script 3

#!/usr/bin/perl


unless ($#ARGV == 0)

{

  print "Usage: deplex_v2.pl SampleList_Pool2_deplex1.txt";

die;
}


open (INLIST, "<$ARGV[0]") || die;

$indir = "/fastdata/bo4rmg/Pool2Scratch";
$outdir = "/fastdata/bo4rmg/Pool2Scratch/demultiplexed";

# Loops through the list for your samples ('SampleList') and performs the commands for each
one
while (<INLIST>) {
$lib = $_;
chomp($lib);

# A shortcut to read or write a file for each of your samples, each file having the same extension
$readids1 = $lib . "_ids.txt";
$fa1 = $lib . ".fasta";


# split fasta read IDs into files grouped by sample ID. Replace 'XX' with the name of you '.groups'
file (output from mothur)
system("grep -w $lib $indir/pool2_aligned.groups | awk '{print \$1}' > $outdir/$readids1");

}

exit;
```

Script 5 – Demultiplex part 2

```
#Script written by Helen Hipperson

#SampleListPool2_demultiplex2.txt is a text file containing #just a list of sample IDs with no ‘a’ or
‘b’ annotations. #This means that the number of rows should be identical to the #sample number.

#!/usr/bin/perl
```

```
unless ($#ARGV == 0)

{

  print "Usage: deplex_v2b.pl SampleListPool2_demultiplex2.txt";

die;
}


open (INLIST, "<$ARGV[0]") || die;

$indir =  "/fastdata/bo4rmg/Pool2Scratch/demultiplexed";
$outdir = "/fastdata/bo4rmg/Pool2Scratch/demultiplexed";

# Loops through the list for your samples ('SampleList') and performs the commands for each
one
while (<INLIST>) {
$lib = $_;
chomp($lib);

# A shortcut to read or write a file for each of your samples, each file having the same extension
$fa1 = $lib . ".fasta";

$readidsa = $lib . "a_ids.txt";
$readidsb = $lib . "b_ids.txt";

$readids2 = $lib . "_ab_ids.txt";


# combine the list of sequence names for 'a' and 'b' matches
system("cat $outdir/$readidsa $outdir/$readidsb >> $outdir/$readids2");

# split the trimmed fasta file into reads specific to each sample. Replace 'XX' with the name of
your trimmed fasta file (output from mothur)
my $command1 = 'perl -ne'."'".'if(/^>(\S+)/){$c=$i{$1}}$c?print:chomp;$i{$_}=1 if'." @ARGV'"."
$outdir/$readids2 $indir/pool2_aligned.trim.fasta > $outdir/$fa1";


system ($command1);

}

exit;
```

Script 6 – USEARCH

```
#Script written by Helen Hipperson

#outputs non-chimeric sequences and discards identical #sequences that occur less than ten
times

#!/usr/bin/perl


unless ($#ARGV == 0)
```

```
{

  print "Usage: usearch.pl SampleListPool2_demultiplex2.txt\n";

die;
}


open (INLIST, "<$ARGV[0]") || die;


$indir = "/fastdata/bo4rmg/Pool2Scratch/demultiplexed";

$outdir = "/fastdata/bo4rmg/Pool2Scratch/demultiplexed";

while (<INLIST>) {
$lib = $_;
chomp($lib);

$fa = $lib . ".fasta";

$usout1 = $lib . "_rc_uniques.fasta";
$usout2 = $lib . "_rc_uniques.out";
$usout3 = $lib . "_rc_uniques_results.uchime";
$usout4 = $lib . "_chimeras.fasta";
$usout5 = $lib . "_nonchimeras.fasta";
$usout6 = $lib . "_uchimealns";


$cent = $lib . "_centroids.fa";
$uc = $lib . "_clusters.uc";
$cons = $lib . "_consout.fa";
$msa = $lib . "_msa.fa";



# removes identical replicates from the fasta input, output for next step =
SampleName_rc_uniques.fasta

system("/usr/local/extras/Genomics/apps/usearch/7.0.1090/usearch -derep_fulllength
$indir/$fa -output $outdir/$usout1 -sizeout -minseqlength 187 -minuniquesize 10 -strand both -
uc $outdir/$usout2");

# chimera detection, output for next step = SampleName_nonchimeras.fasta

system("/usr/local/extras/Genomics/apps/usearch/7.0.1090/usearch -uchime_denovo
$outdir/$usout1 -uchimeout $outdir/$usout3 -uchimealns $outdir/usout6 -chimeras
$outdir/$usout4 -nonchimeras $outdir/$usout5");


}

exit;
```

Script 7 – Edit the header of each sequence

```
#Script written by Helen Hipperson
```

```
#!/bin/bash

#Settings for the Sun Grid Engine

# run time for job in hours:mins:sec (max 168:0:0, jobs with hurt < 8:0:0 have priority)

#$ -l h_rt=7:59:59

# request memory for job (default 4G, max 32G)

#$ -l mem=8G

#number of files
##$ -pe openmp 4

#$ -m e

# give the job a name (optional):

#$-N edit_headers

#

sed 's/^>/>A01_/g' A01_nonchimeras.fasta > A01_nonchimeras_edited.fa
sed 's/^>/>A02_/g' A02_nonchimeras.fasta > A02_nonchimeras_edited.fa
sed 's/^>/>A03_/g' A03_nonchimeras.fasta > A03_nonchimeras_edited.fa
sed 's/^>/>A04_/g' A04_nonchimeras.fasta > A04_nonchimeras_edited.fa
sed 's/^>/>A05_/g' A05_nonchimeras.fasta > A05_nonchimeras_edited.fa
sed 's/^>/>A06_/g' A06_nonchimeras.fasta > A06_nonchimeras_edited.fa
sed 's/^>/>A07_/g' A07_nonchimeras.fasta > A07_nonchimeras_edited.fa
sed 's/^>/>A08_/g' A08_nonchimeras.fasta > A08_nonchimeras_edited.fa
sed 's/^>/>A09_/g' A09_nonchimeras.fasta > A09_nonchimeras_edited.fa

#....for each sample ID
```

Script 8 – Concatenate all sequences into one file

```
cat *_nonchimeras_edited.fa > All.NonChimeras.fa
```

Script 9 – Trim to ITS2 (remove flanking regions)

```
./ITSx -i
/Users/Rosemary/Documents/PhD/NBAF/Pool2/Trimming.to.ITS2_Pool2/All.NonChimeras.fa
-o ITS2Allnonchimeras.fa --reset T
```

Script 10 – Dereplicate: output all unique sequences

```
#Script written by Helen Hipperson

usearch -derep_fulllength ITS2Allnonchimeras.fa -output All_ITS2uniquesnonchimeras.fasta -
sizeout -strand both -uc All_ITS2uniques.out
```

Script 11 –Create a matrix of sample number against unique sequence (OTU) ID in R

```
x <- read.csv(file.choose())
#create this .csv file from the All_ITS2uniques.out file generated in #Script 10

 x_table <- table(x)
 write.table(x_table,"OTU_table.txt")
```

Script 12 – Assign a number to all unique sequences

```
#Script written by Helen Hipperson

#To use:
python fasta_number.py All_ITS2uniquesnonchimeras.fasta >
All_Pool2uniquesnonchimeras_numbered.fasta

#The following is 'fasta_number.py':

#!/usr/bin/python

import sys
#import die

Prefix = ""
if len(sys.argv) > 2:
        Prefix = sys.argv[2]

NeedSize = 0
if len(sys.argv) > 3:
        if sys.argv[3] == "-needsize":
                NeedSize = 1
        elif sys.argv[3] == "-nosize":
                NeedSize = 0
        else:
                die.Die("Must specify -needsize or -nosize")

def GetSize(Label):
        Fields = Label.split(";")
        for Field in Fields:
                if Field.startswith("size="):
                        return int(Field[5:])
        print >> sys.stderr
        print >> sys.stderr, "Size not found in label: " + Label
        sys.exit(1)

File = open(sys.argv[1])
N = 0
while 1:
        Line = File.readline()
        if len(Line) == 0:
                break
        Line = Line[:-1]
        if len(Line) == 0:
                continue
        if Line[0] == '>':
                N += 1
                if NeedSize:
                        Label = Line[1:].strip()
```

```
                Size = GetSize(Label)
                print ">%s%u;size=%u;" % (Prefix, N, Size)
        else:
                print ">%s%u" % (Prefix, N)
    else:
        print Line
```

Script 13 – Fill in the matrix with BLASTn results based on BIT score

```
#Script written by Oliver Remington

#Script fills in the matrix created in Script 11 with BLASTn #results
#For use use:

perl matrix_merge.pl blastfile.txt OTU_table_Non-chimeras.csv duplicates.txt matrix.csv
counts.txt

#duplicates.txt will the be the output file of things that have #multiple matches (more than one
taxa with the same BIT score)

#matrix.csv will be the filled in matrix that you can open again in #excel

#counts.txt will be the list of 'otu' counts


#The following is 'matrix_merge.pl'

use strict;
use warnings;

open BLAST, $ARGV[0] or die $!;

my $oldID = "";
my $oldNam = "";
my $canKeep = 1;

my %otuHash;
my %countHash;
my $cnt = 0;

while(<BLAST>)
{
        if($. > 1)
        {
                chomp;
                my @sps = split(/\t/);
                print $sps[0] . "\t" . $oldID . "\t" . $sps[2] . "\t" . $oldNam . "\n";
                if($sps[0] eq $oldID && $sps[2] ne $oldNam)
                {
                        $canKeep = 0;
                        print $sps[0] . "killed\n";
                }
                if($sps[0] ne $oldID && $cnt > 2)
                {
                        if($canKeep == 1)
                        {
                                $otuHash{$oldID} = $oldNam;
                                if(exists($countHash{$oldNam}))
                                {
```

```
                                        $countHash{$oldNam}++;
                                } else {
                                        $countHash{$oldNam} = 1;
                                }
                        }
                        $canKeep = 1;
                }
                $cnt++;
                $oldID = $sps[0];
                $oldNam = $sps[2];
        }
}
close BLAST;

open BLAST, $ARGV[0] or die $!;
open my $blastout, '>', $ARGV[2] or die $!;

while(<BLAST>)
{
        chomp;
        if($. > 1)
        {
                my @sps = split(/\t/);
                if(!exists $otuHash{$sps[0]})
                {
                        print {$blastout} $sps[0] . "\t" . $sps[2] . "\n";
                }
        }
}

close $blastout;
close BLAST;

open MATRIX, $ARGV[1] or die $!;
open my $matrixout, '>', $ARGV[3] or die $!;

while(<MATRIX>)
{
        chomp;
        my $line = $_;

        if($. > 6)
        {
                my @sps = split(/\t/);
                if(exists $otuHash{$sps[0]})
                {
                        $sps[1] = $otuHash{$sps[0]};
                }
                else {
                        $sps[1] = " ";
                }
                print {$matrixout} join("\t", @sps) . "\n";
        }
        else {
                print {$matrixout} $line . "\n";
        }

        my @sps = split(/\t/)
}
```

```
close MATRIX;
close $matrixout;

open my $countout, '>', $ARGV[4] or die $!;

my @keyset = keys %countHash;

foreach(@keyset)
{
	my $plant = $_;

	print {$countout} $plant . "\t" . $countHash{$plant} . "\n";
}

close $countout;
```

Script 14 – Calculate the total number of reads for each OTU

```
#Script written by Dave Stanton

#Required because unique sequences were grouped both before and after #trimming to ITS2
only

#To use:
python ros_sc1.py

#The following is 'ros_sc1.py':

import pandas
import re

colnames = ['H_S', 'Pro_Lang', 'out_num', 'Seq_info']
data = pandas.read_csv("OUTfileTrimmedtoITS2.csv", names=colnames )
out_num = data.out_num.tolist()
Seq_info = data.Seq_info.tolist()
size = []
size_num = []
ID = []
uniques = 0
cumulative = 0

newlist_out_num = []
newlist_ID = []
newlist_sizenum = []
newlist_ref = []

size = [i.split( ';' )[1:2] for i in Seq_info]

for k in range(0, len(size) ):
   current = str( size[k] )
   size_num.append( re.sub( "\D", "", current ) )

ID = [i.split( '_' )[0] for i in Seq_info]

for k in range(2, len(size) ):
   if ID[k] != ID[k-1] or out_num[k] != out_num[k-1]:
```

```
      uniques = uniques + 1
      newlist_out_num.append( out_num[k-1] )
      newlist_ID.append( ID[k-1] )
      newlist_sizenum.append( int(size_num[k-1]) + int( cumulative ) )
      newlist_ref.append( uniques )
      cumulative = 0
   else:
      cumulative = cumulative + int( size_num[k-1] )

for j in range(0, len(newlist_ref) ):
   print( newlist_ref[j], newlist_out_num[j] , newlist_ID[j], newlist_sizenum[j] )
```

Script 15 – Identify reads occurring due to sequencing artifacts/contamination

