Systematics and Phylogeography of the Cape Parrot (*Poicephalus robustus*)

by

Willem G. Coetzer

Submitted in fulfilment of the academic requirements of

Doctor of Philosophy

in Genetics

School of Life Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg

South Africa

Supervisor:
Dr S. Willows-Munro
Co-supervisor:
Prof C.T. Downs
Prof M.R. Perrin

December 2015

PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Genetics, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by SANBI, NRF and the University of KwaZulu-Natal.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

……..

Signed: Dr S. Willows-Munro

Date: 3 December 2015

DECLARATION 1: PLAGIARISM

I, Willem G. Coetzer, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

……..

Signed: W.G. Coetzer

Date: 3 December 2015

DECLARATION 2: PUBLICATIONS

My role in each paper and presentation is indicated. The * indicates corresponding author.

Chapter 2

1. ***Coetzer, W.G., Downs, C.T., Perrin, M.R. and Willows-Munro, S.** 2015. Molecular systematics of the Cape Parrot (*Poicephalus robustus*): implications for taxonomy and conservation. Paper presentation to the bi-annual Zoological Society of Southern Africa symposium, July 2015, Grahamstown, Eastern Cape, South Africa. Presented by W.G. Coetzer.
2. ***Coetzer, W.G., Downs, C.T., Perrin, M.R. and Willows-Munro, S.** 2015. Molecular systematics of the Cape Parrot (*Poicephalus robustus*): implications for taxonomy and conservation. Paper presentation to the Australasian Ornithological Congress, November 2015, Adelaide, Australia. Presented by M.R. Perrin.
3. **Coetzer, W.G., Downs, C.T., Perrin, M.R. and *Willows-Munro, S.** 2015. Molecular systematics of the Cape Parrot (*Poicephalus robustus*): implications for taxonomy and conservation. *PloS ONE*. 10(8): e0133376. doi: 10.1371/journal.pone.0133376.

Conceived and designed the experiments: WGC CTD MRP SW-M. Performed the experiments: WGC. Analysed the data: WGC. Contributed reagents/materials/analysis tools: CTD MRP SW-M. Wrote the paper: WGC CTD MRP SW-M.

Chapter 3

4. ***Coetzer, W.G., Downs, C.T., Perrin, M.R. and Willows-Munro, S.** 2015. Phylogeography of the South African endemic Cape Parrot (*Poicephalus robustus*). Paper presentation at the annual symposium of the Southern African Wildlife Management Association, September 2015, Kimberley, Northern Cape, South Africa. Presented by W.G. Coetzer.
5. ***Coetzer, W.G., Downs, C.T., Perrin, M.R. and Willows-Munro, S.** 2015. Phylogeography of the South African endemic Cape Parrot (*Poicephalus robustus*). Paper presentation to the Australasian Ornithological Congress, November 2015, Adelaide, Australia. Presented by M.R. Perrin.

Conceived and designed the experiments: WGC CTD MRP SW-M. Performed the experiments: WGC. Analysed the data: WGC. Contributed reagents/materials/analysis tools: CTD MRP SW-M. Prepared the presentations: WGC.

……..

Signed: W.G. Coetzer

Date: 3 December 2015

ABSTRACT

The Cape Parrot (*Poicephalus r. robustus*) is the only endemic parrot found in South Africa. This subspecies is restricted to the mistbelt forests of the Eastern Cape, Kwa-Zulu Natal and Limpopo Provinces. Recent population census estimates suggest that there are fewer than a 1600 Cape Parrots left in the wild. Habitat loss and illegal harvesting for the pet trade are among the major drivers of these low population numbers. The current conservation status of *P. robustus ssp.* does not lend the Cape Parrot sufficient protection national and international conservation agencies, as it is internationally seen as a subspecies of *Poicephalus robustus* i.e. *P. robustus robustus*. A better understanding of the Cape Parrot's taxonomic position within the genus is therefore urgently needed. The three data chapters presented in this thesis address the main aims of this study.

The taxonomic status of the Cape Parrot (*Poicephalus robustus robustus*) has been the focus of much debate over the years. A number of authors have suggested that the Cape Parrot should be viewed as a distinct species separate from the other two *P. robustus* subspecies (*P. r. fuscicollis* and *P. r. suahelicus*). These recommendations were based on previously published morphological, ecological and behavioural assessments. In this chapter the validity of these recommendations were investigated using multilocus DNA analyses. A total of 138 specimens from five *Poicephalus* species (*P. cryptoxanthus, P. gulielmi, P. meyeri, P. robustus* and *P. rueppellii)* were genotyped using 11 microsatellite loci. Additionally, two mitochondrial (cytochrome oxidase I gene and 16S ribosomal RNA) markers and one nuclear intron (intron 7 of the β-fibrinogen gene) marker were amplified and sequenced. Bayesian clustering analysis and pairwise F_{ST} analysis of microsatellite data identified *P. r. robustus* as genetically distinct from the other *P. robustus* subspecies. Phylogenetic analysis on sequence data also supported the microsatellite analyses, placing *P. r. robustus* in a distinct clade separate from the other *P. robustus* subspecies. Molecular clock analysis places the most recent common ancestor of *P. r. robustus* and *P. r. fuscicollis* / *P. r. suahelicus* at 2.13 to 2.67 million years ago. These results all support previous recommendations to elevate the Cape Parrot to species level.

In the second data chapter, an assessment of the historical and contemporary genetic structure of the Cape Parrot was performed. The effect of anthropogenic habitat fragmentation on species, which live in naturally patchy habitats, has rarely been examined in South Africa. The Cape Parrot is a habitat specialist, restricted to forest patches in the Eastern Cape, KwaZulu-Natal and Limpopo Provinces of South Africa. Although current overexploitation of forests in southern Africa is certainly an important driver of fragmentation, this is not solely

responsible for the relictual nature of South African forests. In the Pliocene, periods of climate change driven aridity and increased fire frequency, contributed towards the 'natural' fragmentation of the forests in southern Africa. In this chapter, 85 modern samples, collected from 1951 to 2014, and 29 historical samples, collected from 1870 to 1946, were used to investigate the historical and contemporary genetic structure of Cape Parrots using 16 microsatellite loci. Bayesian clustering analysis identified three geographically correlated genetic clusters: a southern group restricted to forest patches in the Eastern Cape, a central group including birds from KwaZulu-Natal and a genetically distinct northern Limpopo cluster. Results suggest that Cape Parrots have experienced at least two major population bottlenecks. An ancient decline during the mid-Holocene (~1800-3000 years before present) linked to climate change, and a more recent bottleneck, associated with logging of forests during the early 1900's. This chapter highlights the effects of climate change and human activities on this endangered species.

The third data chapter, deals with the use of molecular data in forensic analysis of Cape Parrots. The illicit harvesting of wild Cape Parrots for the pet trade is a significant threat faced by this endemic South African species. Illegal trade in rare wildlife species is a major threat to many parrot species around the world. Wildlife forensics plays an important role in the preservation of endangered or threatened wildlife species. Identification of illegally harvested or traded animals through DNA techniques is one of the many methods implemented during forensic investigations. In this study, 16 microsatellite markers specifically designed for the South African endemic Cape Parrot were assessed for their utility in forensic casework. In addition, this chapter evaluates the genetic diversity of the captive Cape Parrot population and compares this to the wild Cape Parrot population, using these 16 loci. The results showed that the full 16 locus panel has sufficient discriminatory power to be used in parentage analyses of suspected illegally traded Cape Parrots. It was further observed that a panel of 12 loci has sufficient power to assign confiscated birds thought to be illegally removed from the wild to their area of origin. It was recommended that the current reference data sets should be expanded to increase the accuracy of the assignment analyses. The level of genetic diversity observed within the captive data set was comparable to that observed in the wild populations. The captive Cape Parrots did, however, have double the number of private alleles compared to that observed in the most genetically diverse wild population. This was accredited to the presence of rare alleles present in the founder population, which has not been lost due to genetic drift, as many of the specimens tested in this study are F1 to F3 wild descendants.

The results from this comprehensive genetic study on the South African endemic Cape Parrot will have a number of implication for the conservation of this species. The taxonomic assessment of the Cape Parrot clearly supported its elevation to full species. The phylogeographic analysis of these parrots showed that the contemporary population is strongly geographically structured, with a distinct, isolated northern population in the Limpopo Province of South Africa. These results will aid local and international conservation authorities with the planning and implementation of future conservation endeavours focusing on the Cape Parrot. The assessment of a panel of microsatellite markers for their use in forensic analysis will aid conservation and law enforcement authorities to better control legal and illegal trade of this South African endemic. Recommendations were also made with regards to the management of a captive population for possible future reintroduction purposes.

ACKNOWLEDGMENTS

The completion of this thesis would not have been possible without the help and support of numerous people. I would like to express my sincere gratitude to the following people and organisations:

Dr Sandi Willows-Munro, Prof Colleen Downs and Prof Mike Perrin for their supervision, support and guidance throughout the last three years.

All the Cape Parrot breeders for their support by providing specimens for use in my thesis.

The curators of the East London Museum (East London, South Africa), Ditsong National Museum of Natural History (Pretoria, South Africa), Durban Natural Sciences Museum (Durban, South Africa), the British Natural History Museum at Tring (United Kingdom), the National Zoological Gardens (Pretoria, South Africa), the Montecasino Bird Gardens (Johannesburg, South Africa), the Umgeni River Bird Park (Durban, South Africa), the Loro Parque Foundation (Canary Islands, Spain) for their support by supplying me with specimens.

Dr Craig Symes, University of the Witwatersrand, and Dr Steve Boyes, University of Cape Town, for supplying additional field samples.

Dr Riddhika Kalle, Dr T. Ramesh and Dr Lindy Thompson for their assistance during field sampling.

Mr. Cyril Laubsher for the use of his photographs in Chapter 2.

The University of KwaZulu-Natal (UKZN) for academic and financial assistance.

The UKZN Animal Ethics Committee for approval ethical approval for the project.

The National Research Foundation (NRF) for financial assistance.

The South African National Biodiversity Institute (SANBI) for research funding.

Kayleigh Muller for all her support during the research and thesis writing stages of this PhD.

My family for all their support during these last few years.

Thank you all!

TABLE OF CONTENTS

Page

PREFACE ii
DECLARATION 1: PLAGIARISM iii
DECLARATION 2: PUBLICATIONS iv
ABSTRACT v
ACKNOWLEDGMENTS viii
TABLE OF CONTENTS ix
LIST OF TABLES xi
LIST OF FIGURES xiii
CHAPTER 1: INTRODUCTION 15
1.1 Rationale for research 15
1.2 Background to the main issues addressed in this thesis 16
1.3 Thesis structure 20
1.4 References 22
CHAPTER 2: MOLECULAR SYSTEMATICS OF THE CAPE PARROT (*POICEPHALUS ROBUSTUS*): IMPLICATIONS FOR TAXONOMY AND CONSERVATION 26
2.1 Abstract 26
2.2 Introduction 27
2.3 Materials and Methods 31
2.4 Results 36
2.5 Discussion 44
2.6 Conclusion 46
2.7 Acknowledgements 47
2.8 References 48
2.9 Supplementary Information 55
CHAPTER 3: HISTORICAL BIOGEOGRAPHY: THE INFLUENCE OF ANCIENT AND CONTEMPORARY HABITAT CHANGES ON THE ENDEMIC SOUTH AFRICAN PARROT *POICEPHALUS ROBUSTUS* 67
3.1 Abstract 67
3.2 Introduction 68

3.3 Materials and Methods 71
3.4 Results 79
3.5 Discussion 93
3.6 Acknowledgements 98
3.7 References 98
3.8 Supplementary information 110
CHAPTER 4: IDENTIFICATION OF MICROSATELLITE MULTIPLEXES FOR INDIVIDUAL IDENTIFICATION OF CAPE PARROTS (*POICEPHALUS ROBUSTUS*): PATERNITY TESTING AND MONITORING TRADE 125
4.1 Abstract 125
4.2 Introduction 126
4.3 Materials and methods 130
4.4 Results 134
4.5 Discussion 142
4.6 Conclusions 145
4.7 Acknowledgements 146
4.8 References 146
4.9 Supplementary Information 156
CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH 165

LIST OF TABLES

Table **Page**

Table 2-1. Sample details and genetic diversity for each *Poicephalus* species and subspecies analysed. Number of individuals sampled, observed heterozygosity, expected heterozygosity, mean number of alleles and number of private alleles are provided……..…39

Table 2-2. The average pairwise genetic distances estimated in RAxML using the concatenated dataset of all three gene regions (below diagonal) and using COI data only (above diagonal) from the *Poicephalus* specimens from the current study……..……..….43

Table 2-3. Divergence dates of the seven Poicephalus species, with *Psittacus erithacus* as outgroup, analysed with a Bayesian lognormal relaxed-clock model. The mean estimated values and the 95% highest posterior density (HPD) ranges are given for the two dataset partitions. The node numbers correspond to Figure 2-4b. See Supplementary Figure 2-1 for the corresponding maximum clade probability trees…….……...44