```
#Script written by Dave Stanton

#This script identifies OTUs from samples where the read number is #not greater to what was
found in the negative samples for that OUT

#Uses the output from Script 14 and a text file where the first #column is the OTU number and
the second column is the maximum number #of reads for that OTU across all negative samples

import pandas            #Don't think that I actually used this library
import re

colnames = ['ref_ID', 'OTU', 'ID', 'depth']
colnamesNEG = ['OTU_neg', 'depth_neg']
data = pandas.read_table("unique.out", names=colnames, sep=r"\s+")
data_negs = pandas.read_table("OTU_ReadNo_Negatives.txt", names=colnamesNEG, sep=r"\s+")

ref_ID = data.ref_ID.tolist()          #Reading column into list
OTU = data.OTU.tolist()          #Reading column into list
ID = data.ID.tolist()          #Reading column into list
depth = data.depth.tolist()          #Reading column into list

OTU_neg = data_negs.OTU_neg.tolist()          #Reading column into list
depth_neg = data_negs.depth_neg.tolist()          #Reading column into list


for k in range(0, ( len(OTU_neg) - 1) ):
   for j in range(0, ( len(OTU) - 1) ):
      if int( OTU[j] ) == int( OTU_neg[k] ) and int( depth[j] ) < int( depth_neg[k] ):
         print(  OTU[j], ID[j], "original_depth=", depth[j], "threshold_was=", depth_neg[k],
"new_depth= 0" )
```

Script 15 – Modify the matrix to account for artifacts/contamination and collapse

```
#Set of three python scripts written by Dave Stanton

#This set of scripts modify the matrix to remove any spurious OTUs #from each sample based on
the output from Script 14

#The scripts also collapse the matrix: currently multiple haplotypes #for the same taxa are listed.
This script collapses them into one #taxon
```

#For use run the following shell script:

```
#!/bin/bash

python problem3-1.py > out_3-1.out
sed 's/,$//g' out_3-1.out | sed '/^$/d' > out_3-1-f1.out
head -1 out_3-1-f1.out > out_3-1-formatted.out
tail -3557 out_3-1-f1.out | sort --field-separator=',' --key=2 >> out_3-1-formatted.out
python problem3-2.py > out_3-2.out
sed 's/,$//g' out_3-2.out | sed '/^$/d' > out_3-2-f1.out
head -1 out_3-2-f1.out > out_3-2-formatted.out
tail -207 out_3-2-f1.out | sort -n --field-separator=',' --key=1 >> out_3-2-formatted.out
python problem3-3.py > out_3-3.out
sed 's/,$//g' out_3-3.out | sed '/^$/d' > out_3-3-f1.out
head -1 out_3-3-f1.out > out_3-3-formatted.out
tail -207 out_3-3-f1.out | sort -n --field-separator=',' --key=1 >> out_3-3-formatted.out
rm out_3-1-f1.out
rm out_3-2-f1.out
rm out_3-3-f1.out
rm out_3-1.out
rm out_3-2.out
rm out_3-3.out
```

#The following script is 'problem3-1.py':

```
import pandas          #Don't think that I actually used this library
import re
import csv
import numpy

colnames = ['OUT_no', 'taxon_name', 'A01', 'A02', 'A03', 'A04', 'A05', 'A06', 'A07', 'A08', 'A09',
'A10', 'A11', 'A12', 'A13', 'A14', 'A15', 'A16', 'A17', 'A18', 'A19', 'A20', 'A22', 'A23', 'A24', 'A25',
'A26', 'A27', 'A28', 'A29', 'A30', 'A31', 'A32', 'A33', 'A34', 'A35', 'A36', 'A37', 'A38', 'A39', 'A40',
'A41', 'A42', 'A43', 'A44', 'A46', 'A47', 'A48', 'A49', 'A50', 'A51', 'A52', 'A53', 'A54', 'A55', 'A56',
'A57', 'A58', 'A59', 'A60', 'A61', 'A62', 'A63', 'A64', 'A65', 'A66', 'A67', 'A68', 'A70', 'A71', 'A72',
'A73', 'A74', 'A75', 'A76', 'A77', 'A78', 'A79', 'A80', 'A81', 'A82', 'A83', 'A84', 'A85', 'A86', 'A87',
'A88', 'A89', 'A90', 'A91', 'A92', 'A94', 'A95', 'A96', 'B01', 'B02', 'B03', 'B04', 'B05', 'B06', 'B07',
'B08', 'B09', 'B10', 'B11', 'B12', 'B13', 'B14', 'B15', 'B16', 'B17', 'B18', 'B19', 'B20', 'B22', 'B23',
'B24', 'B25', 'B26', 'B27', 'B28', 'B29', 'B30', 'B31', 'B32', 'B33', 'B34', 'B35', 'B36', 'B37', 'B38',
'B39', 'B40', 'B41', 'B42', 'B43', 'B44', 'B46', 'B47', 'B48', 'B49', 'B50', 'B51', 'B52', 'B53', 'B54',
'B55', 'B56', 'B57', 'B58', 'B59', 'B60', 'B61', 'B62', 'B63', 'B64', 'B65', 'B66', 'B67', 'B68', 'B70',
'B71', 'B72', 'B73', 'B74', 'B75', 'B76', 'B77', 'B78', 'B79', 'B80', 'B81', 'B82', 'B83', 'B84', 'B85',
'B86', 'B87', 'B88', 'B89', 'B90', 'B91', 'B92', 'B94', 'B95', 'B96', 'C01', 'C02', 'C03', 'C04', 'C05', 'C06',
'C07', 'C08', 'C09', 'C10', 'C11', 'C12', 'C13', 'C14', 'C15', 'C16', 'C17', 'C18', 'C19', 'C20', 'C22', 'C23',
'C24', 'C25', 'C26', 'C27', 'C28', 'C29', 'C30', 'C31', 'C32', 'C33', 'C34', 'C35', 'C36', 'C37', 'C38', 'C39',
'C40', 'C41', 'C42', 'C43', 'C44', 'C46', 'C47', 'C48', 'C49', 'C50', 'C51', 'C52', 'C53', 'C54', 'C55', 'C56',
'C57', 'C58', 'C59', 'C60', 'C61', 'C62', 'C63', 'C64', 'C65', 'C66', 'C67', 'C68', 'C70', 'C71', 'C72', 'C73',
'C74', 'C75', 'C76', 'C77', 'C78', 'C79', 'C80', 'C81', 'C82', 'C83', 'C84', 'C85', 'C86', 'C87', 'C88', 'C89',
'C90', 'C91', 'C92', 'C94', 'C95', 'C96', 'D01', 'D02', 'D03', 'D04', 'D05', 'D06', 'D07', 'D08', 'D09',
'D10', 'D11', 'D12', 'D13', 'D14', 'D15', 'D16', 'D17', 'D18', 'D19', 'D20', 'D22', 'D23', 'D24', 'D25',
'D26', 'D27', 'D28', 'D29', 'D30', 'D31', 'D32', 'D33', 'D34', 'D35', 'D36', 'D37', 'D38', 'D39', 'D40',
'D41', 'D42', 'D43', 'D44', 'D46', 'D47', 'D48', 'D49', 'D50', 'D51', 'D52', 'D53', 'D54', 'D55', 'D56',
'D57', 'D58', 'D59', 'D60', 'D61', 'D62', 'D63', 'D64', 'D65', 'D66', 'D67', 'D68', 'D70', 'D71', 'D72',
'D73', 'D74', 'D75', 'D76', 'D77', 'D78', 'D79', 'D80', 'D81', 'D82', 'D83', 'D84', 'D85', 'D86', 'D87',
'D88', 'D89', 'D90', 'D91', 'D92', 'D94', 'D95', 'D96', 'E24', 'E25', 'E26', 'E27', 'E28', 'E29', 'E30',
'E31', 'E32', 'E33', 'E34', 'E35', 'E36', 'E37', 'E38', 'E39', 'E40', 'E41', 'E42', 'E43', 'E44', 'E46', 'E47',
'E48', 'E49', 'E50', 'E51', 'E52', 'E53', 'E54', 'E55', 'E56', 'E57', 'E58', 'E59', 'E60', 'E61', 'E62', 'E63',
'E64', 'E65', 'E66', 'E67', 'E68', 'E70', 'E71', 'E72', 'E73', 'E74', 'E75', 'E76', 'E77', 'E78', 'E79', 'E80',
```

```
'E81', 'E82', 'E83', 'E84', 'E85', 'E86', 'E87', 'E88', 'E89', 'E90', 'E91', 'E92', 'E94', 'E95', 'E96', 'F01',
'F02', 'F03', 'F04', 'F05', 'F06', 'F07', 'F08', 'F09', 'F10', 'F11', 'F12', 'F13', 'F14', 'F15', 'F16', 'F17',
'F18', 'F19', 'F20', 'F22', 'F23', 'F24', 'F25', 'F26', 'F27', 'F28', 'F29', 'F30', 'F31', 'F32', 'F33', 'F34',
'F35', 'F36', 'F37', 'F38', 'F39', 'F40', 'F41', 'F42', 'F43', 'F44', 'F46', 'F47', 'F48', 'F49', 'F50', 'F51',
'F52', 'F53', 'F54', 'F55', 'F56', 'F57', 'F58', 'F59', 'F60', 'F61', 'F62', 'F63', 'F64', 'F65', 'F66', 'F67',
'F68', 'F70', 'F71', 'F72', 'F73', 'F74', 'F75', 'F76', 'F77', 'F78', 'F79', 'F80', 'F81', 'F82', 'F83', 'F84',
'F85', 'F86', 'F87', 'F88', 'F89', 'F90', 'F91', 'F92', 'F94', 'F95', 'F96', 'Totals']
#colnames_NEW = [ OUT_no[0, 1, 2], taxon_name[], A01[], A02[], A03[], A04[], A05[], A06[],
A07[], A08[], A09[], A10[], A11[], A12[], A13[], A14[], A15[], A16[], A17[], A18[], A19[], A20[],
A22[], A23[], A24[], A25[], A26[], A27[], A28[], A29[], A30[], A31[], A32[], A33[], A34[], A35[],
A36[], A37[], A38[], A39[], A40[], A41[], A42[], A43[], A44[], A46[], A47[], A48[], A49[], A50[],
A51[], A52[], A53[], A54[], A55[], A56[], A57[], A58[], A59[], A60[], A61[], A62[], A63[], A64[],
A65[], A66[], A67[], A68[], A70[], A71[], A72[], A73[], A74[], A75[], A76[], A77[], A78[], A79[],
A80[], A81[], A82[], A83[], A84[], A85[], A86[], A87[], A88[], A89[], A90[], A91[], A92[], A94[],
A95[], A96[], B01[], B02[], B03[], B04[], B05[], B06[], B07[], B08[], B09[], B10[], B11[], B12[],
B13[], B14[], B15[], B16[], B17[], B18[], B19[], B20[], B22[], B23[], B24[], B25[], B26[], B27[],
B28[], B29[], B30[], B31[], B32[], B33[], B34[], B35[], B36[], B37[], B38[], B39[], B40[], B41[],
B42[], B43[], B44[], B46[], B47[], B48[], B49[], B50[], B51[], B52[], B53[], B54[], B55[], B56[],
B57[], B58[], B59[], B60[], B61[], B62[], B63[], B64[], B65[], B66[], B67[], B68[], B70[], B71[],
B72[], B73[], B74[], B75[], B76[], B77[], B78[], B79[], B80[], B81[], B82[], B83[], B84[], B85[],
B86[], B87[], B88[], B89[], B90[], B91[], B92[], B94[], B95[], B96[], C01[], C02[], C03[], C04[],
C05[], C06[], C07[], C08[], C09[], C10[], C11[], C12[], C13[], C14[], C15[], C16[], C17[], C18[],
C19[], C20[], C22[], C23[], C24[], C25[], C26[], C27[], C28[], C29[], C30[], C31[], C32[], C33[],
C34[], C35[], C36[], C37[], C38[], C39[], C40[], C41[], C42[], C43[], C44[], C46[], C47[], C48[],
C49[], C50[], C51[], C52[], C53[], C54[], C55[], C56[], C57[], C58[], C59[], C60[], C61[], C62[],
C63[], C64[], C65[], C66[], C67[], C68[], C70[], C71[], C72[], C73[], C74[], C75[], C76[], C77[],
C78[], C79[], C80[], C81[], C82[], C83[], C84[], C85[], C86[], C87[], C88[], C89[], C90[], C91[],
C92[], C94[], C95[], C96[], D01[], D02[], D03[], D04[], D05[], D06[], D07[], D08[], D09[], D10[],
D11[], D12[], D13[], D14[], D15[], D16[], D17[], D18[], D19[], D20[], D22[], D23[], D24[], D25[],
D26[], D27[], D28[], D29[], D30[], D31[], D32[], D33[], D34[], D35[], D36[], D37[], D38[], D39[],
D40[], D41[], D42[], D43[], D44[], D46[], D47[], D48[], D49[], D50[], D51[], D52[], D53[], D54[],
D55[], D56[], D57[], D58[], D59[], D60[], D61[], D62[], D63[], D64[], D65[], D66[], D67[], D68[],
D70[], D71[], D72[], D73[], D74[], D75[], D76[], D77[], D78[], D79[], D80[], D81[], D82[], D83[],
D84[], D85[], D86[], D87[], D88[], D89[], D90[], D91[], D92[], D94[], D95[], D96[], E24[], E25[],
E26[], E27[], E28[], E29[], E30[], E31[], E32[], E33[], E34[], E35[], E36[], E37[], E38[], E39[],
E40[], E41[], E42[], E43[], E44[], E46[], E47[], E48[], E49[], E50[], E51[], E52[], E53[], E54[],
E55[], E56[], E57[], E58[], E59[], E60[], E61[], E62[], E63[], E64[], E65[], E66[], E67[], E68[],
E70[], E71[], E72[], E73[], E74[], E75[], E76[], E77[], E78[], E79[], E80[], E81[], E82[], E83[],
E84[], E85[], E86[], E87[], E88[], E89[], E90[], E91[], E92[], E94[], E95[], E96[], F01[], F02[],
F03[], F04[], F05[], F06[], F07[], F08[], F09[], F10[], F11[], F12[], F13[], F14[], F15[], F16[], F17[],
F18[], F19[], F20[], F22[], F23[], F24[], F25[], F26[], F27[], F28[], F29[], F30[], F31[], F32[], F33[],
F34[], F35[], F36[], F37[], F38[], F39[], F40[], F41[], F42[], F43[], F44[], F46[], F47[], F48[], F49[],
F50[], F51[], F52[], F53[], F54[], F55[], F56[], F57[], F58[], F59[], F60[], F61[], F62[], F63[], F64[],
F65[], F66[], F67[], F68[], F70[], F71[], F72[], F73[], F74[], F75[], F76[], F77[], F78[], F79[], F80[],
F81[], F82[], F83[], F84[], F85[], F86[], F87[], F88[], F89[], F90[], F91[], F92[], F94[], F95[], F96[],
Totals[] ]
#colnames_NEW = []

#sc2_colnames = ['OTU_sc2', 'ID_sc2', 'depth_sc2']

sc2out = 'sc2.out'
sc2 = list(csv.reader(open(sc2out)))

poolfile = 'Pool1MatrixForPuzzle3_noHead.csv'
pool = list(csv.reader(open(poolfile)))


for taxon in range( 0, ( len(colnames)) ):
   for k in range( 0, 2523 ):
```

```
      if colnames[taxon] == sc2[k][1]:
         for j in range(1, 3558):
            if int( pool[j][0] ) == int( sc2[k][0] ):
               pool[j][taxon] = sc2[k][2]


for rowprint in range(0, 3558):
   [ print( pool[rowprint][val], end=",") for val in range(0, 533) ]
   print( "\n" )

#                print( sc2[k][1], colnames[taxon] )



#       for j in range( 0, ( len(ID_sc2) - 1) ):
#          if taxon == ID_sc2[j] and OUT_no[k] == OTU_sc2[j]:
#             test
#             print( taxon , ID_sc2[j] , "OTU_sc2=", OTU_sc2[j], "OTU_TestPool=", OUT_no[k] )
#             print( OUT_no[k], taxon_name[k],


#for i in range(0, 10):
#    print( OUT_no[i], taxon_name[i], A01[i] )

#ref_ID = data.ref_ID.tolist()            #Reading column into list
#OTU = data.OTU.tolist()            #Reading column into list
#ID = data.ID.tolist()            #Reading column into list
#depth = data.depth.tolist()            #Reading column into list

#OTU_neg = data_negs.OTU_neg.tolist()            #Reading column into list
#depth_neg = data_negs.depth_neg.tolist()            #Reading column into list


#for k in range(0, ( len(OTU_neg) - 1) ):
#    for j in range(0, ( len(OTU) - 1) ):
#       if int( OTU[j] ) == int( OTU_neg[k] ) and int( depth[j] ) < int( depth_neg[k] ):
#          print(  OTU[j], ID[j], "0" )
```

#The following script is 'problem3-2.py':

```
import csv

colnames = ['OUT_no', 'taxon_name', 'A01', 'A02', 'A03', 'A04', 'A05', 'A06', 'A07', 'A08', 'A09',
'A10', 'A11', 'A12', 'A13', 'A14', 'A15', 'A16', 'A17', 'A18', 'A19', 'A20', 'A22', 'A23', 'A24', 'A25',
'A26', 'A27', 'A28', 'A29', 'A30', 'A31', 'A32', 'A33', 'A34', 'A35', 'A36', 'A37', 'A38', 'A39', 'A40',
'A41', 'A42', 'A43', 'A44', 'A46', 'A47', 'A48', 'A49', 'A50', 'A51', 'A52', 'A53', 'A54', 'A55', 'A56',
'A57', 'A58', 'A59', 'A60', 'A61', 'A62', 'A63', 'A64', 'A65', 'A66', 'A67', 'A68', 'A70', 'A71', 'A72',
'A73', 'A74', 'A75', 'A76', 'A77', 'A78', 'A79', 'A80', 'A81', 'A82', 'A83', 'A84', 'A85', 'A86', 'A87',
'A88', 'A89', 'A90', 'A91', 'A92', 'A94', 'A95', 'A96', 'B01', 'B02', 'B03', 'B04', 'B05', 'B06', 'B07',
'B08', 'B09', 'B10', 'B11', 'B12', 'B13', 'B14', 'B15', 'B16', 'B17', 'B18', 'B19', 'B20', 'B22', 'B23',
'B24', 'B25', 'B26', 'B27', 'B28', 'B29', 'B30', 'B31', 'B32', 'B33', 'B34', 'B35', 'B36', 'B37', 'B38',
'B39', 'B40', 'B41', 'B42', 'B43', 'B44', 'B46', 'B47', 'B48', 'B49', 'B50', 'B51', 'B52', 'B53', 'B54',
'B55', 'B56', 'B57', 'B58', 'B59', 'B60', 'B61', 'B62', 'B63', 'B64', 'B65', 'B66', 'B67', 'B68', 'B70',
'B71', 'B72', 'B73', 'B74', 'B75', 'B76', 'B77', 'B78', 'B79', 'B80', 'B81', 'B82', 'B83', 'B84', 'B85',
'B86', 'B87', 'B88', 'B89', 'B90', 'B91', 'B92', 'B94', 'B95', 'B96', 'C01', 'C02', 'C03', 'C04', 'C05', 'C06',
'C07', 'C08', 'C09', 'C10', 'C11', 'C12', 'C13', 'C14', 'C15', 'C16', 'C17', 'C18', 'C19', 'C20', 'C22', 'C23',
'C24', 'C25', 'C26', 'C27', 'C28', 'C29', 'C30', 'C31', 'C32', 'C33', 'C34', 'C35', 'C36', 'C37', 'C38', 'C39',
'C40', 'C41', 'C42', 'C43', 'C44', 'C46', 'C47', 'C48', 'C49', 'C50', 'C51', 'C52', 'C53', 'C54', 'C55', 'C56',
```

```
'C57', 'C58', 'C59', 'C60', 'C61', 'C62', 'C63', 'C64', 'C65', 'C66', 'C67', 'C68', 'C70', 'C71', 'C72', 'C73',
'C74', 'C75', 'C76', 'C77', 'C78', 'C79', 'C80', 'C81', 'C82', 'C83', 'C84', 'C85', 'C86', 'C87', 'C88', 'C89',
'C90', 'C91', 'C92', 'C94', 'C95', 'C96', 'D01', 'D02', 'D03', 'D04', 'D05', 'D06', 'D07', 'D08', 'D09',
'D10', 'D11', 'D12', 'D13', 'D14', 'D15', 'D16', 'D17', 'D18', 'D19', 'D20', 'D22', 'D23', 'D24', 'D25',
'D26', 'D27', 'D28', 'D29', 'D30', 'D31', 'D32', 'D33', 'D34', 'D35', 'D36', 'D37', 'D38', 'D39', 'D40',
'D41', 'D42', 'D43', 'D44', 'D46', 'D47', 'D48', 'D49', 'D50', 'D51', 'D52', 'D53', 'D54', 'D55', 'D56',
'D57', 'D58', 'D59', 'D60', 'D61', 'D62', 'D63', 'D64', 'D65', 'D66', 'D67', 'D68', 'D70', 'D71', 'D72',
'D73', 'D74', 'D75', 'D76', 'D77', 'D78', 'D79', 'D80', 'D81', 'D82', 'D83', 'D84', 'D85', 'D86', 'D87',
'D88', 'D89', 'D90', 'D91', 'D92', 'D94', 'D95', 'D96', 'E24', 'E25', 'E26', 'E27', 'E28', 'E29', 'E30',
'E31', 'E32', 'E33', 'E34', 'E35', 'E36', 'E37', 'E38', 'E39', 'E40', 'E41', 'E42', 'E43', 'E44', 'E46', 'E47',
'E48', 'E49', 'E50', 'E51', 'E52', 'E53', 'E54', 'E55', 'E56', 'E57', 'E58', 'E59', 'E60', 'E61', 'E62', 'E63',
'E64', 'E65', 'E66', 'E67', 'E68', 'E70', 'E71', 'E72', 'E73', 'E74', 'E75', 'E76', 'E77', 'E78', 'E79', 'E80',
'E81', 'E82', 'E83', 'E84', 'E85', 'E86', 'E87', 'E88', 'E89', 'E90', 'E91', 'E92', 'E94', 'E95', 'E96', 'F01',
'F02', 'F03', 'F04', 'F05', 'F06', 'F07', 'F08', 'F09', 'F10', 'F11', 'F12', 'F13', 'F14', 'F15', 'F16', 'F17',
'F18', 'F19', 'F20', 'F22', 'F23', 'F24', 'F25', 'F26', 'F27', 'F28', 'F29', 'F30', 'F31', 'F32', 'F33', 'F34',
'F35', 'F36', 'F37', 'F38', 'F39', 'F40', 'F41', 'F42', 'F43', 'F44', 'F46', 'F47', 'F48', 'F49', 'F50', 'F51',
'F52', 'F53', 'F54', 'F55', 'F56', 'F57', 'F58', 'F59', 'F60', 'F61', 'F62', 'F63', 'F64', 'F65', 'F66', 'F67',
'F68', 'F70', 'F71', 'F72', 'F73', 'F74', 'F75', 'F76', 'F77', 'F78', 'F79', 'F80', 'F81', 'F82', 'F83', 'F84',
'F85', 'F86', 'F87', 'F88', 'F89', 'F90', 'F91', 'F92', 'F94', 'F95', 'F96', 'Totals']

poolfile2 = 'out_3-1-formatted.out'
pool2 = list(csv.reader(open(poolfile2)))

new_pool2 = [[[] for i in range(533)] for i in range(3558)]

row_index = 0

for firstline in range(0,533):
   new_pool2[0][firstline] = pool2[0][firstline]

for row in range(1, 3558):
   if pool2[row][1] == pool2[row-1][1]:
      new_pool2[row_index][0] = pool2[row][0]
      new_pool2[row_index][1] = pool2[row][1]
      for col in range(2,533):
         new_pool2[row_index][col] = ( int( new_pool2[row_index][col] ) + int( pool2[row][col] ) )
   else:
      row_index = row_index + 1
      for col in range(0,533):
         new_pool2[row_index][col] = pool2[row][col]

for i in range(0, row_index+1):
   [ print( new_pool2[i][val], end=",") for val in range(0, 533) ]
   print( "\n" )
```

#The following script is 'problem3-3.py':