Supplementary Table 2-1. The *Poicephalus* specimens included in the present study. Collection numbers and locations were those assigned to specimens by museums or the collections of researchers. GPS coordinates are taken from the general area of sample collection. The institutions where samples can be accessed are listed. Specimens used for DNA sequencing are indicated by *……..…….…57

Supplementary Table 2-2. GenBank accession numbers for *Poicephalus* sequences generated in the present study and accession numbers for the 27 additional parrot sequences used for molecular clock analyses……..…….....64

Supplementary Table 2-3. The genetic diversity, polymorphic information content and null allele frequencies of each locus over all *Poicephalus* specimens analysed……..…65

Supplementary Table 2-4. The pairwise F_{ST} values for all *Poicephalus* specimens used in this study. The F_{ST} values are below diagonal and probability values (*p-values*) above diagonal……..…….…66

Supplementary Table 2-5. Summary statistics of the COI, 16S rRNA and β-fib sequences generated for the Poicephalus specimens analysed in the current study…….…67

Supplementary Table 2-6. The COI pairwise genetic distances calculated in RaxML for four *Calyptorhynchus* sp. sequences. (Downloaded from GenBank: JF414241.1, JN801424.1, JF414242.1, JF414279.1, JF414274.1)…….……67

Table 3-1. Sampling details and genetic diversity for each *Poicephalus robustus* population analysed in the current study. Standard error values for the mean number of alleles, observed heterozygosity, unbiased expected heterozygosity and allelic richness are provided in parentheses……..…….…..….83

Table 3-2. The pairwise F_{ST} estimates for the combined data set and the historical and contemporary data sets. Comparisons were made between the three sampling regions; South, Central and North. The pairwise F_{ST} values are below the diagonal, with *p-values* above the diagonal. The significance threshold was adjusted for multiple tests: *P*-value = 0.003……..……...……...85

Table 3-3. Bottleneck results (*p-values*) obtained from a signed rank Wilcoxon test for heterozygous excess (one tail) using two mutation models, the Mode-shift test and the *M*-ratio method for bottleneck detection. The two-phase mutation (TPM) and single-step mutation

(SSM) models were used for the heterozygous excess tests. The Bonferroni correction was applied to all *p*-values (*P*-value = 0.003)……...90

Table 3-4. Estimates of effective population size changes over time as calculated in MSVAR using the exponential model. The 95% highest posterior density for each estimate is provided in parentheses……..……91

Supplementary Table 3-1. Sample information for all samples used in Chapter 3…….…..117

Supplementary Table 3-2. SPOTG parameters used in Chapter 3…….....120

Supplementary Table 3-3. Prior settings used for the MSVAR analyses (init_v_file) in Chapter 3……121

Supplementary Table 3-4. Per locus statistics over all samples, contemporary samples only and historical samples only…….....122

Supplementary Table 3-5. The analysis of molecular variance (AMOVA) results from the full, contemporary and historical datasets. Samples were grouped according to region of origin and then according to sampling locality……...…..……123

Supplementary Table 3-6. Results from the migration-drift vs drift only model analysis performed in 2MOD for both the contemporary and historical datasets. *F* - the probability that two genes share a common ancestor within a population; *M* - the number of migrants per generation as calculated from *F*……....……124

Supplementary Table 3-7. Migration rate estimates (*m*), obtained from BayesAss using the contemporary and historical data sets. The 95% confidence interval is provided in parentheses……..…….……..125

Table 4-1. Primer details and genetic diversity estimates per locus as calculated from the captive data set used in Chapter 4. The standard error for the average number of alleles is provided in parentheses…….....137

Table 4-2. The genetic diversity estimates for each of the wild Cape Parrot populations and the captive data set, using 16 microsatellite loci. Standard error for average number of alleles, observed heterozygosity and unbiased expected heterozygosity is provided in parentheses……..…….……143

Supplementary Table 4-1. Sample information for all samples used in Chapter 4 a) identification and origins of all captive specimens used in the current study and b) number of specimens from each of the breeding facilities (BF)……..157

Supplementary Table 4-2. Wild caught Cape Parrot genotypes sourced from Chapter 3 for the captive vs wild Cape Parrot analyses in Chapter 4. Each sample's membership to one of the three genetic clusters identified in Chapter 3 is provided……...……..…159

Supplementary Table 4-3. The eight microsatellite panels tested in Chapter 4, with the polymorphic information content (PIC) and combined probability of identity (P_{IDcom}) for each panel……..……...…….161

Supplementary Table 4-4. The parentage assignment success rates for all parentage assignments performed in Chapter 4. Standard error values are provided in parentheses…..162

Supplementary Table 4-5. The population of origin results for each of the eight microsatellite panels tested in Chapter 4. a) The percentage (%) of correct assignments, with the average assignment probabilities of the correct assignments. Standard error (SE) is given in parentheses; b) The GeneClass2 assignment results for each of the eight panels tested, with correct assignments marked by green and incorrect assignments marked by red..……163

LIST OF FIGURES

Figure **Page**

Figure 2-1. Distribution map of the *Poicephalus* species (and *P. robustus* subspecies) included in the study (maps redrawn from Perrin 2012, photos used with permission from Cyril Laubscher)…….....30

Figure 2-2. The estimated population genetic structure of the *Poicephalus* species/subspecies used in the current study (K = 7). Each individual is represented by a vertical line, and coloured according to each individual's estimated membership probability (*Q*-values). Average *Q*-values for each cluster is depicted above the figure……..……..40

Figure 2-3. A 3D principal coordinates analysis (PCoA), generated in XLSTAT 2014. The pairwise F_{ST} values estimated between species/subspecies of the Poicephalus species included in the study were used to generate the figure. The first three axes explained 70.3% of the estimated variation…….……..41

Figure 2-4. Maximum likelihood phylogeny retrieved of the *Poicephalus* specimens from the current study. The analyses were performed using (left) concatenated data (COI, 16S rRNA and β-fib) and (right) mitochondrial DNA data. Values given above the branches represent maximum likelihood bootstrap values and Bayesian posterior probabilities (in that order). Dated nodes are indicated by numbers next to each node in b) and correspond to Table 2-3. Species/subspecies were highlighted according to the microsatellite clustering analyses. *Psittacus erithacus* was used as an outgroup…….…...…43

Supplementary Figure 2-1. Maximum clade probability trees generated using the concatenated data (16S rRNA, COI and β-fib) and mitochondrial DNA (16S rRNA and COI) data. Separate analyses were conducted using Schweizer et al. 2011 and White et al. 2011 calibration points. Values at nodes indicate the posterior mean divergence dates in millions of years before present. Shaded bars indicate the 95% highest posterior density (HPD) credibility intervals…….....56

Figure 3-1. Sampling sites for Cape Parrot (*Poicephalus robustus*) in South Africa. Grey shading indicates the extent of current forest cover in the region (Mucina and Rutherford, 2006). The black circles indicate sampling sites with detailed locality information, with the white circles representing samples from the Eastern Cape (EC Unknown) and KwaZulu-Natal (KZN Unknown) that do not have precise locality information…….……..……...74

Figure 3-2. The estimated population genetic structure of *Poicephalus robustus* in South Africa using historical, contemporary and combined data sets (left). Each vertical line in the barplot represents an individual and is coloured according to every individual's estimated membership coefficient (*Q*) values. Asterisk indicates historical samples. The mean *Q*-values of each cluster is provided. Maps showing the Mean Bayesian assignment probabilities per locality for historical, contemporary and combined data sets (right). Each colour indicates the mean proportion of individual's genotypes assigned to a particular lineage in each locality……..86

Figure 3-3. A temporal statistical parsimony network displaying the relationships between historical and contemporary genotypes. The network is based on individual 16 loci genotypes. Genotypes are represented by circles (historical) or rectangles (contemporary) colour codes to represent sampling region (blue = South; Green = Central; Red = North), with empty circles and rectangles representing genotypes absent from the given time period. Stippled lines indicate ancestral genotypes……..……..……87

Figure 3-4. Migration rates (*m*), estimated using BayesAss from the contemporary data. Samples were grouped according to (a) the three sampling regions and b) individual sampling locality sites. Higher migration rates are indicated by thicker lines. The 95% confidence interval is provided in parentheses……93

Supplementary data 3-1. Results from the Sample planning tool (SPOTG) simulations for three bottleneck and one temporal simulations performed in Chapter 3. The bargraph below each set of results represents the power of each bottleneck scenario…….....…111

Supplementary Figure 3-1. The delta K and Evanno table output for a) the historical and b) the contemporary STRUCTURE analyses……...116

Figure 4-1. The probability of identity (P_{ID}) and probability of exclusion (one parent known, P_{E2}) estimates for the eight microsatellite panels tested in the current study. It can be observed that the full 16 locus panel has the most optimum P_{ID} and P_{E2} values compared to the other seven panels tested in this study……..…….....……138

Figure 4-2. The parent pair and individual parentage assignment success of the eight microsatellite panels tested in the current study. The bars corresponds to the percentage known parents correctly assigned to each offspring, with high probability; the lines are representative of the probability values for each assignment type…….140

Figure 4-3. The assignment success rates for the eight microsatellite panels tested for their ability to correctly assign specimens to their area of origin in the current study. The black line with the dots represents the average assignment probability calculated from the correctly assigned specimens' probability values. The exact probability values for the assignments conducted with each of the eight panels are available in Supplementary Table 4-4…….……...……...…141

CHAPTER 1: INTRODUCTION

1.1 Rationale for research

The Cape Parrot (*Poicephalus r. robustus*) is found exclusively in South Africa. It is a very scarce avian species, restricted to the mistbelt forests of the Eastern Cape, KwaZulu-Natal and Limpopo Provinces (Downs, 2005a, Perrin, 2012, Wirminghaus et al., 2000a). There is much debate surrounding the taxonomic position of *P. r. robustus*. Previous authors have suggested based on morphological, ecological and behavioural research that *P. r. robustus* represents a distinct species (Clancey, 1997, Perrin, 2005, Wirminghaus et al., 2002a). Additional multi-locus molecular data were needed to provide further clarity on this issue.

Currently, *P. r. robustus* is listed as critically endangered under South African legislation (Minister of Environmental Affairs and Tourism, 2007). As a subspecies of *Poicephalus robustus* (together with *P. r. fuscicollis* and *P. r. suahelicus*) it is, however, listed by the International Union for Conservation of Nature (IUCN) as of 'Least Concern' and is not listed under the Convention of International Trade in Endangered Species (CITES) as endangered. *Poicephalus robustus* is listed as an Appendix II CITES species, allowing some control of trade. No distinction is however made between the three *P. robustus ssp.* The conservation of this South African endemic is therefore not considered during issuing of permits for trade, making it challenging for local authorities to monitor trade in *P. r. robustus*. Careful examination of the species status of *P. r. robustus* is therefore required to better clarify their taxonomic status. Elevation to species level would provide additional legislative protection to the taxon and it would be easier to enforce conservation legislation and to control legal and illegal trade. The current distribution of *P. r. robustus* is highly fragmented and examination of the genetic connectivity of this species' different populations (Eastern Cape, KwaZulu-Natal and Limpopo) will aid in the conservation management of these geographic populations. Besides habitat fragmentation, illegal trade is another threat to this South African endemic parrot. The identification of a microsatellite panel, with sufficient individual identification power, is therefore of utmost importance to aid in the control of trade of these endemic birds.

1.2 Background to the main issues addressed in this thesis

1.2.1 Taxonomy of the genus Poicephalus

The order Psittaciformes which includes about 350 extant parrot species is divided into 82 genera (Forshaw, 2010, Wright et al., 2008). The genera are grouped into three superfamilies, namely Strigopoidea (including all New Zealand genera), Cacatuoidea (including all cockatoos) and Psittacoidea (all other parrots). The family Psittacidae includes the true parrots, and is grouped under the superfamily Psittacoidea. The subfamily Psittacinae (Psittacidae) consists of two extant genera, namely *Psittacus* and *Poicephalus* (Joseph et al., 2012). Both of these genera are African but *Poicephalus* is the most species rich. The genus consists of nine species and is generally divided into two clades (Forshaw, 1978, Fry et al., 1988). These are the *P. robustus* clade, including the Cape Parrot (*P. robustus*) and the Red-fronted Parrot (*P. gulielmi*), and the *P. meyeri* clade, consisting of the Yellow-faced Parrot (*P. flavifrons*), the Senegal Parrot (*P. senegalus*), the Red-bellied Parrot (*P. rufiventrisi)*, the Niam-niam Parrot *(P. crassus*), the Brown-headed Parrot (*P. cryptoxanthus*), Meyer's Parrot (*P. meyeri*) and Ruppell's Parrot (*P. rueppellii*; Massa et al., 2000). Many of these species are further subdivided into subspecies. In particular, *P. robustus*. is currently divided into three recognised subspecies, namely *P. r. robustus* (Cape Parrot), *P. r. suahelicus* (Grey-headed Parrot) and *P. r. fuscicollis* (Brown-necked Parrot). The taxonomic status of these three subspecies has been the topic of debate for over two decades (Clancey, 1997, Perrin, 2012, 2005, Wirminghaus et al., 2002a). Within the *P. robustus* group, *P. r. fuscicollis* (Brown-necked Parrot) prefer Red Mangrove forests, mature wooded savanna and palm woodland, while *P. r. suahelicus* (Grey-headed Parrot), utilizes a variety of woodland habitat types (See Chapter 2 for more information). The South African *P. r. robustus* is almost exclusively restricted to yellowwood forests (*Afrocarpus*/*Podocarpus*) of South Africa (Perrin, 2012). *Poicephalus r. robustus* is morphologically different from the other two subspecies, with the *P. r. robustus* generally being smaller (Wirminghaus et al., 2002a). It has also been shown that these subspecies have distinct behaviour and show differences in vocalisations (Symes and Perrin, 2004, Wirminghaus et al., 2000b), feeding and breeding behaviour (Symes and Perrin, 2003, Wirminghaus et al., 2002b). International conservation organizations, such as BirdLife International have, however, highlighted the need for additional genetic data for full species status to be recognized (see Chapter 2). The second chapter of this thesis will focus on the taxonomy of the three *P. robustus* subspecies.