```
import csv

colnames = ['OUT_no', 'taxon_name', 'A01', 'A02', 'A03', 'A04', 'A05', 'A06', 'A07', 'A08', 'A09',
'A10', 'A11', 'A12', 'A13', 'A14', 'A15', 'A16', 'A17', 'A18', 'A19', 'A20', 'A22', 'A23', 'A24', 'A25',
'A26', 'A27', 'A28', 'A29', 'A30', 'A31', 'A32', 'A33', 'A34', 'A35', 'A36', 'A37', 'A38', 'A39', 'A40',
'A41', 'A42', 'A43', 'A44', 'A46', 'A47', 'A48', 'A49', 'A50', 'A51', 'A52', 'A53', 'A54', 'A55', 'A56',
'A57', 'A58', 'A59', 'A60', 'A61', 'A62', 'A63', 'A64', 'A65', 'A66', 'A67', 'A68', 'A70', 'A71', 'A72',
'A73', 'A74', 'A75', 'A76', 'A77', 'A78', 'A79', 'A80', 'A81', 'A82', 'A83', 'A84', 'A85', 'A86', 'A87',
'A88', 'A89', 'A90', 'A91', 'A92', 'A94', 'A95', 'A96', 'B01', 'B02', 'B03', 'B04', 'B05', 'B06', 'B07',
'B08', 'B09', 'B10', 'B11', 'B12', 'B13', 'B14', 'B15', 'B16', 'B17', 'B18', 'B19', 'B20', 'B22', 'B23',
'B24', 'B25', 'B26', 'B27', 'B28', 'B29', 'B30', 'B31', 'B32', 'B33', 'B34', 'B35', 'B36', 'B37', 'B38',
'B39', 'B40', 'B41', 'B42', 'B43', 'B44', 'B46', 'B47', 'B48', 'B49', 'B50', 'B51', 'B52', 'B53', 'B54',
'B55', 'B56', 'B57', 'B58', 'B59', 'B60', 'B61', 'B62', 'B63', 'B64', 'B65', 'B66', 'B67', 'B68', 'B70',
```

```
'B71', 'B72', 'B73', 'B74', 'B75', 'B76', 'B77', 'B78', 'B79', 'B80', 'B81', 'B82', 'B83', 'B84', 'B85',
'B86', 'B87', 'B88', 'B89', 'B90', 'B91', 'B92', 'B94', 'B95', 'B96', 'C01', 'C02', 'C03', 'C04', 'C05', 'C06',
'C07', 'C08', 'C09', 'C10', 'C11', 'C12', 'C13', 'C14', 'C15', 'C16', 'C17', 'C18', 'C19', 'C20', 'C22', 'C23',
'C24', 'C25', 'C26', 'C27', 'C28', 'C29', 'C30', 'C31', 'C32', 'C33', 'C34', 'C35', 'C36', 'C37', 'C38', 'C39',
'C40', 'C41', 'C42', 'C43', 'C44', 'C46', 'C47', 'C48', 'C49', 'C50', 'C51', 'C52', 'C53', 'C54', 'C55', 'C56',
'C57', 'C58', 'C59', 'C60', 'C61', 'C62', 'C63', 'C64', 'C65', 'C66', 'C67', 'C68', 'C70', 'C71', 'C72', 'C73',
'C74', 'C75', 'C76', 'C77', 'C78', 'C79', 'C80', 'C81', 'C82', 'C83', 'C84', 'C85', 'C86', 'C87', 'C88', 'C89',
'C90', 'C91', 'C92', 'C94', 'C95', 'C96', 'D01', 'D02', 'D03', 'D04', 'D05', 'D06', 'D07', 'D08', 'D09',
'D10', 'D11', 'D12', 'D13', 'D14', 'D15', 'D16', 'D17', 'D18', 'D19', 'D20', 'D22', 'D23', 'D24', 'D25',
'D26', 'D27', 'D28', 'D29', 'D30', 'D31', 'D32', 'D33', 'D34', 'D35', 'D36', 'D37', 'D38', 'D39', 'D40',
'D41', 'D42', 'D43', 'D44', 'D46', 'D47', 'D48', 'D49', 'D50', 'D51', 'D52', 'D53', 'D54', 'D55', 'D56',
'D57', 'D58', 'D59', 'D60', 'D61', 'D62', 'D63', 'D64', 'D65', 'D66', 'D67', 'D68', 'D70', 'D71', 'D72',
'D73', 'D74', 'D75', 'D76', 'D77', 'D78', 'D79', 'D80', 'D81', 'D82', 'D83', 'D84', 'D85', 'D86', 'D87',
'D88', 'D89', 'D90', 'D91', 'D92', 'D94', 'D95', 'D96', 'E24', 'E25', 'E26', 'E27', 'E28', 'E29', 'E30',
'E31', 'E32', 'E33', 'E34', 'E35', 'E36', 'E37', 'E38', 'E39', 'E40', 'E41', 'E42', 'E43', 'E44', 'E46', 'E47',
'E48', 'E49', 'E50', 'E51', 'E52', 'E53', 'E54', 'E55', 'E56', 'E57', 'E58', 'E59', 'E60', 'E61', 'E62', 'E63',
'E64', 'E65', 'E66', 'E67', 'E68', 'E70', 'E71', 'E72', 'E73', 'E74', 'E75', 'E76', 'E77', 'E78', 'E79', 'E80',
'E81', 'E82', 'E83', 'E84', 'E85', 'E86', 'E87', 'E88', 'E89', 'E90', 'E91', 'E92', 'E94', 'E95', 'E96', 'F01',
'F02', 'F03', 'F04', 'F05', 'F06', 'F07', 'F08', 'F09', 'F10', 'F11', 'F12', 'F13', 'F14', 'F15', 'F16', 'F17',
'F18', 'F19', 'F20', 'F22', 'F23', 'F24', 'F25', 'F26', 'F27', 'F28', 'F29', 'F30', 'F31', 'F32', 'F33', 'F34',
'F35', 'F36', 'F37', 'F38', 'F39', 'F40', 'F41', 'F42', 'F43', 'F44', 'F46', 'F47', 'F48', 'F49', 'F50', 'F51',
'F52', 'F53', 'F54', 'F55', 'F56', 'F57', 'F58', 'F59', 'F60', 'F61', 'F62', 'F63', 'F64', 'F65', 'F66', 'F67',
'F68', 'F70', 'F71', 'F72', 'F73', 'F74', 'F75', 'F76', 'F77', 'F78', 'F79', 'F80', 'F81', 'F82', 'F83', 'F84',
'F85', 'F86', 'F87', 'F88', 'F89', 'F90', 'F91', 'F92', 'F94', 'F95', 'F96', 'Totals']

allonesfile = 'out_3-2-formatted.out'
allones = list(csv.reader(open(allonesfile)))

#new_pool2 = [[[] for i in range(533)] for i in range(3558)]


for i in range( 1, 208 ):
   for j in range( 2, 533 ):
      if int( allones[i][j] ) > 1:
         allones[i][j] = 1

for i in range( 0, 208 ):
   [ print( allones[i][val], end="," ) for val in range(0, 533) ]
   print( "\n" )
```

Appendix 2.3 – Taxa removed from the Pink Pigeon and Telfair's skink metabarcoding datasets

Table A2.3.1 Taxa removed from the Pink Pigeon and Telfair's skink datasets

Taxa	Reason for removal from dataset	Dataset present in
Abelmoschus esculentus	Field staff food	Pigeon, Skink
Actinidia sp.	Field staff food	Skink
Anacardium occidentale	Field staff food	Pigeon
Anthoxanthum sp.	UK pollen	Skink

Table A2.3.1 Taxa removed from the Pink Pigeon and Telfair's skink datasets

Taxa	Reason for removal from dataset	Dataset present in
Archis sp.	Field staff food	Pigeon
Avena sp.	Field staff food/UK pollen	Pigeon, Skink
Azadirachta indica	Field staff food	Pigeon, Skink
Betula sp.	UK pollen	Pigeon, Skink
Brassica sp.	Field staff food	Pigeon, Skink
Camellia chekiangoleosa	UK pollen	Skink
Camellia sinensis	Field staff food	Skink
Camellia sp	Field staff food	Skink
Cannabis sativa	Silo contamination	Pigeon, Skink
Capsicum annuum	Field staff food	Skink
Capsicum sp.	Field staff food	Skink
Chamaecyparis lawsoniana	UK pollen	Pigeon
Chenopodium sp.	Silo contamination	Pigeon
Citrus sp.	Field staff food/UK pollen	Pigeon
Coriandrum sativum	Field staff food	Pigeon
Cucumis sp.	Field staff food	Pigeon
Cucurbita pepo	Field staff food	Pigeon, Skink
Cucurbita sp.	Field staff food	Pigeon, Skink
Cupressus	UK pollen	Pigeon
Datura inoxia	Silo contamination	Pigeon, Skink
Daucus sp.	UK pollen	Skink
Diplotaxis sp.	Field staff food	Pigeon, Skink
Fagopyrum sp.	UK pollen	Skink
Fraxinus excelsior	UK pollen	Skink
Helianthus annuus	Silo contamination/UK pollen	Skink
Helianthus sp.	Silo contamination	Pigeon, Skink
Helminthotheca sp.	Silo contamination/UK pollen	Skink
Hordeum vulgare	Silo contamination	Pigeon, Skink
Impatiens glandulifera	UK pollen/UK lab contamination	Skink
Ixoroideae	Field staff food	Pigeon
Juglans regia	UK pollen	Pigeon
Leucaena sp.	No match to *L. leucocephala*	Pigeon, Skink
Linum usitatissimum/Tetradium ruticarpum	Silo contamination	Pigeon, Skink
Lolium perenne	UK pollen	Skink
Lolium sp.	UK pollen	Skink
Malus sp.	Field staff food/UK pollen/captive skink supplementary feed	Skink
Mimusops sp.	Field staff food	Pigeon, Skink
Musa acuminata	Field staff food	Pigeon, Skink
Musa sp.	Field staff food	Skink
Nasturtium sp.	Field staff food	Pigeon, Skink

Table A2.3.1 Taxa removed from the Pink Pigeon and Telfair's skink datasets

Taxa	Reason for removal from dataset	Dataset present in
Oenanthe sp.	Field staff food	Pigeon
Phaseolus sp./Theobroma sp.	Field staff food	Skink
Pisum sativum	Field staff food	Pigeon, Skink
Platanus orientalis	UK pollen	Skink
Platanus sp.	UK pollen	Skink
Potentilla sp.	UK pollen	Skink
Prunus sp.	Field staff food/UK pollen	Pigeon, Skink
Pyrus elaeagrifolia	UK pollen	Skink
Pyrus salicifolia	UK pollen	Pigeon
Pyrus sp.	Field staff food/UK pollen	Pigeon, Skink
Quercus sp.	UK pollen	Pigeon
Ranunculus sp.	UK pollen	Skink
*Salvadora oleoides**	Not belived to grow on Ile aix Aigrettes	Skink
Sambucus nigra	UK pollen	Pigeon
*Senna alexandrina**	Not belived to grow on Ile aix Aigrettes/fungicide/medicinal tea	Skink
Sesamum indicum	Silo contamination	Pigeon, Skink
Solanum lycopersicum	Field staff food/lab consumables	Pigeon, Skink
Solanum sp.	Field staff food/lab consumables	Pigeon, Skink
Solanum tuberosum	Field staff food/lab consumables	Pigeon, Skink
Spinacia sp.	Field staff food	Pigeon, Skink
Taraxacum sp.	UK pollen	Skink
Taxus sp.	UK pollen	Pigeon
Tilia platyphyllos	UK pollen	Pigeon
Tilia sp.	UK pollen	Skink
Triglochin maritima	UK pollen	Pigeon
Triglochin palustris	UK pollen	Pigeon
Vitis vinifera	Field staff food/Passerine sup. Feed	Pigeon, Skink

*May be new introductions on Ile aux Aigrettes.

Appendix Three – Supplementary information relating to Chapter Five

Appendix 3.1 – Determining the percentage of Aldabra giant tortoise relocations to use in Minimum Convex Polygon home-range calculations

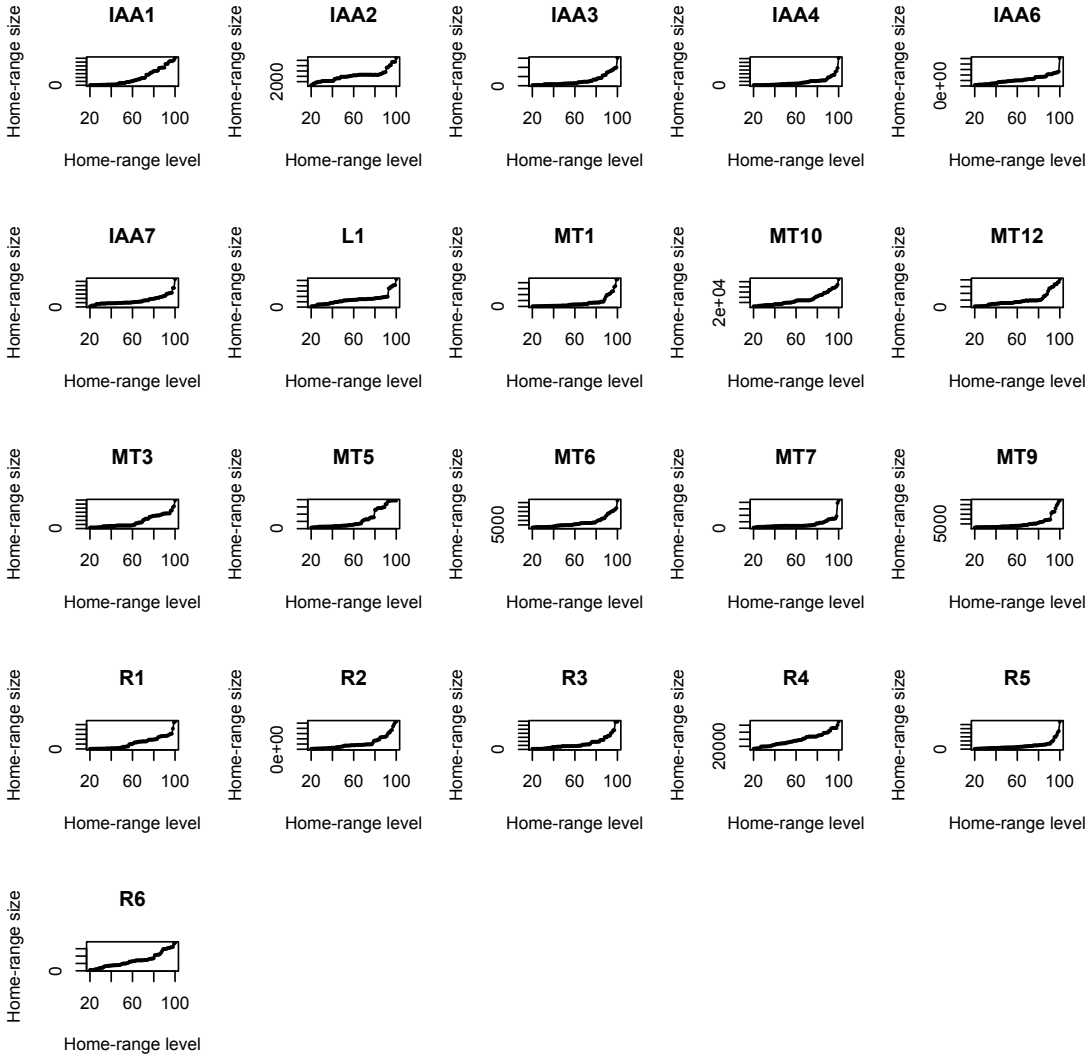


Figure A3.1 Determining the percentage of extreme tortoise relocations to discard before calculating Minimum Convex Polygon (MCP) home-ranges using the adehabitat package (Calenge 2006). Any relocations found after an asymptote was reached, were classified as extreme relocations and removed. For example, to calculate the home-range of IAA2, 95% of relocations were used. Graph titles refer to tortoise ID.

Appendix 3.2 – Ile aux Aigrettes natural food availability data

Table A3.1 Dry season food availability data on Ile aux Aigrettes. Values are the sum of the percentage cover estimates across 172 grid squares.

Plant taxon	Sum of percentage cover			
	Ground (<0.5 m)	Understory (0.5 - 2 m)	Canopy (>2 m)	Total
Acalypha indica	0.65	0	0	0.65
Albizia lebbeck	11.1	5	0	16.1
Aloe tormentorii	2.7	0	0	2.7
Angraecum eburneum	0	22.3	0	22.3
Asparagus setaceus	263.8	103.5	67	434.3
Asparagus umbellatus	36.1	28	4	68.1
Asystasia gangetica	12.85	1	0	13.85
Bryophyllum pinnatum	0.65	0	0	0.65
Caesalpinia bonduc	0.1	0	0	0.1
Cassine orientalis	51.7	94	157.5	303.2
Cassytha filiformis	3	131.1	115	249.1
Catharanthus roseus	0.55	0	0	0.55
Clerodendrum heterophyllum	0.25	10	5	15.25
Coffea myrtifolia	0.95	0.25	0	1.2
Coptosperma borbonica	548.85	1150.95	3811	5510.8
Cynanchum staubii	8.25	70.5	94.75	173.5
Cyperus dubius	363.85	0	0	363.85
Dendrolobium umbellatum	0	0.25	0	0.25
Dictyosperma album var. conjugatum	1	5	4	10
Diospyros egrettarum	455.25	905	3274	4634.25
Dodonaea viscosa	23.95	195.9	907	1126.85
Dracaena concinna	56.8	656.1	734.25	1447.15
Erythroxylum sideroxyloides	3.65	0.6	0	4.25
Eugenia lucida	198.22	758.75	1543	2499.97
Euphorbia hirta	0.3	0	0	0.3
Euphorbia thymifolia	0.75	0	0	0.75
Fenelia buxifolia	0.75	0.5	0	1.25
Ficus reflexa	1.55	22	439.75	463.3
Ficus rubra	0.8	10	325	335.8
Fimbristylis cymosa	8	0	0	8
Flacourtia indica	144.85	106.4	5	256.25
Foetidia mauritiana	0.25	0	0	0.25
Gagnebina pterocarpa	0.3	0	0	0.3
Hibiscus tiliaceus	3.3	84.7	678	766
Hilsenbergia petiolaris	26.8	570.85	2001	2598.65
Hyophorbe lagenicaulis	0	24.5	11	35.5
Ipomoea obscura	1.05	6	0	7.05

Table A3.1 Dry season food availability data on Ile aux Aigrettes. Values are the sum of the percentage cover estimates across 172 grid squares.

Plant taxon	Sum of percentage cover			
	Ground (<0.5 m)	Understory (0.5 - 2 m)	Canopy (>2 m)	Total
Ipomoea violacea	25.6	15.25	7.8	48.65
Lantana camara	1	0	0	1
Latania loddigesii	104.35	165.25	40	309.6
Leucaena leucocephala	188.05	66.8	87.25	342.1
Margaritaria anomala	0	6.75	12	18.75
Maytenus pyria	169.28	1376.05	446	1991.33
Morinda citrifolia	14.95	22.3	7	44.25
Oeoniella polystachys	21.85	56.35	3	81.2
Pandanus vandermeerschii	37.95	50	37	124.95
Passiflora suberosa	60.16	66.15	33.3	159.61
Phyllanthus casticum	3.05	0	1	4.05
Phymatodes scolopendria	3780.85	146.85	4	3931.7
Pithecellobium dulce	28.75	8.8	0	37.55
Polyscias maraisiana	8.2	116.45	688.5	813.15
Premna serratifolia	5.5	64.05	107	176.55
Rhynchosia viscosa	0.7	0	0	0.7
Rivina humilis	9.7	1.1	0	10.8
Santalum album	18.85	9.5	0.5	28.85
Scaevola taccada	293.3	554.5	224	1071.8
Schinus terebinthifolius	2.6	8.8	6	17.4
Secamone dilapidens	0.2	0	0	0.2
Secamone volubilis	0	0.3	0	0.3
Sideroxylon boutonianum	0.25	1	24	25.25
Stachytarpheta jamaicensis	961	0	0	961
Stenotaphrum dimidiatum	690.25	0	0	690.25
Stenotaphrum micranthum	13.45	0	0	13.45
Tabebuia pallida	0.5	6	0	6.5
Thespesia populnea	0.7	1.25	0	1.95
Triphasia trifolia	0.05	0	0	0.05
Trochetia boutoniana	0.25	0	0	0.25
Turnera angustifolia	96.2	21.05	0	117.25
Turraea thouarsiana	4.65	26.25	42	72.9
Tylophora coriacea	11.1	13.5	1.25	25.85
Vetiveria arguta	21.55	0	0	21.55
Wikstroemia indica	110.4	55	0	165.4

Table A3.2 Wet season food availability data on Ile aux Aigrettes. Values are the sum of the percentage cover estimates across 130 grid squares.

Plant taxon	Sum of percentage cover			
	Ground (<0.5 m)	Understory (0.5 - 2 m)	Canopy (>2 m)	Total
Acalypha indica	2.75	0	0.3	3.05
Albizia lebbeck	23.53	9	20	52.53
Aloe tormentorii	2.3	0	0	2.3
Asparagus setaceus	373.65	97.25	92	562.9
Asparagus umbellatus	37.37	7	27.6	71.97
Asystasia gangetica	79.15	0	0.5	79.65
Caesalpinia bonduc	3.25	0.3	6.3	9.85
Carica papaya	0.25	0	0	0.25
Cassine orientalis	8.5	89	43.2	140.7
Cassytha filiformis	0.3	21	7	28.3
Catharanthus roseus	1.1	0	0	1.1
Chloris barbata	35.25	0	0	35.25
Coffea myrtifolia	1.6	0	0.55	2.15
Colubrina asiatica	0.3	0	2	2.3
Coptosperma borbonica	588.56	3615	1118.6	5322.16
Cynanchum staubii	3	31	15.6	49.6
Cyperus dubius	272.49	0	0.25	272.74
Dactyloctenium aegyptium	1.35	0	0	1.35
Dendrolobium umbellatum	7.2	13	18	38.2
Dictyosperma album var. conjugatum	0	2	5	7
Diospyros egrettarum	371.81	2755	748.1	3874.91
Dodonaea viscosa	6.41	500	105	611.41
Dracaena concinna	42.02	419.8	337.7	799.52
Eragrostis amabilis	16.85	0	0	16.85
Erythroxylum sideroxyloides	0.95	2	0.25	3.2
Eugenia lucida	90.69	1440.75	526.32	2057.76
Euphorbia hirta	18.12	0	0	18.12
Euphorbia thymifolia	0.25	0	0	0.25
Ficus reflexa	0.25	271	11.5	282.75
Ficus rubra	3	392	35.45	430.45
Fimbristylis cymosa	6.3	0	0	6.3
Flacourtia indica	310.75	10	218	538.75
Gagnebina pterocarpa	0.45	58	5	63.45
Heteropogon contortus	5	0	0	5
Hibiscus tiliaceus	7.75	602	46.45	656.2
Hilsenbergia petiolaris	33.55	1714	387.65	2135.2
Hyophorbe lagenicaulis	0	17.5	5.2	22.7
Ipomoea violacea	48.51	112	39.55	200.06
Ipomoea obscura	6.25	0	1	7.25
Lantana camara	11.6	3	16	30.6
Latania loddigesii	56.75	62.5	149.5	268.75
Lepturus repens	0.8	0	0	0.8
Leucaena leucocephala	781.95	185.5	401	1368.45

Table A3.2 Wet season food availability data on Ile aux Aigrettes. Values are the sum of the percentage cover estimates across 130 grid squares.

Plant taxon	Sum of percentage cover			
	Ground (<0.5 m)	Understory (0.5 - 2 m)	Canopy (>2 m)	Total
Margaritaria anomala	12.64	57	37.7	107.34
Maytenus pyria	184.76	318	766.6	1269.36
Morinda citrifolia	10.1	1	11.8	22.9
Oeoniella polystachys	4.25	0.55	28.6	33.4
Oxalis corniculata	0.25	0	0	0.25
Pandanus vandermeerschii	42.5	14.5	52	109
Passiflora suberosa	86.3	147.5	67.4	301.2
Phyllanthus amarus	0.33	0	0	0.33
Phyllanthus casticum	13	0	7.55	20.55
Phyllanthus mauritianus	1.14	0	0	1.14
Phymatodes scolopendria	3396.25	2	285	3683.25
Pithecellobium dulce	31.18	4	7.55	42.73
Polyscias maraisiana	2.22	488.7	72	562.92
Poupartia borbonica	5.14	8	1.1	14.24
Premna serratifolia	2.04	143	70.95	215.99
Rhynchosia viscosa	18.3	1	1	20.3
Ricinus communis	0.5	0	0	0.5
Rivina humilis	46.5	0	3.3	49.8
Santalum album	47.53	15.5	54.4	117.43
Scaevola taccada	259.6	180.5	343.3	783.4
Schinus terebinthifolius	0.7	0.5	2.5	3.7
Scutia myrtina	0.3	0	0	0.3
Secamone dilapidens	0	0	0.3	0.3
Secamone volubilis	0	0	1	1
Sporobolus virginicus	11.85	0	0	11.85
Stachytarpheta jamaicensis	1433.61	0	0.01	1433.62
Stenotaphrum dimidiatum	545.75	0	0	545.75
Stenotaphrum micranthum	172.06	0	0	172.06
Stillingia lineata	0.3	1	1.5	2.8
Tabebuia pallida	2.56	6.25	8.8	17.61
Thespesia populnea	0.25	10	6	16.25
Turnera angustifolia	213.41	0	23.8	237.21
Turraea thouarsiana	0.62	50	20.5	71.12
Tylophora coriacea	14.9	6	13.5	34.4
Unknown	0.4	0	0	0.4
Vetiveria arguta	20.75	0	0	20.75
Wikstroemia indica	444.26	0	186.8	631.06
Zanthoxylum heterophyllum	0.25	0	0	0.25

Appendix 3.3 – Plant species recorded on Ile aux Aigrettes during vegetation surveys (see Appendix 3.2) but never detected in the metabarcoding dietary studies

Table A3.3. Plant taxa not recorded in the diets of Aldabra giant tortoises, Telfair's skinks and Pink Pigeons

Family name	Species name
Anacardiaceae	*Schinus terebinthifolius*