1.2.2 Cape Parrot biology, range and distribution

Poicephalus species exhibit an allopatric geographical distribution. This highly fragmented distribution coupled with findings of a recent phylogenetic study (Massa et al., 2000) suggest that the current distribution pattern of the members of this clade can be linked to environmental changes driven by the changing Quaternary glacial periods (Moreau, 1966). Glacial periods produced drier environments which caused fragmentation of forests (Diamond and Hamilton, 1980, Hewitt, 2000). Both taxa belonging to the *P. robustus* clade, i.e. *P. robustus* and *P. gulielmi*, are restricted to forest habitats and may be particularly sensitive to climate driven vegetation changes (Massa et al., 2000).

Poicephalus r. robustus is a medium sized bird (body length of 283 ± 19 mm; Perrin, 2012). They have dark olive-green heads and neck, with adult females possessing an orange-red forehead, the rest of the plumage varying from olive- to dark-green with adults of both sexes having red on the edge of the wings (Wirminghaus, 1997, Wirminghaus et al., 2002a). The taxon has a highly fragmented distribution, located in the mistbelt forests of the Eastern Cape, KwaZulu-Natal and a relic population of fewer than 100 birds in the Limpopo Province (Downs, 2005a, Perrin, 2012, Wirminghaus et al., 2000a). Overall population size of *P. r. robustus* have drastically contracted over the last century, particularly in the northern parts of KZN and along the escarpment of Mpumalanga (Downs, 2005a, Downs et al., 2014, Symes et al., 2004, Wirminghaus et al., 2000a; See Chapter 3 for more information). The distribution of *P. r. robustus* is limited by the availability of fragmented yellowwood forests, as the birds feed almost exclusively on the fruits of these trees at certain times of the year (Perrin, 2005, Wirminghaus et al., 2002b) and prefer these trees for roostin and nesting (Downs and Symes, 2004, Wirminghaus et al., 2001). However, there have been observations of *P. r. robustus* occasionally feeding on fruiting trees other than yellowwood trees, and on agricultural land, such as pecan-nut orchards. This shift in feeding behaviour occurs seasonally when fruit production of yellowwood species are particularly low (Symes and Downs, 2002, Wirminghaus et al., 2002b).

1.2.3 Population decline and fragmentation

Current estimates suggest that there are fewer than 1600 *P. r. robustus* individuals remaining in the wild (Downs et al., 2014). Wirminghaus et al. (1999) reported several factors which

likely play a role in the decline of these endemic parrots. Decreasing food and nesting site availability, fragmentation of natural forests, the removal of wild birds for the caged bird trade, low breeding success, and the prevalence of psittacine beak and feather disease have all played a role in population decline (Wirminghaus et al., 1999, Wirminghaus et al., 2000b).

Commercial harvesting of *Afrocarpus/Podocarpus* trees during the late 1800's to early 1900's and the current subsistence harvesting of pole-sized trees in more rural areas (Lawes et al., 2007), are prominent factors leading to the decreased quality of South African yellowwood forests. The shortage of forest fruits is the main driver for *P. r. robustus* to use other non-natural food sources including pecan-nut orchards in agricultural areas (Downs, 2005a) and exotics and commercial trees like black wattle (*Acacia mearnsii*), syringa (*Melia azedarach*) and gum trees (*Eucalyptus* spp.; Wirminghaus et al., 2002b). *P. r. robustus* is not only reliant on *Afrocarpus/Podocarpus* species for food, but also for hollows in dead trees for roosting and nesting sites (Downs and Symes, 2004, Wirminghaus et al., 2001). Selective harvesting of these dead trees decrease the number of possible nesting sites for the birds, adding to the low breeding success of this species (Downs, 2005b, Wirminghaus et al., 2001). Although the use of artificial nest boxes might help alleviate the problem of decreasing nesting site availability, *P. r. robustus* very seldom makes use of them (Downs, 2005b).

The current fragmented state of southern African forests is not solely due to anthropogenic factors, but is also strongly linked to prevailing climate changes (Eeley et al., 1999). Climate driven changes in aridity and increased fire frequency, led to the 'natural' fragmentation of the southern African forests since the start of the Pliocene (Geldenhuys, 1989, Scott et al., 1997). These ancient climate driven fragmentation events would clearly also have influenced the Cape Parrot populations of that time (see Chapter 3 for more information). The loss of the natural habitats causes high levels of population fragmentation, which can greatly influence the levels of gene flow between individual populations (Segelbacher et al., 2003). Lack of gene flow among disjunct populations can result in low genetic diversity and inbreeding, resulting in inbreeding depression which can adversely affect population fitness (Andersen et al., 2004, Hemmings et al., 2012). It was reported that *P. r. robustus* individuals can fly distances of up to a 100 km in search of foraging locations (Skead, 1964), however, the over exploitation of yellowwood forests in South Africa, means that this habitat is increasingly fragmented which could limit gene flow: for example, the Limpopo and KwaZulu-Natal populations are more than 600 km apart (Chapter 3, Figure 3-1). It is therefore necessary to

assess the role played by long-term factors such as climate change and recent human-mediated habitat destruction on the genetic status of this South African parrot to better understand dispersion, dispersal and gene exchange among geographically fragmented populations.

A further threat to the South African *P. r. robustus* is Psittacine beak and feather disease (PBFD) caused by the beak and feather disease virus (BFDV). In 1999, it was suggested that some wild *P. r. robustus* populations might be infected with PBFD after several confiscated illegally caught birds tested positive for the BFDV (Wirminghaus et al., 1999). South African bird breeders lose between 10 - 20 % of their parrot stock each year to PBFD (Heath et al., 2004). This disease can spread to wild bird populations when captive birds escape their aviaries. Psittacine beak and feather disease is also prevalent among captive Cape Parrots, with high losses observed in numerous breeding facilities (C.T. Downs pers. comm.). It is unclear to what extent the wild parrot populations of southern Africa are affected by the disease, but it does pose a significant threat. The illegal trade of wild-caught birds is a significant mechanism through which this disease can spread to wild bird populations (Heath et al., 2004).

1.2.4 Cape Parrots and wildlife trade

Understanding the illegal bird trade is an important part of Cape Parrot conservation. The financial worth of the annual number of traded wild plants and animals, both legally and illegally, is in the billions of dollars (Broad et al., 2002, Interpol, 2014). Fifty-five percent of the world's threatened or near-threatened parrot species are affected by wildlife trading (Pain et al., 2006). The value of exotic birds tends to increase as they become scarcer in natural environments (Cooney and Jepson, 2006, White et al., 2012). There is also no notion of sustainable harvesting or animal welfare with regards to illegal harvesting (Bush et al., 2014, Cooney and Jepson, 2006). Illegal poaching of *P. r. robustus* nestlings and eggs, is an ongoing problem in South Africa and is a major threat to the integrity of the wild populations (Martin et al., 2014, Wirminghaus et al., 1999). Unsustainable harvesting of nestlings and eggs leads to a decrease in nesting success. In other parts of the world an increase in national and/or local protection of wild endemic parrots had a reduction in illegal harvesting and increased nesting success (Pain et al., 2006). The impact of illegal trade on South African *P. r. robustus* populations is further complicated by officials finding it difficult to morphologically distinguish *P. r. robustus* from *P. r. suahelicus* individuals. It is difficult for untrained personnel to identify the subtle morphological differences between the two taxa. Such

difficulties are well known in other closely related parrot species (Abe et al., 2012) and can lead to the misidentification of species. Another problem with illegal trade is that it limits the ability of conservation specialists to identify species of concern, through the monitoring of changes in trading intensities (Cooney and Jepson, 2006, Martin et al., 2014). It is therefore important to have sufficient methods in place to verify the legality of a trade, one such method being DNA-based parentage analyses. The parental identity of suspected illegally birds can be confirmed through the implementation of genetic fingerprinting techniques. The use of molecular techniques, in conjunction with an up to date studbook, is a well-established method of managing captive populations and controlling trade in rare species (Ferrie et al., 2013, Presti et al., 2015, White et al., 2012).

1.3 Thesis structure

Three main research questions were addressed in this thesis. In each case multilocus molecular data were used to answer these questions. Each chapter is formatted as a manuscript for publication in peer reviewed journals, so some overlap in content is unavoidable.

Chapter 2: Molecular Systematics of the Cape Parrot (Poicephalus robustus): Implications for Taxonomy and Conservation

Chapter 2 followed the careful examination of the taxonomic status of *P. r. robustus* using multilocus DNA data. Microsatellite and sequencing data (one nuclear and two mitochondrial genes) were used to examine the taxonomy of *P. r. robustus, P. r. fuscicollis* and *P. r. suahelicus*. Previously published behavioural, ecological and morphological data were used in conjunction with the molecular data to clarify the higher-level taxonomic status of the Cape Parrot. It was found that *P. r. robustus* forms a distinct lineage, separate from *P. r. fuscicollis* and *P. r. suahelicus.* It was recommended that *P. r. robustus* should be elevated to full species status, namely *Poicephalus robustus* senso stricto. This recognition will allow conservation authorities to better protect this parrot species, and will allow greater control over legal trade and better policing of illegal trade.

Chapter 3: Historical biogeography: The influence of ancient and contemporary habitat changes on the endemic South African parrot Poicephalus robustus

The current fragmented state of the South African mistbelt forests are both due to historical climate changes and more recent anthropogenic factors such as logging of Yellowwood trees.

Chapter 3 focuses on comparing and contrasting the historical and contemporary genetic structure of wild populations of Cape Parrots, and to identify the effects of ancient and more recent habitat fragmentation events. Multilocus nuclear DNA data were amplified from historical (1870 to 1946) and recently collected (1951 to 2014) material. The genetic data were combined with geographical data in a phylogeographic study to assess what impact long-term processes and recent anthropogenic driven habitat changes have had on the genetic differentiation of the *P. robustus* populations in South Africa. Ancient and more recent (early 1900's) bottleneck events were identified. These events played a major role in the current Cape Parrot population structure. Three distinct genetic clusters (South, Central and North) were identified, with the North cluster showing signs of isolation from the other clusters. It was observed that the South cluster forms a source population for immigration into the Central population, highlighting the importance of this southern genetic cluster. This information will help conservation authorities to better plan and implement appropriate conservation efforts to protect the Cape Parrot.

Chapter 4: Validation of microsatellite multiplexes for individual identification of Cape Parrots (Poicephalus robustus): paternity testing and use against illegal trade

The illegal trade of wild Cape Parrots is a major problem for the conservation of this species. It is therefore important to develop tools that can improve the monitoring of legal and illegal trade in Cape Parrots. Chapter 4 addresses this issue by identifying a set of microsatellite markers suited for individual identification of captive-bred Cape Parrots and for use in identifying the area of origin of possible illegally harvested individuals. Additionally, the genetic differentiation among the three wild Cape Parrot populations identified in Chapter 3 and the captive populations was assessed to evaluate the genetic health of the captive populations. Two microsatellite panels were identified for use in parentage analysis and assignment of confiscated individuals to their population of origin. These results will assist conservation and law enforcement agencies to better police trade of this South African endemic and will also aid in the management of the captive population.

1.4 References

ABE, H., HAYANO, A. & INOUE-MURAYAMA, M. 2012. Forensic species identification of large macaws using DNA barcodes and microsatellite profiles. *Molecular biology reports,* 39**,** 693-699.

ANDERSEN, L. W., FOG, K. & DAMGAARD, C. 2004. Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). *Proceedings of the Royal Society of London, Series B: Biological Sciences,* 271**,** 1293-1302.

BROAD, S., MULLIKEN, T. & ROE, D. 2002. The nature and extent of legal and illegal trade in wildlife. *In:* OLDFIELD, S. (ed.) *The trade in wildlife: regulation for conservation.* London: Earthscan Publications Ltd.

BUSH, E. R., BAKER, S. E. & MACDONALD, D. W. 2014. Global Trade in Exotic Pets 2006–2012. *Conservation Biology,* 28**,** 663-676.

CLANCEY, P. A. 1997. The Cape Parrot: an additional valid species. *Honeyguide,* 43**,** 61-62.

COONEY, R. & JEPSON, P. 2006. The international wild bird trade: what's wrong with blanket bans? *Oryx,* 40**,** 18-23.

DIAMOND, A. W. & HAMILTON, A. C. 1980. The distribution of forest passerine birds and quaternary climatic change in tropical Africa. *Journal of Zoology,* 191**,** 379-402.

DOWNS, C. & SYMES, C. 2004. Snag dynamics and forest structure in Afromontane forests in KwaZulu-Natal, South Africa: implications for the conservation of cavity-nesting avifauna. *South African Journal of Botany,* 70**,** 265-276.

DOWNS, C. T. 2005a. Abundance of the endangered Cape parrot, *Poicephalus robustus*, in South Africa: implications for its survival. *African Zoology,* 40**,** 15-24.

DOWNS, C. T. 2005b. Artificial nest boxes and wild Cape Parrots *Poicephalus robustus*: persistence pays off. *Ostrich,* 76**,** 222-224.

DOWNS, C. T., PFEIFFER, M. & HART, L. A. 2014. Fifteen years of annual Cape Parrot *Poicephalus robustus* censuses: current population trends and conservation contributions. *Ostrich,* 85**,** 273-280.

EELEY, H. A., LAWES, M. J. & PIPER, S. E. 1999. The influence of climate change on the distribution of indigenous forest in KwaZulu-Natal, South Africa. *Journal of Biogeography,* 26**,** 595-617.

FERRIE, G. M., COHEN, O. R., SCHUTZ, P., LEIGHTY, K. A., PLASSE, C., BETTINGER, T. L. & HOFFMAN, E. A. 2013. Identifying parentage using molecular markers: Improving accuracy of studbook records for a captive flock of marabou storks (*Leptoptilus crumeniferus*). *Zoo biology,* 32**,** 556-564.

FORSHAW, J. M. 1978. *Parrots of the world,* Newton Abbot, London, David & Charles.

FORSHAW, J. M. 2010. *Parrots of the world*, Princeton University Press.

FRY, C. H., KEITH, S. & URBAN, E. K. 1988. *The Birds of Africa, Vol. 3,* New York, London, Academic Press.

GELDENHUYS, C. J. 1989. Biogeography of the mixed evergreen forests of southern Africa. *Ecosystems Programmes Occasional Report no. 45.* Pretoria: Foundation for Research Development.

HEATH, L., MARTIN, D. P., WARBURTON, L., PERRIN, M., HORSFIELD, W., KINGSLEY, C., RYBICKI, E. P. & WILLIAMSON, A. L. 2004. Evidence of unique genotypes of beak and feather disease virus in southern Africa. *Journal of Virology,* 78**,** 9277-9284.

HEMMINGS, N. L., SLATE, J. & BIRKHEAD, T. R. 2012. Inbreeding causes early death in a passerine bird. *Nature Communications,* 3**,** 863.

HEWITT, G. 2000. The genetic legacy of the Quaternary ice ages. *Nature,* 405**,** 907-913.

INTERPOL. 2014. *Illegal trade in wildlife and timber products finances criminal and militia groups, threatening security and sustainable development* [Online]. Available: http://www.interpol.int/News-and-media/News/2014/N2014-119 [Accessed 20 August 2015].

JOSEPH, L., TOON, A., SCHIRTZINGER, E. E., WRIGHT, T. F. & SCHODDE, R. 2012. A revised nomenclature and classification for family-group taxa of parrots (Psittaciformes). *Zootaxa,* 3205**,** 26-40.

LAWES, M. J., GRIFFITHS, M. E. & BOUDREAU, S. 2007. Colonial logging and recent subsistence harvesting affect the composition and physiognomy of a podocarp dominated Afrotemperate forest. *Forest Ecology and Management,* 247**,** 48-60.

MARTIN, R. O., PERRIN, M. R., BOYES, R. S., ABEBE, Y. D., ANNORBAH, N. D., ASAMOAH, A., BIZIMANA, D., BOBO, K. S., BUNBURY, N. & BROUWER, J. 2014. Research and conservation of the larger parrots of Africa and Madagascar: a review of knowledge gaps and opportunities. *Ostrich,* 85, 205-233.

MASSA, R., SARA, M., PIAZZA, M., DI GAETANO, C., RANDAZZO, M. & COGNETTI, G. 2000. A molecular approach to the taxonomy and biogeography of African parrots. *Italian Journal of Zoology,* 67**,** 313-317.

MINISTER OF ENVIRONMENTAL AFFAIRS AND TOURISM 2007. National Environmental Management: Biodiversity Act (ACT 10 of 2004): Publication of Lists of Critically Endangered, Endangered, Vulnerable and Protected Species. *In:* DEAT (ed.). Cape Town, South Africa.

MOREAU, R. E. 1966. *The bird faunas of Africa and its islands,* New York, Academic Press.

PAIN, D., MARTINS, T., BOUSSEKEY, M., DIAZ, S., DOWNS, C., EKSTROM, J., GARNETT, S., GILARDI, J., MCNIVEN, D. & PRIMOT, P. 2006. Impact of protection on nest take and nesting success of parrots in Africa, Asia and Australasia. *Animal Conservation,* 9**,** 322-330.

PERRIN, M. R. 2005. A review of the taxonomic status and biology of the Cape Parrot *Poicephalus robustus*, with reference to the Brown-necked Parrot *P. fuscicollis fuscicollis* and the Grey-headed Parrot *P. f. suahelicus*. *Ostrich,* 76**,** 195-205.

PERRIN, M. R. 2012. *Parrots of Africa, Madagascar and the Mascarene Islands,* Johannesburg, Wits University Press.

PRESTI, F. T., GUEDES, N. M., ANTAS, P. T. & MIYAKI, C. Y. 2015. Population genetic structure in Hyacinth Macaws (*Anodorhynchus hyacinthinus*) and identification of the probable origin of confiscated individuals. *Journal of Heredity,* 106**,** 491-502.

SCOTT, L., ANDERSON, H., ANDERSON, J., COWLING, R., RICHARDSON, D. & PIERCE, S. 1997. Vegetation history. *Vegetation of southern Africa***,** 62-84.

SEGELBACHER, G., HÖGLUND, J. & STORCH, I. 2003. From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. *Molecular Ecology,* 12**,** 1773-1780.

SKEAD, C. J. 1964. The overland flights and the feeding habits of the Cape Parrot, *Poicephalus robustus* (Gmelin) in the Eastern Cape Province. *Ostrich,* 35**,** 202-223.

SYMES, C., BROWN, M., WARBURTON, L., PERRIN, M. & DOWNS, C. 2004. Observations of Cape Parrot, *Poicephalus robustus*, nesting in the wild. *Ostrich,* 75**,** 106-109.

SYMES, C. & DOWNS, C. 2002. Occurrence of Cape parrot *Poicephalus robustus* at non-forest feeding sites in South Africa: threats to a declining population. *Bulletin of the African Bird Club,* 9**,** 27-31.

SYMES, C. T. & PERRIN, M. R. 2003. Feeding biology of the Grey-headed Parrot, *Poicephalus fuscicollis suahelicus* (Reichenow), in Northern Province, South Africa. *Emu,* 103**,** 49-58.

SYMES, C. T. & PERRIN, M. R. 2004. Behaviour and some vocalisations of the Grey-headed Parrot *Poicephalus fuscicollis suahelicus*. *Durban Museum Novitates,* 29**,** 5-13.

WHITE, N. E., DAWSON, R., COGHLAN, M. L., TRIDICO, S. R., MAWSON, P. R., HAILE, J. & BUNCE, M. 2012. Application of STR markers in wildlife forensic casework involving Australian black-cockatoos (*Calyptorhynchus* spp.). *Forensic Science International: Genetics,* 6**,** 664-670.

WIRMINGHAUS, J. O. 1997. Cape Parrot *Poicephalus robustus*. *In:* HARRISON, J. A., ALAN, D. G., UNDERHILL, L. L., HERREMANS, M., TREE, A. J., PARKER, V. & BROWN, C. J. (eds.) *The Atlas of Southern African Birds.* Johannesburg: BirdLife South Africa.

WIRMINGHAUS, J. O., DOWNS, C. T., PERRIN, M. R. & SYMES, C. T. 2001. Breeding biology of the Cape Parrot, *Poicephalus robustus*. *Ostrich,* 72**,** 159-164.

WIRMINGHAUS, J. O., DOWNS, C. T., PERRIN, M. R. & SYMES, C. T. 2002a. Taxonomic relationships of the subspecies of the Cape Parrot *Poicephalus robustus* (Gmelin). *Journal of Natural History,* 36**,** 361-378.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 1999. Conservation of the Cape parrot in southern Africa. *South African Journal of Wildlife Research,* 29**,** 118-129.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 2000a. Abundance of the Cape Parrot in South Africa. *South African Journal of Wildlife Research,* 30**,** 43-52.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 2002b. Diet of the Cape Parrot, *Poicephalus robustus*, in Afromontane forests in KwaZulu-Natal, South Africa. *Ostrich,* 73**,** 20-25.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T., PERRIN, M. R. & DEMPSTER, E. R. 2000b. Vocalisations, and some behaviours of the Cape Parrot *Poicephalus robustus*. *Durban Museum Novitates,* 25**,** 12-17.

WRIGHT, T. F., SCHIRTZINGER, E. E., MATSUMOTO, T., EBERHARD, J. R., GRAVES, G. R., SANCHEZ, J. J., CAPELLI, S., MULLER, H., SCHARPEGGE, J., CHAMBERS, G. K. & FLEISCHER, R. C. 2008. A multilocus molecular phylogeny of the parrots (Psittaciformes): Support for a Gondwanan origin during the cretaceous. *Molecular Biology and Evolution,* 25**,** 2141-2156.

CHAPTER 2: MOLECULAR SYSTEMATICS OF THE CAPE PARROT (*POICEPHALUS ROBUSTUS*): IMPLICATIONS FOR TAXONOMY AND CONSERVATION

2.1 Abstract

The taxonomic position of the Cape Parrot (*Poicephalus robustus robustus*) has been the focus of much debate. A number of authors suggest that the Cape Parrot should be viewed as a distinct species separate from the other two *P. robustus* subspecies (*P. r. fuscicollis* and *P. r. suahelicus*). These recommendations were based on morphological, ecological and behavioural assessments. In this study we investigated the validity of these recommendations using multilocus DNA analyses. We genotyped 138 specimens from five *Poicephalus* species (*P. cryptoxanthus, P. gulielmi, P. meyeri, P. robustus* and *P. rueppellii)* using 11 microsatellite loci. Additionally, two mitochondrial (cytochrome oxidase I gene and 16S ribosomal RNA) and one nuclear intron (intron 7 of the β-fibrinogen gene) markers were amplified and sequenced. Bayesian clustering analysis and pairwise F_{ST} analysis of microsatellite data identified *P. r. robustus* as genetically distinct from the other *P. robustus* subspecies. Phylogenetic and molecular clock analyses on sequence data also supported the microsatellite analyses, placing *P. r. robustus* in a distinct clade separate from the other *P. robustus* subspecies. Molecular clock analysis places the most recent common ancestor between *P. r. robustus* and *P. r. fuscicollis* / *P. r. suahelicus* at 2.13 to 2.67 million years ago. Our results all support previous recommendations to elevate the Cape Parrot to species level. This will facilitate better planning and implementation of international and local conservation management strategies for the Cape Parrot.

Key words: Cape Parrot, *Poicephalus*, microsatellite, mitochondrial, molecular clock, species, subspecies

Citation: COETZER, W. G., DOWNS, C. T., PERRIN, M. R. & WILLOWS-MUNRO, S. 2015. Molecular Systematics of the Cape Parrot (*Poicephalus robustus*): Implications for Taxonomy and Conservation. *PLoS ONE,* 10**,** e0133376. doi: 10.1371/journal.pone.0133376.

2.2 Introduction

Accurate species delimitation plays an important role in effective conservation of biodiversity and assisting conservation authorities with the planning and implementation of appropriate conservation strategies. The utility of subspecies in conservation has been a subject of controversy for a long time (Barrowclough, 1980, Gippoliti and Amori, 2007, Mayr, 1982, Phillimore and Owens, 2006, Ryder, 1986, Zink, 2004). It has been shown that in some cases subspecies do not form separate phylogenetic clusters and classifying taxa to subspecies rank can be misleading (Zink, 2004). Consequently, subspecies are not always given the same conservation consideration as species, especially with less well studied taxa, which can hinder protection of genetically distinct lineages (Gippoliti and Amori, 2007, Hazevoet, 1996). This is particularly relevant to birds where Phillimore and Owens (2006) estimated that roughly 36% of traditionally defined avian subspecies from North America and Eurasia form distinct phylogenetic clusters. It is therefore important to critically identify such phylogenetically distinct lineages and where appropriate elevate subspecies to species so that they can be given adequate conservation consideration.