Apocynaceae	*Catharanthus roseus*
Apocynaceae	*Secamone dilapidens*
Apocynaceae	*Secamone volubilis*
Arecaceae	*Dictyosperma album var. conjugatum*
Arecaceae	*Hyophorbe lagenicaulis*
Asparagaceae	*Dracaena concinna*
Erythroxylaceae	*Erythroxylum sideroxyloides*
Euphorbiaceae	*Stillingia lineata*
Fabaceae	*Albizia lebbeck*
Fabaceae	*Dendrolobium umbellatum*
Lauraceae	*Cassytha filiformis*
Lecythidaceae	*Foetidia mauritiana*
Malvaceae	*Trochetia boutoniana*
Orchidaceae	*Angraecum eburneum*
Orchidaceae	*Oeoniella polystachys*
Oxalidaceae	*Oxalis corniculata*
Pandanaceae	*Pandanus vandermeerschii*
Poaceae	*Lepturus repens*
Poaceae	*Sporobolus virginicus*
Poaceae	*Vetiveria arguta*
Ricinus communis	*Ricinus communis*
Rubiaceae	*Coffea myrtifolia*
Rubiaceae	*Fernelia buxifolia*
Rutaceae	*Zanthoxylum heterophyllum*
Sapotaceae	*Sideroxylon boutonianum*
Xanthorrhoeaceae	*Aloe tormentorii*

Appendix 3.4 – Plant taxa removed from the Aldabra giant tortoise metabarcoding dataset

Table A3.4. Taxa removed from the Aldabra giant tortoise metabarcoding dataset

Taxa	Reason for removal form dataset
Abelmoschus esculentus	Field staff food
Allium sativum	Field staff food

Table A3.4. Taxa removed from the Aldabra giant tortoise metabarcoding dataset

Taxa	Reason for removal form dataset
Alopecurus myosuroides	UK pollen
Alopecurus sp.	UK pollen
Azadirachta indica	Field staff food
Betula papyrifera	UK pollen
Betula sp.	UK pollen
Brassica sp.	Field staff food
Caltha palustris	UK pollen
Cannabis sativa	Silo contamination
Capsicum annuum	Field staff food
Carex nigra	UK pollen
Carya sp.	UK pollen
Chamaecyparis lawsoniana	UK pollen
Cicer sp.	Field staff food
Cirsium sp.	UK pollen
Coriandrum sativum	Field staff food
Corylus avellana/Embryophyte	UK pollen
Cucurbita pepo	Field staff food
Cucurbita sp.	Field staff food
Cuminum cyminum	Field staff food
Cyperus sp.	UK pollen
Cupressaceae	UK pollen
Dactylis glomerata	UK pollen
Datura inoxia	Silo contamination
Diplotaxis sp.	Field staff food
Fagopyrum sp.	UK pollen
Fagus sp.	UK pollen
Filipendula ulmaria	UK pollen
Fraxinus angustifolia	UK pollen
Fraxinus/embryophyte	UK pollen
Glyceria maxima	UK pollen
Fabaceae*	UK pollen/or unknown sp.ecies on Ile aux Aigrettes
Helianthus annuus	Silo contamination
Hesp.erocyparis sp.	UK pollen
Hiptage benghalensis*	Not believed to grow on Ile aux Aigrettes
Hordeum vulgare	Silo contamination
Ixoroideae	Field staff food
Jacobaea aquatica	UK pollen
Juglans regia	UK pollen
Leontodon saxatilis	UK pollen
Leucaena sp.	No match to *L. leucocephala*
Linum usitatissimum/Tetradium ruticarpum	Silo contamination

Table A3.4. Taxa removed from the Aldabra giant tortoise metabarcoding dataset

Taxa	Reason for removal form dataset
Lolium perenne	UK pollen
Lolium sp.	UK pollen
Mimusops sp.	Field staff food
Musa acuminata	Field staff food
Myosotis sp.	UK pollen
Nasturtium sp.	Field staff food
Papaver rhoeas	UK pollen
Pisum sativum	Field staff food
Poaceae*	UK pollen or unknown grass present on Ile aux Aigrettes
Polygonaceae	Field staff food/UK pollen
Prunus avium	Field staff food/UK pollen
Prunus sp.	Field staff food/UK pollen
Quercus sp.	UK pollen
Ranunculus sp.	UK pollen
Rumex acetosa	UK pollen
Salix sp.	UK pollen
Salvadora oleoides*	Not believed to grow on Ile aux Aigrettes
Sambucus nigra	UK pollen
Scorzoneroides sp.	UK pollen
Sechium sp.	Field staff food
Sesamum indicum	Silo contamination
Solanum sp.	Field staff food/lab consumables
Solanum tuberosum	Field staff food/lab consumables
Solanum lycopersicum	Field staff food/lab consumables
Solanum sp.	Field staff food/lab consumables
Sophora japonica	UK pollen
Thesp.esia sp.*	Unknown origin
Tilia sp.	UK pollen
Trifolium occidentale	UK pollen
Triglochin palustris	UK pollen
Turnera sp. *	Unknown origin
Vitis vinifera	Field staff food/passerine sup. Feed

*Possible new introductions to Ile aux Aigrettes

Bibliography

Alcover JA, Sans A, Palmer M (1998) The extent of extinctions of mammals on islands. *Journal of Biogeography*, **25**, 913-918.

Alonso H, Granadeiro JP, Waap S*, et al.* (2014) An holistic ecological analysis of the diet of Cory's shearwaters using prey morphological characters and DNA barcoding. *Molecular Ecology*, **23**, 3719-3733.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology*, **215**, 403-410.

Ando H, Setsuko S, Horikoshi K*, et al.* (2013) Diet analysis by next-generation sequencing indicates the frequent consumption of introduced plants by the critically endangered red-headed wood pigeon (Columba janthina nitens) in oceanic island habitats. *Ecology and Evolution*, **3**, 4057-4069.

Ankenbrand MJ, Keller A, Wolf M, Schultz J, Forster F (2015) ITS2 Database V: twice as much. *Molecular Biology and Evolution*, **32**, 3030-3032.

Arnold EN (1980) Recently extinct reptile populations from Mauritius and Réunion, Indian Ocean. *Journal of Zoology*, **191**, 33-47.

Athens JS, Tuggle HD, Ward JV, Welch DJ (2002) Avifaunal extinctions, vegetation change, Polynesian impacts in prehistoric Hawai'i. *Archaeology in Oceania*, **37**, 57-78.

Baamrane MAA, Shehzad W, Ouhammou A*, et al.* (2012) Assessment of the food habits of the Moroccan Dorcas Gazelle in M'Sabih Talaa, West Central Morocco, using the trnL approach. *Plos One*, **7**, e35643.

Bakker ES, Gill JL, Johnson CN*, et al.* (2016) Combining paleo-data and modern exclosure experiments to assess the impact of megafauna extinctions on woody vegetation. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 847-855.

Barnosky AD, Matzke N, Tomiya S*, et al.* (2011) Has the Earth's sixth mass extinction already arrived? *Nature*, **471**, 51-57.

Bates D, Machler M, Bolker BM, Walker SC (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1-48.

Beheregaray LB, Ciofi C, Caccone A, Gibbs JP, Powell JR (2003) Genetic divergence, phylogeography and conservation units of giant tortoises from Santa Cruz and Pinzon, Galapagos Islands. *Conservation Genetics*, **4**, 31-46.

Bellemain E, Carlsen T, Brochmann C*, et al.* (2010) ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. *Bmc Microbiology*, **10**, 189.

Bengtsson-Palme J, Ryberg M, Hartmann M*, et al.* (2013) Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other

eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution*, **4**, 914-919.
Benson DA, Clark K, Karsch-Mizrachi I*, et al.* (2014) GenBank. *Nucleic Acids Research*, **42**, D32-D37.
BirdLife International (2016) *Nesoenas mayeri. The IUCN Red List of Threatened Species 2016: e.T22690392A93271827.* 25 October 2017
Birnie-Gauvin K, Peiman KS, Raubenheimer D, Cooke SJ (2017) Nutritional physiology and ecology of wildlife in a changing world. *Conservation Physiology*, **5**, cox030.
Blake S, Guezou A, Deem SL, Yackulic CB, Cabrera F (2015) The Dominance of Introduced Plant Species in the Diets of Migratory Galapagos Tortoises Increases with Elevation on a Human-Occupied Island. *Biotropica*, **47**, 246-258.
Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, **30**, 2114-2120.
Boyer AG, Jetz W (2014) Extinctions and the loss of ecological function in island bird communities. *Global Ecology and Biogeography*, **23**, 679-688.
Boyer F, Mercier C, Bonin A*, et al.* (2016) OBITOOLS: a UNIX-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, **16**, 176-182.
Breheny P, Burchett W Visualization of regression models using visreg. R Package, 1-15,
Britton JR, Pegg J, Shepherd JS, Toms S (2006) Revealing the prey items of the otter Lutra lutra in South West England using stomach contents analysis. *Folia Zoologica*, **55**, 167-174.
Broveak T (1996) Sequencher. *Biotechnology Software & Internet Journal*, **13**, 7-&.
Brown DS, Burger R, Cole N*, et al.* (2014) Dietary competition between the alien Asian Musk Shrew (*Suncus murinus*) and a re-introduced population of Telfair's Skink (*Leiolopisma telfairii*). *Molecular Ecology*, **23**, 3695-3705.
Bullock DJ (1986) The ecology and conservation of reptiles on Round Island and Gunner's Quoin, Mauritius. *Biological Conservation*, **37**, 135-156.
Burgess KS, Fazekas AJ, Kesanakurti PR*, et al.* (2011) Discriminating plant species in a local temperate flora using the rbcL plus matK DNA barcode. *Methods in Ecology and Evolution*, **2**, 333-340.
Cade TJ, Jones CG (1993) Progress in restoration of the Mauritius kestrel. *Conservation Biology*, **7**, 169-175.
Calenge C (2006) The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling*, **197**, 516-519.
Camacho C, Coulouris G, Avagyan V*, et al.* (2009) BLAST plus : architecture and applications. *Bmc Bioinformatics*, **10**, 421.
Caro T (2007) The Pleistocene re-wilding gambit. *Trends in Ecology & Evolution*, **22**, 281-283.
Chapin FS, Zavaleta ES, Eviner VT*, et al.* (2000) Consequences of changing biodiversity. *Nature*, **405**, 234-242.

Check E (2006) Treasure island: pinning down a model ecosystem. *Nature*, **439**, 378-379.
Cheke A, Hume J (2008) *Lost Land of the Dodo* T & AD Poyser, UK.
Chen S, Yao H, Han J*, et al.* (2010) Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *Plos One*, **5**, e8613.
Cheng T, Xu C, Lei L*, et al.* (2016) Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. *Molecular Ecology Resources*, **16**, 138-149.
Clare EL (2014) Molecular detection of trophic interactions: emerging trends, distinct advantages, significant considerations and conservation applications. *Evolutionary Applications*, **7**, 1144-1157.
Clare EL, Schiestl FP, Leitch AR, Chittka L (2013) The promise of genomics in the study of plant-pollinator interactions. *Genome Biology*, **14**, 11. 207.
Coghlan ML, White NE, Murray DC*, et al.* (2013) Metabarcoding avian diets at airports: implications for birdstrike hazard management planning. *Investigative genetics*, **4**, 27-27.
Coissac E, Riaz T, Puillandre N (2012) Bioinformatic challenges for DNA metabarcoding of plants and animals. *Molecular Ecology*, **21**, 1834-1847.
Cole N, Goder M, Vencatasamy D*, et al.* (2013) Restoration of Island Ecosystems in Mauritius: The Mauritius Reptile Recovery Programme Annual Report 2013. Durrell Wildlife Conservation Trust, Jersey, Channel Islands.
Cole N, Goder M, Vencatasamy D*, et al.* (2014) Restoration of Island Ecosystems in Mauritius: The Mauritius Reptile Recovery Programme Annual Report 2014. Durrell Wildlife Conservation Trust, Jersey, Channel Islands.
Cole N, Jones CG, Buckland S*, et al.* (2009) The reintroduction of endangered Mauritian reptiles, Mauritian Wildlife Foundation, Vacoas, Mauritius.
Cole NC, Jones CG, Harris S (2005) The need for enemy-free space: The impact of an invasive gecko on island endemics. *Biological Conservation*, **125**, 467-474.
Coleman AW (2003) ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends in Genetics*, **19**, 370-375.
Concannon L (2014) *Managing threatened species: understanding the factors limiting the recovery of the endangered Pink Pigeon*, University of Reading.
Corlett RT (2013) The shifted baseline: Prehistoric defaunation in the tropics and its consequences for biodiversity conservation. *Biological Conservation*, **163**, 13-21.
Corlett RT (2016) Restoration, Reintroduction, and Rewilding in a Changing World. *Trends in Ecology & Evolution*, **31**, 453-462.
Crutzen PJ (2002) Geology of mankind. *Nature*, **415**, 23-23.
de Vere N, Jones LE, Gilmore T*, et al.* (2017) Using DNA metabarcoding to investigate honey bee foraging reveals limited flower use despite high floral availability. *Scientific Reports*, **7**, 42838.