The genus *Poicephalus* (Psittaciformes) is the most species rich and widely distributed parrot genus in Africa. *Poicephalus* consists of nine species which are divided into two clades (Forshaw, 1978, Fry et al., 1988). These are the *P. robustus* clade, including the Cape Parrot (*P. robustus*) and the Red-fronted Parrot (*P. gulielmi*), and the *P. meyeri* clade, consisting of the Yellow-faced Parrot (*P. flavifrons*), the Senegal Parrot (*P. senegalus*), the Red-bellied Parrot (*P. rufiventrisi)*, the Niam-niam Parrot *(P. crassus*), the Brown-headed Parrot (*P. cryptoxanthus*), Meyer's Parrot (*P. meyeri*) and Ruppell's Parrot (*P. rueppellii*) (Massa et al., 2000). Several of the species (*P. robustus, P. gulielmi*, *P. senegalus, P. flavifrons* and *P. meyeri*) are further divided into subspecies. This study will focus on *P. robustus* which is currently divided into three recognised subspecies, namely the Cape Parrot (*P. r. robustus*), the Grey-headed Parrot (*P. r. suahelicus*) and the Brown-necked Parrot (*P. r. fuscicollis*).

The recognition of the South African taxon *P. r. robustus* as a species separate from *P. r. fuscicollis* and *P. r. suahelicus* has been a controversial subject over the last few decades (Clancey, 1997, Perrin, 2012, 2005, Wirminghaus et al., 2002a). The *P. robustus* clade exhibits an allopatric geographical distribution, with most species restricted to forest habitats (Massa et al., 2000). Within the *P. robustus* group, the west African *P. r. fuscicollis* is found in Red Mangrove forests, mature wooded savannah and palm woodlands (Clancey, 1997, Perrin, 2012). This species was once widely distributed from Angola through to West Africa but is

now primarily found in Gambia (Wirminghaus et al., 2002a). The subspecies *P. r. suahelicus* occurs in a wide variety of woodland habitats and is widely distributed in the south-eastern region of the Democratic Republic of Congo, south-western Uganda, Rwanda, north-western Tanzania, Malawi, Zambia, Mozambique, Zimbabwe and the northern Limpopo Province of South Africa (Forshaw, 1989, Fry et al., 1988, Snow, 1978). The South African subspecies *P. r. robustus*, however, is a habitat specialist and is almost exclusively restricted to the southern mistbelt (*Afrocarpus*/*Podocarpus*) forests of southern Africa (Fig. 2-1; Perrin, 2012). The distribution of the subspecies *P. r. robustus* and *P. r. suahelicus* are reported to overlap in the Limpopo Province of South Africa, but there is strong evidence that the two taxa are ecologically separated by habitat and altitude, with *P. r. robustus* found in mixed *Afrocarpus/Podocarpus* mistbelt forests above 1000 m and *P. r. suahelicus* preferring mixed woodland habitats below 800 m (Perrin, 2005).

Morphologically *P. r. robustus* differs significantly from the other two *P. robustus* subspecies. In addition to differences in plumage colouration (Clancey, 1997, Forshaw, 1989, Wirminghaus et al., 2002a), *P. r. robustus* is the smallest of the three subspecies and has a more lightly structured bill than either *P. r. fuscicollis* or *P. r. suahelicus* (Fig. 2-1). Wirminghaus et al. (2002a) showed that there are significant differences in skull dimensions between *P. r. robustus* and *P. r. suahelicus*. The two taxa also have quite distinct behaviour and show differences in vocalisations (Symes and Perrin, 2004a, Wirminghaus et al., 2000), feeding and breeding behaviour (Symes and Perrin, 2003, Wirminghaus et al., 2002b). *P. r. robustus* is a dietary specialist, feeding predominantly on *Afrocarpus/Podocarpus* fruits, they also prefer these trees for nesting during the breeding season (August to February) (Wirminghaus et al., 2001, Wirminghaus et al., 2002b). *P. r. suahelicus*, however, is a dietary generalist feeding on a range of forest fruits and seeds, they prefer to nest in *Adansonia* trees, and breed from April to August (Symes and Perrin, 2004b, 2003).

Given the morphological, ecological and behavioural differentiation of the three *Poicephalus* subspecies, several authors have proposed full species status for the South African *P. r. robustus* (Clancey, 1997, Perrin, 2005, Wirminghaus et al., 2002a). However, international conservation organizations, such as BirdLife International, have highlighted the need for genetic data for full species status to be recognized. In particular genetic data are needed to clarify if overlapping populations of *P. r. robustus* and *P. r. suahelicus* are reproductively isolated.

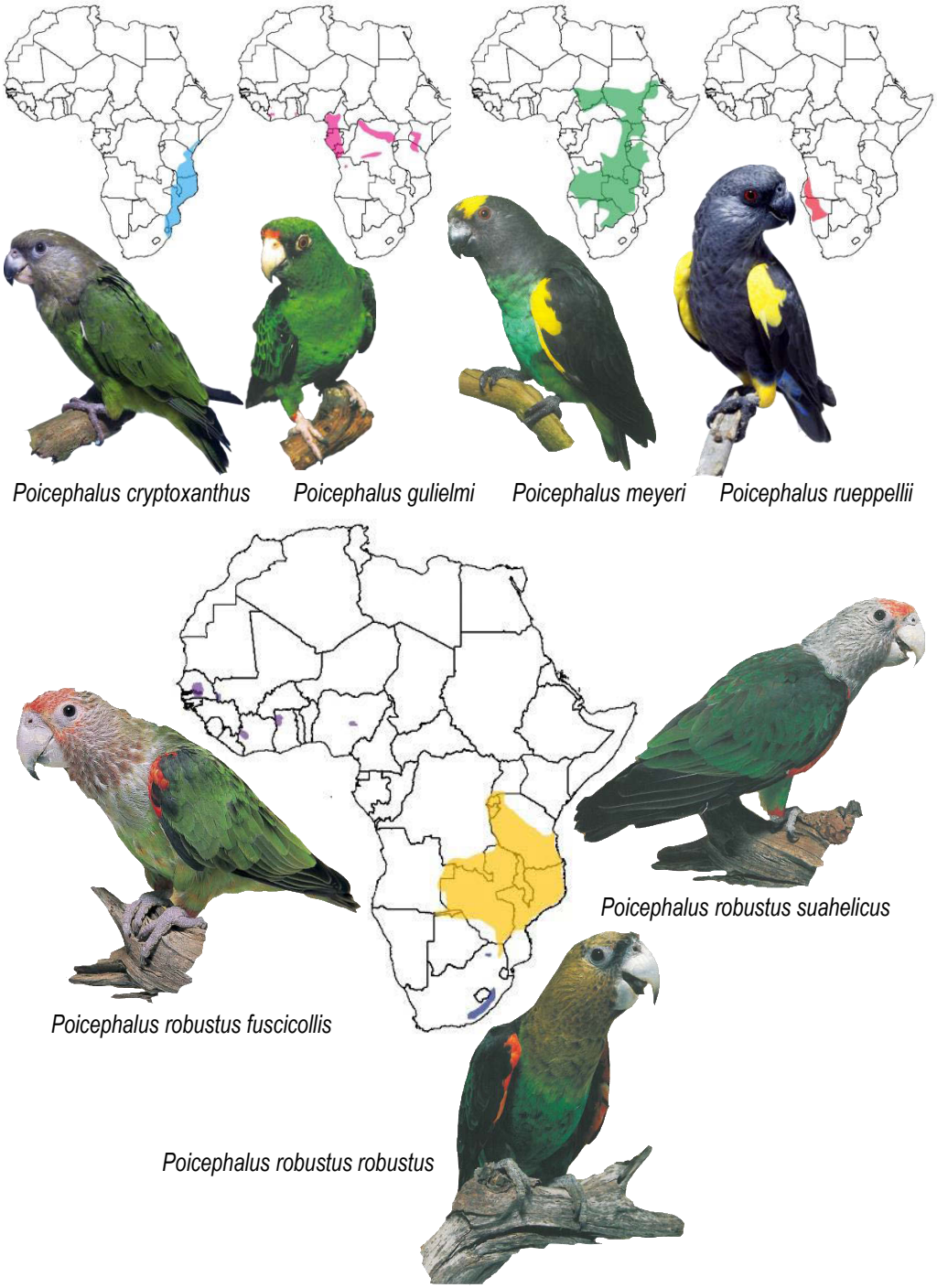


Figure 2-1. Distribution map of the *Poicephalus* species (and *P. robustus* subspecies) included in the study (maps redrawn from Perrin 2012, photos used with permission from Cyril Laubscher).

The conservation of subspecies is a contentious issue, especially given the limited resources currently available for biodiversity conservation. The International Union for Conservation of Nature (IUCN) considers the taxonomic rank of species as the primary unit for conservation (Wenner et al., 2012) and rarely assesses the status of subspecies (Gippoliti

and Amori, 2007). This is problematic for *P. r. robustus*, given that the population has undergone a dramatic decline in the last century with an estimated current population size of less than 1600 birds in the wild (Downs et al., 2014). Up to five decades ago Cape Parrots were recorded in many of the forests of KwaZulu-Natal, but these birds are now only rarely sighted (Wirminghaus et al., 1999). This population decline has been attributed to various factors, including habitat loss, illegal harvesting of wild birds and psittacine beak and feather disease (Downs et al., 2014, Wirminghaus et al., 1999, Wirminghaus et al., 2000).

Based on previously published morphological, behavioural and ecological data (Clancey, 1997, Perrin, 2005, Wirminghaus et al., 2002a), *P. r. robustus* is considered a distinct conservation unit by the International Ornithologists' Union and BirdLife South Africa and is accordingly listed for protection under South African legislation (Minister of Environmental Affairs and Tourism, 2007). It is also listed as Endangered in the Red Data Book of Birds of South Africa, Lesotho and Swaziland (Taylor, 2014). The Cape Parrot is, however, only listed as Least Concern in the IUCN Red List of Threatened Species (BirdLife International, 2012) as the Red List assessment does not recognize *P. r. robustus* as a species separate from the more widespread *P. r. suahelicus* and *P. r. fuscicollis* (Perrin, 2012). In accordance with the IUCN Red List and the position taken by BirdLife International, international trade of *P. r. robustus* under CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) is regulated at the species level, whereby no distinction is made between the three *P. robustus* subspecies, effectively ignoring the poor conservation status of this South African endemic. Effective regulation and monitoring of the international trade in *P. r. robustus* is therefore impossible. To ensure effective conservation of *P. r. robustus*, both locally and internationally, it is important to provide convincing scientific evidence that *P. r. robustus* warrants species status. Our main aim was to assess the taxonomic status of *P. r. robustus* using both multilocus microsatellites and mitochondrial data. We predicted that *P. r. robustus* would be genetically distinct from *P. r. fuscicollis* and *P. r. suahelicus*, thus supporting elevation to full species status that has already been accepted by some authorities on the basis of morphological, ecological and behavioural data (Minister of Environmental Affairs and Tourism, 2007, Taylor, 2014). The use of multilocus analyses is a well-established method of separating closely related bird species (Calderón et al., 2014, Eberhard and Bermingham, 2005, Russello et al., 2010, Tavares et al., 2006) and investigating within species relationships (Eberhard et al., 2004, Faria et al., 2008, Masello et al., 2011, Wenner et al., 2012).

2.3 Materials and Methods

2.3.1 Ethics

We obtained ethical approval for this study from the University of KwaZulu-Natal Animal Ethics sub-committee (Ref numbers: 074/13/Animal, 017/14/Animal). The sampling permits for KwaZulu-Natal were obtained through Ezemvelo KZN Wildlife (Permit number: OP 1546/2014). All necessary import permits were obtained through Ezemvelo KZN Wildlife (Permit numbers: OP 1230/2014 and OP 878/2014) or the National Department of Environmental Affairs (Permit Number: 133120).

2.3.2 Sampling

We included a total of 138 *Poicephalus* specimens representing the four southern African species (*P. cryptoxanthus, P. meyeri, P. robustus* and *P. rueppellii*) and one central African species (*P. gulielmi*; Supplementary Table 2-1) in our analyses. These species were selected based on their distribution ranges either overlapping or being in close proximity to *P. robustus ssp*. We included representatives of all three *P. robustus* subspecies (*P. r. fuscicollis, P. r. robustus* and *P. r. suahelicus*) and two of the *P. gulielmi* subspecies (*P. g. gulielmi* and *P. g. massaicus*). We had multiple representatives of *P. r. robustus* (n = 32) drawn from all three of the isolated South African populations (Eastern Cape = 10, KwaZulu-Natal = 13 and Limpopo = 9). We did this to ensure the inclusion of as much geographic representation across the *P. r. robustus* distribution range as possible.

We used a variety of different tissue types. Whole blood collected from wild trapped and captive bred birds was stored on Whatman FTA Elute or Classic Cards. Clean needles were used for each individual to avoid cross-contamination of blood samples. Feathers were collected from the field, and muscle tissue samples were taken from dead birds. Archival museum toe pad samples were sourced from various local and international museums (Supplementary Table 2-1).

2.3.3 DNA extraction

For the whole blood stored on Whatman FTA Elute cards, we followed the standard DNA extraction protocol as suggested by the manufacturer. The DNA was eluted with 30 μl ultrapure water and stored at -20 °C. We extracted DNA from the muscle tissue samples using the NucleoSpin Tissue kit (Macherey-Nagel), following the manufacturers standard protocol.

Modified extraction protocols were used for the toe pad and feather samples. In order to minimize surface contamination, we performed three washing steps (with 95% ethanol, 70% ethanol and ultrapure water) prior to extraction, followed by a final hydration step where samples were soaked in 1 ml ultrapure water for 60 min. Thereafter we extracted DNA using the NucleoSpin Tissue kit. The lysis step was extended until the samples were completely lysed. The final elution step was also modified, such that after 40 µl of preheated elution buffer was added to the spin column the samples were incubated at 70 °C for 10 min. After centrifuging the samples at 11 000 x g the elution buffer was placed back into the same spin column and an additional 40 µl warmed elution buffer added to each tube. We incubated the samples at 70 °C for 5 min, after which we centrifuged them at 11 000 x g to obtain the final DNA product.

2.3.4 Microsatellite amplification

We chose a panel of 11 microsatellite loci (*Prob06, Prob15, Prob18, Prob23, Prob25, Prob26, Prob28, Prob29, Prob30, Prob34* and *Prob35*), previously described by Pillay et al. (2010), for amplification by polymerase chain reaction (PCR). In each case the forward primers were synthesized with a fluorescent dye on the 5' end. We divided the microsatellite panel into four multiplex sets (Multiplex 1: *Prob06*, *Prob15* and *Prob26*; Multiplex 2: *Prob29*, *Prob34* and *Prob35*; Multiplex3: *Prob18* and *Prob25*; Multplex4: *Prob23* and *Prob28*), with the locus *Prob30* in a single reaction. The PCR reactions for the fresh samples (blood and muscle tissue samples) consisted of: ~2-30 ng template DNA, 5 µl KAPA2G Fast Multiplex mix (KAPA Biosystems), 0.2 µM of each primer and dH_2O to give a final reaction volume of 10 µl. The PCR reaction mixtures for the feather and archival toe pad samples consisted of: ~20-200 ng template DNA, 5 µl KAPA2G Fast Multiplex mix, 0.2 µM of each primer, 0.3 µl of 1 mg/ml BSA and dH_2O to give a final reaction volume of 10 µl. We used identical PCR cycle parameters for all multiplex reactions and included an initial denaturation step at 94 °C for 3 min followed by 30 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, with a final extension step at 72 °C for 5 min. The PCR cycles were increased to 40 cycles for the museum and feather samples to ensure sufficient amplification. PCR setup prior to addition of the DNA was done in a DNA free area to avoid contamination of reagents.

We sent all amplified products to the Central Analytical Facility at Stellenbosch University, South Africa for fragment analysis. The software program Gene Marker v2.4.0 (Soft Genetics) was used for subsequent genotype scoring. To ensure genotyping consistency,

we reamplified the archival museum samples and analysed each locus three times. In addition we reamplified 20% of the fresh samples to check for consistency in genotype scoring.

2.3.5 DNA sequencing

In addition to the microsatellite analysis, we amplified and sequenced two mitochondrial (mtDNA) markers and one nuclear intron (nucDNA) marker: cytochrome oxidase I (COI using the primers BirdF1/BirdR1; Hebert et al., 2004), 16S ribosomal RNA (16S rRNA using the primers 16Sa/16SB; Palumbi et al., 1991) and a nuclear intron of the β-fibrinogen gene (β-fib using the primers FIB-BI7U/FIB-BI7L; Prychitko and Moore, 1997). Where possible, these three markers were amplified for five representative *P. r. robustus* samples (Eastern Cape, KwaZulu-Natal and Limpopo) and two samples for each of the other species and subspecies included in the microsatellite analysis (See Supplementary Table 2-1). PCR reactions for COI and 16S rRNA consisted of: ~20-150 ng template DNA, 2.5 µl 10 x KAPA buffer, 1 U KAPA Taq DNA polymerase, 200 µM dNTPs, 0.2 µM of each primer and 18.4 µl dH_2O to give a final reaction volume of 25 μl. We added an additional 0.5 mM $MgCl_2$ to the reaction mixture for β-fib. A touchdown PCR protocol was used. The PCR cycle parameters for COI and 16S rRNA included an initial denaturation step at 95 °C for 3 min followed by 10 cycles at 95 °C for 30 s, 60-50 °C for 30 s, 72 °C for 30 s, 25 cycles at 95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s with a final extension step at 72 °C for 5 min. The touchdown temperature range and annealing temperature for β-fib was 65 - 55 °C and 55 °C.

We sent all PCR products which showed positive amplification for sequencing. Cycle sequencing was performed using the BigDye Chemistry, v3.1 and sequencing products were analyzed on an Applied Biosystems 3730xl Genetic Analyzer (Applied Biosystematics, Perkin Elmer). All heterozygous sites in the nuclear intron were coded using the International Union of Biochemistry (IUB) codes. All raw sequence data were viewed and edited in BioEdit v7.1.11 (Hall, 1999). The edited sequences were aligned using ClustalW (Thompson et al., 1994) as implemented in MEGA v6 (Tamura et al., 2013) and then checked manually to ensure homology. We deposited all new sequences in GenBank (Supplementary Table 2-2). *Psittacus erithacus,* the Grey Parrot, was included as an outgroup with sequences downloaded from GenBank (Supplementary Table 2-2).

2.3.6 Data analysis

2.3.6.1 Microsatellite analysis

We estimated null allele frequencies for each marker using the software program FreeNA (Chapuis and Estoup, 2007) using the Expectation Maximization algorithm (EM) (Dempster et al., 1977). We compared the null allele corrected and uncorrected global F_{ST} values using the excluding null alleles (ENA) method (Chapuis and Estoup, 2007). Summary statistics (average number of alleles, observed and expected heterozygosity and the number of private alleles), polymorphic information content (PIC), pairwise F_{ST} and analysis of molecular variance (AMOVA) were estimated using GenAlEx v6.5 (Peakall and Smouse, 2012) and Cervus v3.0.7 (Kalinowski et al., 2007). Two AMOVA analyses were conducted. One grouping individuals into five species (*P. cryptoxanthus, P. gulielmi, P. meyeri, P. robustus* and *P. rueppellii)*. The three *P. robustus* subspecies and the two *P. gulielmi* subspecies were placed into *P. robustus* and *P. gulielmi* respectively. A second AMOVA was conducted in which the subspecies (*P. g. gulielmi, P. g. massaicus, P. r. fuscicollis, P. r. robustus* and *P. r. suahelicus)* were placed into individual groups. We used the program XLSTAT 2014 (XLSTAT, 2014) to generate a 3D principal coordinates analysis (PCoA) figure using pairwise F_{ST} values. Arlequin v3.5 (Excoffier et al., 2005) was used to test for linkage disequilibrium and deviation from Hardy–Weinberg equilibrium. We performed Bayesian clustering analysis in STRUCTURE v2.3.4 (Pritchard et al., 2010). Ten independent runs were performed. Each STRUCTURE run consisted of 1,000,000 Markov chain Monte Carlo (MCMC) replicates with a burn-in of 100,000 with the proposed number of clusters (K) ranging from 2 to 10. The no admixture model with correlated allele frequencies was selected for all runs. Sampling locality information was incorporated using the LOCPRIOR model. We used the program STRUCTURE harvester (Earl, 2009) to estimate the most probable number of genetic clusters using the method implemented by Evanno et al. (2005). The STRUCTURE figure and the membership probabilities (Q-values) for each individual and for each cluster were estimated using ClumpAK (http://clumpak.tau.ac.il).

2.3.6.2 DNA sequence analysis

We initially analyzed the three gene regions (COI, 16S rRNA and β-fib) separately and then combined them into a single data matrix. In addition, we analyzed the sequence data from each gene according to origin of marker (mtDNA or nucDNA). Number of variable sites, number of observed transitions, number of observed transversions and number of observed indels were

estimated using MEGA and Arlequin. Phylogenies were constructed using both maximum likelihood (ML) conducted in Garli v2 (Zwickl, 2006) and Bayesian inference (BI) using MrBayes v3.2 (Ronquist et al., 2012). For these analyses the optimal model of nucleotide substitution for each gene region was used. This was selected using the Akaike information criterion (AIC) (Akaike, 1974) in jModelTest v.2.1 (Darriba et al., 2012). In the combined analyses, data were partitioned by gene with model parameters unlinked across partitions. In ML analyses branch support was assessed using 1000 bootstraps replicates with consensus topologies generated using PHYLIP v3.695 (Felsenstein, 1989, Felsenstein, 2009). Each Bayesian run consisted of three heated chains at default temperature of 0.2 and one cold chain and was run for 10 million generations with the sampling frequency of 1000 and a burn-in of 0.25 (25,000 trees). To ensure that MCMC chains had reached convergence, Tracer v1.5 (Rambaut and Drummond, 2007) was used to verify that the appropriate estimated sample sizes (ESS) for all parameters were above 200 (Drummond et al., 2006). A 50% majority rule consensus tree was constructed in PHYLIP after burn-in was removed. We viewed trees in FigTree v1.4.0 (Rambaut, 2012). We calculated pairwise genetic distances in RAxMLGUI v.1.3.1 (Silvestro and Michalak, 2012) using the general time reversible nucleotide substitution model with gamma distribution and invariant sites (GTRGAMMAI).

2.3.6.3 Molecular clock analysis

There are no fossil calibration points available within the genus *Poicephalus*. Molecular clock analysis was performed using secondary calibration dates from two other studies (Schweizer et al., 2011, White et al., 2011) to estimate divergence times of *Poicephalus* species. Schweizer et al. (2011), using three nuclear genes, used three avian fossil records outside Psittaciformes as calibration points to estimate diversification times. These authors estimated that the separation of Strigopidae from the rest of the parrot taxa occurred ~58.6 million years ago (Mya). White et al. (2011) used full mitochondrial genomes and six avian fossil records as calibration points to study the evolutionary history of the Cacatuidae and estimated Strigopidae and the other parrot taxa split ~47.4 Mya.

Given that the divergence dates estimated by Schweizer et al. (2011) and White et al. (2011) are quite different, we used the calibration points from these two studies in separate analyses. Five calibration points were used from Schweizer et al. (2011) and included the split between *Nestor* and the rest of the parrot taxa (58.59 Mya; SD: 8.2), the split between the Australasian Cacatuidae and Psittacidae (47.38 Mya; SD: 7), the split between *Psittacus*-*Poicephalus* and Arini (35.16 Mya; SD: 5.6), the split between *Amazona*/*Pionus* and

Ara/*Deroptyus* (25.26 Mya; SD: 5) and the split between *Psittacus* and *Poicephalus* (12.92 Mya; SD: 3.5). We used three calibration points from White et al. (2011) and included the split between *Nestor* and the rest of the parrot taxa (47.4 Mya; SD: 7), the split between the Australasian Cacatuidae and Psittacidae (40.7; SD: 7) and the split between *Cacatuninae* and *Calyptorhynchinae* (27.9 Mya; SD: 6).

We downloaded sequences from 27 parrot species covering 21 genera, used by Schweizer et al. (2011), from GenBank (Supplementary Table 2-2) and included them in the molecular clock analyses. Divergence times were calculated using BEAST v1.8 (Drummond et al., 2012). We conducted analyses on two datasets, one containing sequences from all three gene regions (COI, 16S rRNA and β-fib) and then to limit the inclusion of missing data we also analysed a dataset containing only the mtDNA genes. In all analyses we partitioned the data by gene with the parameters of the substitution models unlinked. The GTR + Γ + I substitution model was used for COI and the GTR + I model was used for β-fib as the best-fit models suggested by jModelTest (TPM2uf + Γ + I and TPM1uf + I) are not currently implemented in BEAST. The GTR + Γ + I model was, however, identified as the best fit model for 16S rRNA. A lognormal relaxed-clock approach was implemented following Schweizer et al. (2011) with a Yule speciation model set as tree prior.

For each dataset (three gene and mtDNA only), we conducted two independent simulations for each set of calibration points. Each BEAST run consisted of 400 million generations, with a sampling frequency of 10000 trees. The program Tracer was used to confirm that MCMC chains had reached stationarity and ESS of all parameters were greater than 200. We used TreeAnnotator v1.8.1 (in Beast v1.8.1) to estimate the maximum clade probability tree which we viewed in TreeGraph v2 (Stover and Muller, 2010).