de Vere N, Rich TCG, Ford CR*, et al.* (2012) DNA Barcoding the Native Flowering Plants and

Conifers of Wales. *Plos One*, **7**, e37945.
Deagle BE, Kirkwood R, Jarman SN (2009) Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology*, **18**, 2022-2038.
Dirzo R, Miranda A (1991) *Plant-animal interactions: Evolutionary ecology in tropical and temperate regions* Wiley, New York, USA.
Dirzo R, Young HS, Galetti M*, et al.* (2014) Defaunation in the Anthropocene. *Science*, **345**, 401-406.
Donlan CJ, Berger J, Bock CE*, et al.* (2006) Pleistocene rewilding: An optimistic agenda for twenty-first century conservation. *American Naturalist*, **168**, 660-681.
Donlan J, Berger J, Bock CE*, et al.* (2005) Re-wilding North America. *Nature*, **436**, 913-914.
Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, **26**, 2460-2461.
Edmunds K, Bunbury N, Sawmy S, Jones CG, Bell DJ (2008) Restoring avian island endemics: use of supplementary food by the endangered Pink Pigeon (Columba mayeri). *Emu*, **108**, 74-80.
Elbrecht V, Leese F (2015) Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass-Sequence Relationships with an Innovative Metabarcoding Protocol. *Plos One*, **10**, e0130324.
Eskildsen LI, Olesen JM, Jones CG (2004) Feeding response of the Aldabra giant tortoise (Geochelone gigantea) to island plants showing heterophylly. *Journal of Biogeography*, **31**, 1785-1790.
Estes JA, Terborgh J, Brashares JS*, et al.* (2011) Trophic Downgrading of Planet Earth. *Science*, **333**, 301-306.
Fernandez N, Navarro LM, Pereira HM (2017) Rewilding: A Call for Boosting Ecological Complexity in Conservation. *Conservation Letters*, **10**, 276-278.
Fitter AH, Peat HJ (1994) The ecological flora database. *Journal of Ecology*, **82**, 415-425.
Ford MJ, Hempelmann J, Hanson MB*, et al.* (2016) Estimation of a Killer Whale (Orcinus orca) Population's Diet Using Sequencing Analysis of DNA from Feces. *Plos One*, **11**, e0144956.
Galetti M (2004) Parks of the Pleistocene: recreating the Cerrado and the Pantanal with megafauna. *Natureza & Conservação*, **2**
Garcia-Robledo C, Erickson DL, Staines CL, Erwin TL, Kress WJ (2013) Tropical Plant-Herbivore Networks: Reconstructing Species Interactions Using DNA Barcodes. *Plos One*, **8**, e52967.
Garcia-Robledo C, Kuprewicz EK, Staines CL, Kress WJ, Erwin TL (2013) Using a comprehensive DNA barcode library to detect novel egg and larval host plant associations in a Cephaloleia rolled-leaf beetle (Coleoptera: Chrysomelidae). *Biological Journal of the Linnean Society*, **110**, 189-198.
Gebremedhin B, Flagstad O, Bekele A*, et al.* (2016) DNA metabarcoding reveals diet overlap between the endangered Walia Ibex and domestic goats - implications for

conservation. *Plos One*, **11**, e0159133.
Geiger MF, Astrin JJ, Borsch T*, et al.* (2016) How to tackle the molecular species inventory for an industrialized nation-lessons from the first phase of the German Barcode of Life initiative GBOL (2012-2015). *Genome*, **59**, 661-670.
Gibson CWD, Hamilton J (1983) Feeding ecology and seasonal movements of giant tortoises on aldabra atoll. *Oecologia*, **56**, 84-92.
Goder M, Bhagan D, Moorhouse-Gann RJ, Cole N (2017) Photo library of the flora of Round Island and Ile aux Aigrettes. Mauritian Wildlife Foundation.
Gonzalez MA, Baraloto C, Engel J*, et al.* (2009) Identification of Amazonian Trees with DNA Barcodes. *Plos One*, **4**, e7483.
Griffiths CJ, Hansen DM, Jones CG, Zuel N, Harris S (2011) Resurrecting Extinct Interactions with Extant Substitutes. *Current Biology*, **21**, 762-765.
Griffiths CJ, Jones CG, Hansen DM*, et al.* (2010) The use of extant non-indigenous tortoises as a restoration tool to replace extinct ecosystem engineers. *Restoration Ecology*, **18**, 1-7.
Griffiths CJ, Zuel N, Jones CG, Ahamud Z, Harris S (2013) Assessing the Potential to Restore Historic Grazing Ecosystems with Tortoise Ecological Replacements. *Conservation Biology*, **27**, 690-700.
Griffiths CJ, Zuel N, Tatayah V*, et al.* (2012) The Welfare Implications of Using Exotic Tortoises as Ecological Replacements. *Plos One*, **7**, e39395.
Grubb P (1971) Growth, ecology and population structure of giant tortoises on aldabra. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **260**, 327-&.
Gwiazdowski RA, Foottit RG, Maw HEL, Hebert PDN (2015) The Hemiptera (Insecta) of Canada: Constructing a Reference Library of DNA Barcodes. *Plos One*, **10**, e0125635.
Han J, Zhu Y, Chen X*, et al.* (2013) The short ITS2 sequence serves as an efficient taxonomic sequence tag in comparison with the full-length ITS. *BioMed Research International*, **2013**, article no. 741476.
Hansen DM, Donlan CJ, Griffiths CJ, Campbell KJ (2010) Ecological history and latent conservation potential: large and giant tortoises as a model for taxon substitutions. *Ecography*, **33**, 272-284.
Hansen DM, Galetti M (2009) The Forgotten Megafauna. *Science*, **324**, 42-43.
Hansen I, Brimer L, Molgaard P (2004) Herbivore-deterring secondary compounds in heterophyllous woody species of the Mascarene Islands. *Perspectives in Plant Ecology Evolution and Systematics*, **6**, 187-203.
Hawkins J, de Vere N, Griffith A*, et al.* (2015) Using DNA metabarcoding to identify the floral composition of honey: A new tool for investigating honey bee foraging preferences. *Plos One*, **10**, e0134735.
Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B-Biological Sciences*, **270**, 313-321.

Hebert PDN, Gregory TR (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology*, **54**, 852-859.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 14812-14817.

Hibert F, Taberlet P, Chave J*, et al.* (2013) Unveiling the diet of elusive rainforest herbivores in Next Generation Sequencing era? The Tapir as a case study. *Plos One*, **8**, e60799.

Hofreiter M, Betancourt JL, Sbriller AP, Markgraf V, McDonald HG (2003) Phylogeny, diet, and habitat of an extinct ground sloth from Cuchillo Cura, Neuquen Province, southwest Argentina. *Quaternary Research*, **59**, 364-378.

Holechek JL, Vavra M, Pieper RD (1982) Botanical composition determination of range herbivore diets - a review. *Journal of Range Management*, **35**, 309-315.

Hollingsworth PM (2011) Refining the DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 19451-19452.

Hollingsworth PM, Forrest LL, Spouge JL*, et al.* (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 12794-12797.

Hollingsworth PM, Graham SW, Little DP (2011) Choosing and using a plant DNA barcode. *Plos One*, **6**, e19254.

Hollingsworth PM, Li DZ, van der Bank M, Twyford AD (2016) Telling plant species apart with DNA: from barcodes to genomes. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **371**, 20150338.

Houston D, McInnes K, Elliott G*, et al.* (2007) The use of a nutritional supplement to improve egg production in the endangered kakapo. *Biological Conservation*, **138**, 248-255.

Hume JP (2011) Systematics, morphology, and ecology of pigeons and doves (Aves: Columbidae) of the Mascarene Islands, with three new species. *Zootaxa*, 1-+.

Hume JP (2014) Systematics, morphology, and ecological history of the Mascarene starlings (Aves: Sturnidae) with the description of a new genus and species from Mauritius. *Zootaxa*, **3849**, 1-75.

Hume JP (2015) A new subfossil bulbul (Aves: Passerines: Pycnonotidae) from Rodrigues Island, Mascarenes, south-western Indian Ocean. *Ostrich*, **86**, 247-260.

Hunter EA, Gibbs JP, Cayot LJ, Tapia W (2013) Equivalency of Galapagos Giant Tortoises Used as Ecological Replacement Species to Restore Ecosystem Functions. *Conservation Biology*, **27**, 701-709.

IUCN (1998) Guidelines for Re-introductions (ed. Group ISR-iS), p. 10. IUCN, Gland, Switzerland and Cambridge, UK.

IUCN/SSC (2013) Guidelines for Reintroductions and Other Conservation Translocations, p. Viiii + 57, Gland, Switzerland: IUCN Species Survival Commission.

Iwanowicz DD, Vandergast AG, Cornman RS*, et al.* (2016) Metabarcoding of fecal samples to determine herbivore diets: A case study of the endangered Pacific Pocket Mouse. *Plos One*, **11**, e0165366.

Ji YQ, Ashton L, Pedley SM*, et al.* (2013) Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology Letters*, **16**, 1245-1257.

Jones C, Banbury N, Zuel N, Tatayah V, Swinnerton KJ (2013) *Pink pigeon Nesoenas mayeri. Birds of Africa Volume 8: the Malagasy region* Bloomsbury Publishing, London, UK.

Jones CG (1993) The ecology and conservation of Mauritian skinks. *Proceedings of the Royal Society of the Arts and Sciences of Mauritius*, **5**

Jones CG (2002) Reptiles and amphibians. *Handbook of ecological restoration. Principles of restoration (Volume 1); Restoration in practice (Volume 2)*, 355-375.

Jones CG, Hartley J (1995) A conservation project on Mauritius and Rodrigues: An overview and bibliography. *Dodo-Journal of the Wildlife Preservation Trusts*, 40-65.

Jones CG, Swinnerton KJ (1997) A summary of conservation status and research for the Mauritius kestrel Falco punctatus, Pink pigeon Columba mayeri and Echo parakeet Psittacula eques. *The Dodo Journal of The Wildlife Preservation Trusts*, **33**, 72-75.

Jorgensen D (2015) Rethinking rewilding. *Geoforum*, **65**, 482-488.

Jung TS, Stotyn SA, Czetwertynski SM (2015) Dietary overlap and potential competition in a dynamic ungulate community in Northwestern Canada. *Journal of Wildlife Management*, **79**, 1277-1285.

Jurado-Rivera JA, Vogler AP, Reid CAM, Petitpierre E, Gomez-Zurita J (2009) DNA barcoding insect-host plant associations. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 639-648.

Kaiser-Bunbury CN, Traveset A, Hansen DM (2010) Conservation and restoration of plant-animal mutualisms on oceanic islands. *Perspectives in Plant Ecology Evolution and Systematics*, **12**, 131-143.

King RA, Read DS, Traugott M, Symondson WOC (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology*, **17**, 947-963.

King RA, Vaughan IP, Bell JR, Bohan DA, Symondson WOC (2010) Prey choice by carabid beetles feeding on an earthworm community analysed using species- and lineage-specific PCR primers. *Molecular Ecology*, **19**, 1721-1732.

Kowalczyk R, Taberlet P, Coissac E*, et al.* (2011) Influence of management practices on large herbivore diet-Case of European bison in Bialowieza Primeval Forest (Poland). *Forest Ecology and Management*, **261**, 821-828.

Kress WJ, Erickson DL, Jones FA*, et al.* (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 18621-18626.

Krook K, Bond WJ, Hockey PAR (2007) The effect of grassland shifts on the avifauna of a South African savanna. *Ostrich*, **78**, 271-279.

Kruuk H (1995) *Wild otters: predation and populations* Oxford University Press Inc., New

York, USA.
Lahaye R, Van der Bank M, Bogarin D*, et al.* (2008) DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 2923-2928.
Larkin MA, Blackshields G, Brown NP*, et al.* (2007) Clustal W and clustal X version 2.0. *Bioinformatics*, **23**, 2947-2948.
Leal MC, Ferrier-Pagès C, Calado R*, et al.* (2013) Coral feeding on microalgae assessed with molecular trophic markers. *Molecular Ecology*, **23**, 3870–3876.
Li DZ, Gao LM, Li HT*, et al.* (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 19641-19646.
Little DP (2014) A DNA mini-barcode for land plants. *Molecular Ecology Resources*, **14**, 437-446.
Lorimer J, Sandom C, Jepson P*, et al.* (2015) Rewilding: Science, Practice, and Politics. *Annual Review of Environment and Resources, Vol 40*, **40**, 39-62.