2.4 Results

2.4.1 Microsatellite analysis

In this study we genotyped 138 individuals using 11 microsatellite loci. Individuals in this data set had minimal missing data, with only 3.03% missing data included. Mean null allele frequencies ranged from 0.4 % -14.6 % across species (Na; Supplementary Table 2-3). In particular the error rate in the data collected from four loci in the *P. rueppellii* dataset (*Prob18*, Na = 23.3%; *Prob26*, Na = 22.9%; *Prob29*, Na = 30.2%; *Prob34*, Na = 26.0%) was high, although below values reported in other studies (Dakin and Avise, 2004). No loci showed null

allele frequencies higher than 30.2%. The detection of null alleles can be biased in natural populations which deviate from Hardy-Weinberg Equilibrium (HWE), as is the case in this study where all loci except *Prob35* deviated from Hardy-Weinberg equilibrium in at least one species/subspecies. The presence of null alleles can inflate F_{ST} values (Carlsson, 2008), but we found that there was little difference between the global F_{ST} values using the ENA corrected (F_{ST} = 0.25) and the uncorrected (F_{ST} = 0.26) data. The effects of any null alleles present in the *Poicephalus* data set are likely minimal and we performed all future analysis using data from all loci.

Estimates of the mean number of alleles, private alleles, observed and expected heterozygosity are reported in Table 2-1. All loci were polymorphic in all species/subspecies with the exception of *Prob18* and *Prob30* which were monomorphic in *P. g. gulielmi* and *Prob25* and *Prob35* which were monomorphic in *P. g. massaicus* (Supplementary Table 2-3). Ascertainment bias could influence genetic diversity analyses, as allele numbers might be higher in the focal species from which the markers were developed (Brandström and Ellegren, 2008). Noticeably lower allele numbers were only observed in two species (*P. rueppellii* and *P. gulielmi*). One locus in *P. rueppellii* (*Prob6*), two loci in *P. g. massaicus* (*Prob30* and *Prob25*), and four loci in *P. g. gulielmi* (*Prob6*, *Prob15*, *Prob18* and *Prob30*) showed allele numbers < 50% of that observed in *P. robustus ssp.* Low sample number is also a consideration in the case of *P. g. gulielmi* (n = 4; Supplementary Table 2-3).

Nine of the eleven loci used were highly informative with PIC values > 0.7. The PIC values for each locus range from 0.513 (*Prob35*) to 0.895 (*Prob23*) with a mean PIC value of 0.794. (Supplementary Table 2-3). The highest mean number of alleles was recorded for *P. r. suahelicus* (N_A = 7.091). Private alleles (P_A) were identified for all species and subspecies. The most distinct species *P. rueppellii* has nine private alleles. The number of private alleles observed in the *P. robustus* subspecies ranged from one to six alleles, with *P. r. suahelicus* possessing the highest number of private alleles (P_A = 6). The observed heterozygosity (H_O) ranged from 0.368 to 0.632, and the expected heterozygosity (H_E) range from 0.457 to 0.705 over all species and subspecies. Of the three *P. robustus* subspecies, *P. r. suahelicus* showed the highest level of genetic diversity (H_E = 0.701; Table 2-1) and is comparable to previous observations (H_E = 0.76) (Taylor, 2011). *P. r. fuscicollis* has a relatively small and fragmented distribution range, and comparatively has the lowest level of genetic diversity among the *P. robustus* subspecies (H_E = 0.557). This lower genetic diversity estimate might be an artefact of sample size or that the majority of these samples were from captive bred birds, given that

Taylor (2011) observed a higher level of genetic diversity for *P. r. fuscicollis* (H_E = 0.77). The observed heterozygosity of *P. r. robustus* (H_O = 0.622) was comparable to previous estimates (H_O = 0.63) (Pillay et al., 2010).

Table 2-1. Sample details and genetic diversity for each *Poicephalus* species and subspecies analysed. Number of individuals sampled, observed heterozygosity, expected heterozygosity, mean number of alleles and number of private alleles are provided.

Species:	Number of samples:	Average number of alleles:	Observed Heterozygosity:	Expected Heterozygosity:	Number of private alleles:	Polymorphic information content:
P. robustus robustus	32	5.455	0.622	0.619	1	0.567
P. r. suahelicus	23	7.091	0.632	0.701	6	0.673
P. r. fuscicollis	26	5	0.485	0.557	1	0.519
P. meyeri	12	5.727	0.515	0.705	6	0.664
P. rueppellii	16	4.818	0.368	0.581	9	0.536
P. cryptoxanthus	14	5.909	0.504	0.612	3	0.582
P. gulielmi gulielmi	4	2.818	0.545	0.457	4	0.412
P. g. massaicus	11	4.364	0.471	0.498	4	0.472
Total:	**138**	**5.148**	**0.518**	**0.591**	**-**	**0.794**

2.4.2 Species delimitation

2.4.2.1 Microsatellite analysis

The Bayesian clustering analysis identified seven genetic clusters (K = 7, mean LnP(K) = -4222.4; Fig. 2) as the most likely number of clusters following Evanno et al. [50]. These clusters corresponds to the species *P. cryptoxanthus, P. gulielmi* (with *P. g. gulielmi* and *P. g. massaicus* clustering together; Q = 1*)*, *P. meyeri* and *P. rueppelli*. The three *P. robustus* subspecies were assigned to separate clusters with only two *P. r. fuscicollis* individuals assigned to the *P. r. suahelicus* cluster with high probability (Q = 0.99).

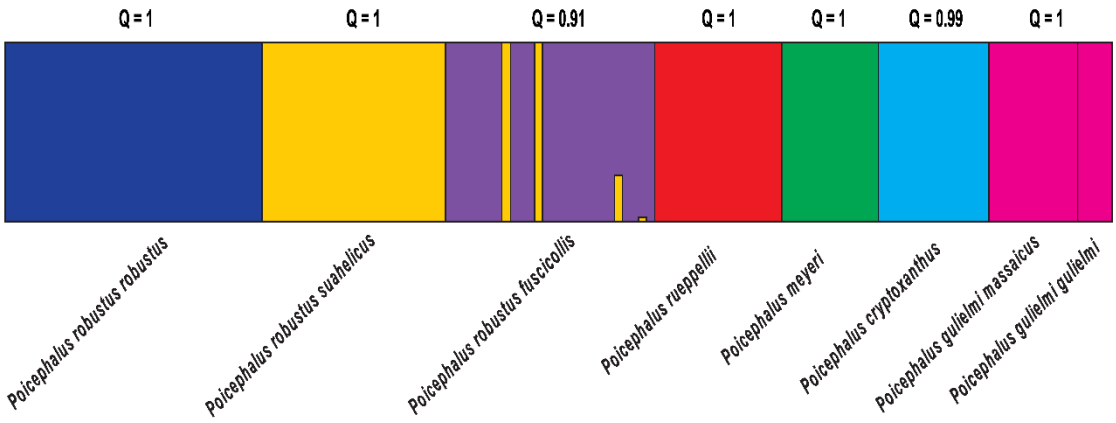


Figure 2-2. The estimated population genetic structure of the *Poicephalus* species/subspecies used in the current study (K = 7). Each individual is represented by a vertical line, and coloured according to each individual's estimated membership probability (Q-values). Average Q-values for each cluster is depicted above the figure.

The STRUCTURE clustering was supported by pairwise F_{ST} values which were highly significant between all species and subspecies ($0.13 \leq F_{ST} \leq 0.41$; *P*-value < 0.05). The pairwise F_{ST} values between *P. r. robustus* and *P. r. suahelicus* ($F_{ST} = 0.14$; *P*-value = 0.001), and *P. r. robustus* and *P. r. fuscicollis* ($F_{ST} = 0.22$: *P*-value = 0.001) were comparable to the pairwise values between the other *Poicephalus* species, for example between *P. cryptoxanthus* and *P. meyeri* ($F_{ST} = 0.14$; *P*-value = 0.001) and *P. cryptoxanthus* and *P. rueppelli* ($F_{ST} = 0.21$; *P*-value = 0.001; Supplementary Table 2-4). These relationships can also be clearly seen in the 3D PCoA drawn from the pairwise F_{ST} values (Fig. 3). The global F_{ST} value (subspecies assigned to species) was significantly different from zero ($F_{ST} = 0.21$; *P*-value = 0.001) and the analysis of molecular variance (AMOVA) indicated that 21% of the observed genetic variation occurred between species with 58% occurring within individuals and 21% among individuals that belong to the same species/subspecies. High F_{ST} values were also recovered when individuals were assigned to subspecies ($F_{ST} = 0.25$; *P*-value = 0.001). The AMOVA analysis indicated that 25% of the variation occurs between species and subspecies, with 14% variation among individuals. The majority of the genetic variation occurred within individuals (61%).

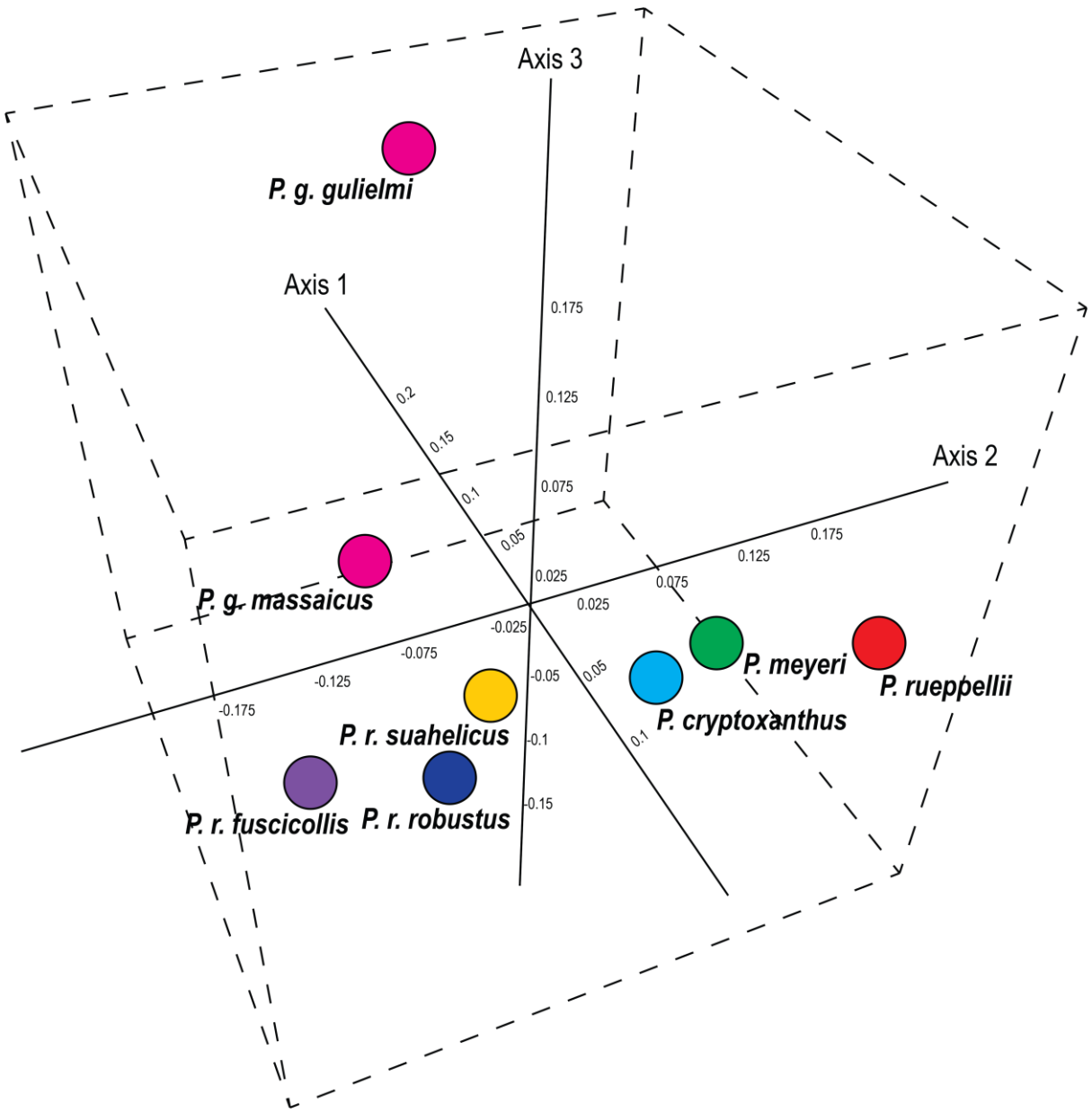


Figure 2-3. A 3D principal coordinates analysis (PCoA), generated in XLSTAT 2014. The pairwise F_{ST} values estimated between species/subspecies of the *Poicephalus* species included in the study were used to generate the figure. The first three axes explained 70.3% of the estimated variation.

2.4.2.2 Phylogenetic analysis

The two mtDNA markers were successfully amplified for all 18 specimens; unfortunately β-fib was only successfully sequenced from 10 specimens (Supplementary Table 2-5). The data matrices for each marker included (Supplementary Table 2-5): COI (592 bp; 66 variable sites), 16S rRNA (707 bp; 32 variable sites) and β-fib (707 bp; 4 variable sites). To reduce the effects of missing data we conducted two analyses. First, the data from all three markers including missing data were concatenated and analysed. Second, only data from the two mtDNA markers

were analysed (no missing data included). There was no significant conflict among the topologies produced when each marker was analysed independently and the data were concatenated (concatenated: 1834 bp, 102 variable characters; mtDNA only: 1127 bp, 98 variable characters). The *P. robustus* clade formed a distinct monophyletic group separate from the *P. meyeri* clade in both the concatenated (ML bootstrap, 87; Bayes' posterior probability, 1.00) and mtDNA topologies (ML bootstrap, 94; Bayes' posterior probability, 1.00), supporting hypotheses proposed by Forshaw (1978) and Fry et al. (1988).