Louzao M, Igual JM, McMinn M*, et al.* (2006) Small pelagic fish, trawling discards and breeding performance of the critically endangered Balearic shearwater: improving conservation diagnosis. *Marine Ecology Progress Series*, **318**, 247-254.
Mack RN, Simberloff D, Lonsdale WM*, et al.* (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689-710.
Madagascar Reptile & Amphibian Specialist Group (1996) *Leiolopisma telfairii. The IUCN Red List of Threatened Species 1996.* **e.T11409A3276976**. http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T11409A3276976.en Downloaded on 04 October 2017
Maggs G, Nicoll M, Zuel N*, et al.* (2015) Rattus management is essential for population persistence in a critically endangered passerine: Combining small-scale field experiments and population modelling. *Biological Conservation*, **191**, 274-281.
Magoc T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, **27**, 2957-2963.
Marris E (2009) Reflecting the past. *Nature*, **462**, 30-32.
Mickleburgh S, Hutson AM, Bergmans W, Howell K (2008) *Pteropus subniger. The IUCN Red List of Threatened Species 2008: e.T18761A8580195.* http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T18761A8580195.en 26 October 2017
Murray MH, Becker DJ, Hall RJ, Hernandez SM (2016) Wildlife health and supplemental feeding: A review and management recommendations. *Biological Conservation*, **204**, 163-174.
Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853-858.

Navarro J, Louzao M, Igual JM*, et al.* (2009) Seasonal changes in the diet of a critically endangered seabird and the importance of trawling discards. *Marine Biology*, **156**, 2571-2578.

Newfield M, Khadun A, Atkinson R, Mauremootoo J (2003) Ile aux Aigrettes weed management strategy. Mauritian Wildlife Foundation, Vacoas, Mauritius.

North SG, Bullock DJ (1986) Changes in the vegetation and populations of introduced mammals of Round Island and Gunner's Quoin, Mauritius. *Biological Conservation*, **37**, 99-117.

North SG, Bullock DJ, Dulloo ME (1994) Changes in the vegetation and reptile populations on Round Island, Mauritius, following eradication of rabbits. *Biological Conservation*, **67**, 21-28.

Owen-Smith RN (1987) Pleistocene extinctions - the pivotal role of megaherbivores. *Paleobiology*, **13**, 351-362.

Owen-Smith RN (1988) *Megaherbivores. The influence of very large body size on ecology* Cambridge University Press, New York, USA.

Page W (1988) *Dictyosperma album var. conjugatum. The IUCN Red List of Threatened Species 1998: e.T38510A10125739. .* http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T38510A10125739.en. Downloaded on 11 August 2017

Paine RT (1969) Pisaster-Tegula interaction - prey patches, predator food preference, and intertidal community structure. *Ecology*, **50**, 950-&.

Paine RT (1980) Food webs - linkage, interaction strength and community infrastructure - the 3rd tansley lecture. *Journal of Animal Ecology*, **49**, 667-685.

Pang X, Song J, Zhu Y, Xie C, Chen S (2010) Using DNA Barcoding to Identify Species within Euphorbiaceae. *Planta Medica*, **76**, 1784-1786.

Peixoto PV, Franca TN, Cunha BM, Tavares D, Brito MF (2008) Spontaneous poisoning by Leucaena leucocephala in a goat from Rio de Janeiro State, Brazil. *Ciencia Rural*, **38**, 551-555.

Pernetta AP, Bell DJ, Jones CG (2005) Macro- and microhabitat use of Telfair's skink (Leiolopisma telfairii) on Round Island, Mauritius: implications for their translocation. *Acta Oecologica-International Journal of Ecology*, **28**, 313-323.

Perring MP, Standish RJ, Price JN*, et al.* (2015) Advances in restoration ecology: rising to the challenges of the coming decades. *Ecosphere*, **6**, 131.

Pompanon F, Deagle BE, Symondson WOC*, et al.* (2012) Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology*, **21**, 1931-1950.

Prior J, Ward KJ (2016) Rethinking rewilding: A response to Jorgensen. *Geoforum*, **69**, 132-135.

Pumarino L, Alomar O, Agusti N (2011) Development of specific ITS markers for plant DNA identification within herbivorous insects. *Bulletin of Entomological Research*, **101**, 271-276.

QGISDevelopmentTeam QGIS Geographic Information System, Open Source Geospatial Foundation Project http://qgis.osgeo.org

R Core Team R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Randall RS, Sornay E, Dewitte W, Murray JAH (2015) AINTEGUMENTA and the D-type cyclin CYCD3;1 independently contribute to petal size control in *Arabidopsis*: evidence for organ size compensation being an emergent rather than a determined property. *Journal of Experimental Botany*, **66**, 3991-4000.

Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes*, **7**, 355-364.

Raye G, Miquel C, Coissac E*, et al.* (2011) New insights on diet variability revealed by DNA barcoding and high-throughput pyrosequencing: chamois diet in autumn as a case study. *Ecological Research*, **26**, 265-276.

Rewilding Europe (2015) *Making Europe a Wilder Place.* www.rewildingeurope.com

Ricciardi A, Simberloff D (2009) Assisted colonization is not a viable conservation strategy. *Trends in Ecology & Evolution*, **24**, 248-253.

Ricciardi A, Simberloff D (2009) Assisted colonization: good intentions and dubious risk assessment. *Trends in Ecology & Evolution*, **24**, 476-477.

Richman AD, Case TJ, Schwaner TD (1988) Natural and unnatural extinction rates of reptiles on islands. *American Naturalist*, **131**, 611-630.

Ripple WJ, Beschta RL (2007) Restoring Yellowstone's aspen with wolves. *Biological Conservation*, **138**, 514-519.

Ripple WJ, Beschta RL, Fortin JK, Robbins CT (2014) Trophic cascades from wolves to grizzly bears in Yellowstone. *Journal of Animal Ecology*, **83**, 223-233.

Ripple WJ, Larsen EJ, Renkin RA, Smith DW (2001) Trophic cascades among wolves, elk and aspen on Yellowstone National Park's northern range. *Biological Conservation*, **102**, 227-234.

Rubenstein DR, Rubenstein DI, Sherman PW, Gavin TA (2006) Pleistocene park: Does re-wilding North America represent sound conservation for the 21st century? *Biological Conservation*, **132**, 232-238.

Saarela JM, Sokoloff PC, Gillespie LJ, Consaul LL, Bull RD (2013) DNA Barcoding the Canadian Arctic Flora: Core Plastid Barcodes (rbcL plus matK) for 490 Vascular Plant Species. *Plos One*, **8**, UNSP e77982.

Sanderson EW, Jaiteh M, Levy MA*, et al.* (2002) The human footprint and the last of the wild. *Bioscience*, **52**, 891-904.

Schloss PD, Westcott SL, Ryabin T*, et al.* (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology*, **75**, 7537-7541.

Seddon PJ, Griffiths CJ, Soorae PS, Armstrong DP (2014) Reversing defaunation: Restoring

species in a changing world. *Science*, **345**, 406-412.
Sequencher® version 5.4.6 DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA http://www.genecodes.com
Seymour A, Varnham K, Roy S*, et al.* (2005) Mechanisms underlying the failure of an attempt to eradicate the invasive Asian musk shrew Suncus murinus from an island nature reserve. *Biological Conservation*, **125**, 23-35.
Sikes DS, Bowser M, Morton JM*, et al.* (2017) Building a DNA barcode library of Alaska's non-marine arthropods. *Genome*, **60**, 248-259.
Smith CI (2005) Re-wilding: introductions could reduce biodiversity. *Nature*, **437**, 318-318.
Soininen EM, Valentini A, Coissac E*, et al.* (2009) Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. *Frontiers in Zoology*, **6**, 16.
Sonstebo JH, Gielly L, Brysting AK*, et al.* (2010) Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Molecular Ecology Resources*, **10**, 1009-1018.
Soorae PS (2016) Global re-introduction perspectives : 2016. Case-studies from around the globe. IUCN SSC Re-introduction Specialist Group, Gland, Switzerland.
Spatz DR, Zilliacus K, Holmes ND*, et al.* (2017) Globally Threatened Vertebrates on Islands With Invasive Species *Science Advances*, **3**
Suding KN (2011) Toward an Era of Restoration in Ecology: Successes, Failures, and Opportunities Ahead. *Annual Review of Ecology, Evolution, and Systematics, Vol 42*, **42**, 465-487.
Suttle KB, Thomsen MA, Power ME (2007) Species interactions reverse grassland responses to changing climate. *Science*, **315**, 640-642.
Svenning JC, Pedersen PBM, Donlan CJ*, et al.* (2016) Science for a wilder Anthropocene: Synthesis and future directions for trophic rewilding research. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 898-906.
Swinnerton KJ, Greenwood AG, Chapman RE, Jones CG (2005) The incidence of the parasitic disease trichomoniasis and its treatment in reintroduced and wild Pink Pigeons Columba mayeri. *Ibis*, **147**, 772-782.
Swinnerton KJ, Peirce MA, Greenwood A, Chapman RE, Jones CG (2005) Prevalence of Leucocytozoon marchouxi in the endangered Pink Pigeon Columba mayeri. *Ibis*, **147**, 725-737.
Symondson WOC (2002) Molecular identification of prey in predator diets. *Molecular Ecology*, **11**, 627-641.
Taberlet P, Coissac E, Pompanon F*, et al.* (2007) Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Research*, **35**, e14.
Tamura K, Peterson D, Peterson N*, et al.* (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**, 2731-2739.

ThePlantList (2013) *The Plant List.* **1.1**. http://www.theplantlist.org/

Valentini A, Miquel C, Nawaz MA*, et al.* (2009) New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. *Molecular Ecology Resources*, **9**, 51-60.

van Baalen M, Krivan V, van Rijn PCJ, Sabelis MW (2001) Alternative food, switching predators, and the persistence of predator-prey systems. *American Naturalist*, **157**, 512-524.

Vaughan IP, Gotelli NJ, Memmott J*, et al.* (2017) econullnetr: An r package using null models to analyse the structure of ecological networks and identify resource selection. *Methods in Ecology and Evolution*, **00:1-6**

Vinson J, Vinson J (1969) The Saurian fauna of the Mascarene islands. *The Mauritius Institute Bulletin*, **6**

Waibel A, Griffiths CJ, Zuel N, Schmid B, Albrecht M (2013) Does a giant tortoise taxon substitute enhance seed germination of exotic fleshy-fruited plants? *Journal of Plant Ecology*, **6**, 57-63.

Wake DB, Vredenburg VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 11466-11473.

Waldram MS, Bond WJ, Stock WD (2008) Ecological engineering by a mega-grazer: White Rhino impacts on a South African savanna. *Ecosystems*, **11**, 101-112.

Wallinger C, Staudacher K, Schallhart N*, et al.* (2013) The effect of plant identity and the level of plant decay on molecular gut content analysis in a herbivorous soil insect. *Molecular Ecology Resources*, **13**, 75-83.

Warnes GR, Bolker B, Bonebakker L*, et al.* gplots: Various R programming tools for plotting data. https://CRAN.R-project.org/package=gplots

,

Wortley L, Hero JM, Howes M (2013) Evaluating Ecological Restoration Success: A Review of the Literature. *Restoration Ecology*, **21**, 537-543.

Zimov SA (2005) Pleistocene park: Return of the mammoth's ecosystem. *Science*, **308**, 796-798.

Zimov SA, Chuprynin VI, Oreshko AP*, et al.* (1995) Steppe-tundra transition - a herbivore-driven biome shift at the end of the Pleistocene. *American Naturalist*, **146**, 765-794.

Zuel N (2009) *Ecology and conservation of an endangered reptile community on Round Island, Mauritius*, PhD thesis. Universität Zürich.

Zuel N, Griffiths C, Hector A*, et al.* (2012) Ingestion by an endemic frugivore enhances seed germination of endemic plant species but decreases seedling survival of exotics. *Journal of Biogeography*, **39**, 2021-2030.