The phylogenetic analysis confirmed the monophyly of all species with the exception of *P. robustus* (Fig. 2-4). Phylogenetic analysis of the mtDNA markers cluster together the subspecies *P r. fuscicollis* and *P. r. suahelicus* (ML bootstrap, 54; Bayes' posterior probability, 0.92). The phylogenetic position of *P. r. robustus* is not well resolved. In the concatenated analysis *P. r. robustus* is placed sister to a clade containing the two *P. gulielmi* subspecies although this association is only weakly supported (ML bootstrap, 58; Bayes' posterior probability, 0.62). In the mtDNA phylogeny the three *P. robustus* subspecies are clustered together (ML bootstrap, 68; Bayes' posterior probability, 0.89).

The COI sequence differentiation among the *P. robustus* subspecies was comparable to that observed among other well-established parrot species. For example, the average pairwise genetic distance for *P. r. robustus* vs. *P. r. fuscicollis* (D = 4.5%) and *P. r. robustus* vs. *P. r. suahelicus* (D = 4.9%; Table 2-2) was greater than the genetic difference between three well-established cockatoo species [69]: *Calyptorhynchus funereus* vs. *C. latirostris* (D = 3.0%) and *C. funereus* vs. *C. baudinii* (D = 3.6%; Supplementary Table 2-6). Comparable genetic distance values were observed by Rocha et al. [70] using COI sequences to investigate the taxonomic relationship between two closely related Amazon parrot species, *Amazona pretrei* and *A. tucumana* (D = 2.2%).

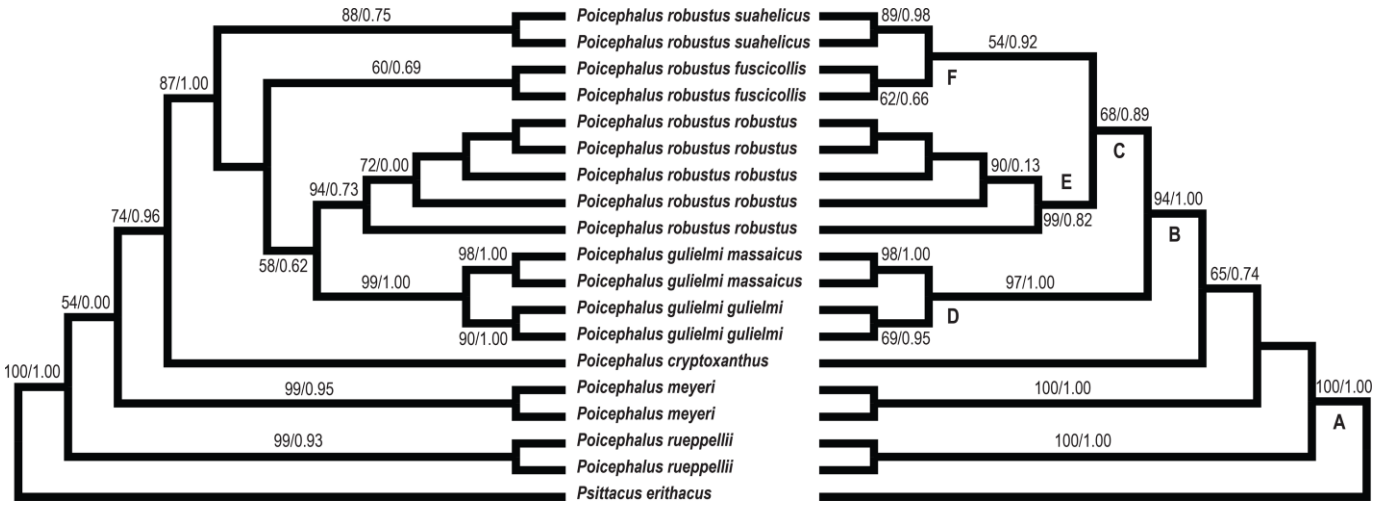


Figure 2-4. Maximum likelihood phylogeny retrieved of the *Poicephalus* specimens from the current study. The analyses were performed using (left) concatenated data (COI, 16S rRNA and β-fib) and (right) mitochondrial DNA data. Values given above the branches represent maximum likelihood bootstrap values and Bayesian posterior probabilities (in that order). Dated nodes are indicated by letters next to each node in the right hand tree and correspond to Table 2-3. *Psittacus erithacus* was used as an outgroup.

Table 2-2. The average pairwise genetic distances estimated in RAxML using the concatenated dataset of all three gene regions (below diagonal) and using COI data only (above diagonal) from the *Poicephalus* specimens used in the current study.

	P. r. robustus	*P. r. suahelicus*	*P. r. fuscicollis*	*P.g. gulielmi*	*P.g. massaicus*	*P. meyeri*	*P. rueppellii*	*P. cryptoxanthus*
P. robustus robustus	*	0.049	0.045	0.237	0.277	0.26	0.281	0.238
P. r. suahelicus	0.036	*	0.009	0.27	0.313	0.242	0.239	0.211
P. r. fuscicollis	0.045	0.009	*	0.261	0.289	0.262	0.258	0.217
P. gulielmi gulielmi	0.175	0.185	0.189	*	0.074	0.406	0.285	0.316
P. g. massaicus	0.164	0.164	0.186	0.044	*	0.485	0.376	0.432
P. meyeri	0.271	0.243	0.247	0.35	0.392	*	0.113	0.124
P. rueppellii	0.288	0.243	0.248	0.271	0.325	0.104	*	0.115
P. cryptoxanthus	0.241	0.208	0.205	0.263	0.325	0.11	0.105	*

2.4.2.3 Molecular clock analysis

The molecular clock analyses conducted with the concatenated (COI, 16S rRNA and β-fib) and mtDNA (COI and 16S rRNA) datasets produced similar maximum clade probability trees, which suggests that the β-fib missing data did not negatively bias the molecular clock analysis. The estimated divergence dates obtained from the Schweizer et al. (2011) and White et al. (2011) calibration points were similar (all fall within the 95% highest posterior density (HPD) range of each other; Table 2-3). The most recent common ancestor of the *Poicephalus* species included in the present study dated to 10.27 to 10.63 Mya. The origin of the *P. robustus* clade is estimated at 6.16 to 6.72 Mya. The maximum clade probability tree suggests that the *P. r. fuscicollis* and *P. r. suahelicus* lineage (0.57 to 0.69 Mya) is younger than the most recent common ancestor of the three *P. r. robustus* populations (Eastern Cape, KwaZulu-Natal and Limpopo; 1.16 to 1.44 Mya). It is clear that *P. r. robustus* represents a distinct evolutionary lineage, having diverged from the other species during the late Pliocene to early Pleistocene (2.13 to 2.67 Mya; Table 2-3). Similar divergence dates were estimated for two recognised cockatoo species, *Calyptorhynchus funereus* and *Calyptorhynchus latirostris* (2.49 to 2.83 Mya; Supplementary Table 2-6).

Table 2-3. Divergence dates of the seven *Poicephalus* species, with *Psittacus erithacus* as outgroup, analysed with a Bayesian lognormal relaxed-clock model. The mean estimated values and the 95% highest posterior density (HPD) ranges are given for the two dataset partitions. The node numbers correspond to Figure 2-4. See Supplementary Figure 2-1 for the corresponding maximum clade probability trees.

	Divergence times using mtDNA and nuclear gene regions following Schweizer et al. (2011):		Divergence times using mtDNA gene regions only following Schweizer et al. (2011):		Divergence times using mtDNA and nuclear gene regions following White et al. (2011):		Divergence times using mtDNA gene regions only following White et al. (2011):	
Node number/ID:	Mean:	95% HPD (Mya):	Mean:	95% HPD (Mya):	Mean:	95% HPD (Mya):	Mean:	95% HPD (Mya):
a / *Poicephalus*	10.4	7.32-13.87	10.34	7.11-14.03	10.27	6.55-15.04	10.63	6.58-15.73
b / *P. robustus* complex	6.36	4.12-9.14	6.64	4.14-9.61	6.16	3.68-9.53	6.72	3.88-10.33
c / *P. robustus ssp.*	2.24	1.15-3.60	2.67	1.35-4.37	2.13	1.03-3.15	2.62	1.27-4.45
d / *P. gulielmi ssp.*	1.9	0.90-3.26	2.01	0.91-3.50	1.81	0.80-3.21	1.97	0.86-3.53
e / *P. robustus* population	1.23	0.52-2.16	1.44	0.60-2.63	1.16	0.48-2.11	1.4	0.55-2.61
f / *P. r. suahelicus-fuscicollis*	0.6	0.16-1.25	0.69	0.17-1.46	0.57	0.14-1.20	0.66	0.16-1.44

2.5 Discussion

The multilocus nuclear and mtDNA results obtained from the current study along with previous morphological, ecological and behavioural data (Clancey, 1997, Perrin, 2012, 2005, Wirminghaus et al., 2002a) provide strong support for the classification of *P. r. robustus* as a distinct species separate from *P. r. fuscicollis* and *P. r. suahelicus*, namely *P. robustus* sensu stricto. Our results showed no hybrids or signs of genetic introgression between *P. r. robustus* and *P. r. suahelicus*, even in the Limpopo Province of South Africa where these subspecies occur in close proximity.

Multilocus molecular data are often used to investigate taxonomic issues within Psittaciformes. Wenner et al. (2012) performed a taxonomic analysis of the *Amazona farinose* species complex using four mtDNA and two non-coding nuclear intron fragments. The authors found support for distinct Central and South American Mealy Amazon clades. It was suggested that these clades should be split into separate species to allow for the implementation of appropriate conservation planning (Wenner et al., 2012). In another study, the phylogenetic relationships within the *Ampazona ochrocephala* species complex were investigated by Eberhard et al. (2004) using four mtDNA markers. The authors found no support for the division of the complex into three species as proposed by others (Eberhard et al., 2004, Juniper and Parr, 1998, Sibley and Monroe, 1990, American Ornithologists' Union, 1998). Molecular data have also been used in the past to resolve taxonomic problems in other avian species. For example, microsatellite and mtDNA data were used to assess taxonomic questions within the widespread plover species, *Charadrius alexandrinus* (Rheindt et al., 2011). The authors confirmed the recommendations by Küpper et al. (2009) that *C. nivosus* should be considered a separate species. Vilaça and Santos (2010) investigated the taxonomic status of the *Basileuterus culicivorus* species complex using an mtDNA (cytochrome *b*), a nuclear intron (β-fibrinogen intron 5) and six microsatellite markers. The two species from the species complex were found to be genetically indistinguishable and it was recommended that these taxa should be grouped into a single species, namely *Basileuterus culicivorus*.

2.5.1 Evolution of the Cape Parrot

The divergence of *P. robustus* senso stricto from *P. r. fuscicollis* and *P. r. suahelicus* coincides with the start of the Quaternary Period (early Pleistocene) about 2.4 Mya. The Quaternary period consisted of a series of ice ages which led to major global climatic changes resulting in drastic vegetation and habitat changes. These climatic changes led to numerous cycles of

grassland expansions and forest contractions and *vice versa.* The expansion of grasslands in Africa and subsequent forest contraction happened at around 2.4 Mya (Bobe and Behrensmeyer, 2003). It has been estimated that the Afromontane forests of southern Africa have been expanding and contracting over the last 100 000 years in accordance with glacial cycles (Lawes, 1990). South African Afromontane forest is the oldest of the two major forest types found in southern Africa, and has been present prior to the last glacial maximum (~18000 BP) (Moreau, 1963). The discovery of parrot fossils in the Western Cape Province, South Africa, dating to the early Pliocene indicates the presence of woodlands in this area and signifies the substantial changes in habitat during the Plio-Pleistocene (Manegold, 2013). The contraction of forests during the arid glacial periods would have driven ancestral forest dwelling species, for example the *P. robustus* ancestor, into forest refugia (Hewitt, 2000). These fragmented subpopulations would have started to differentiate under adaptive pressures (such as dietary constraints), leading to speciation events (Massa et al., 2000, Perrin, 2005). It is proposed that about 1-2 million years (Myr) is sufficient time for speciation to occur (Hewitt, 2000). Using *cytochrome b* sequence data, Kundu et al. (2012) estimated that speciation events occurred within the Afro-Asian parakeet genus *Psittacula* about every 1-2 Myr, and our data suggests that *Poicephalus* show similar short periods of cladogenesis. Comparable molecular clock estimates were obtained for the two extant New Zealand *Nestor* species (*Nestor meridionalis* and *Nestor notabili*; Rheindt et al., 2014). The authors suggest that the separation between *N. meridionalis* and *N. notabili* occurred between 2.3 to 2.5 Mya, using a multilocus dataset and calibration points from Schweizer et al. (2011) and White et al. (2011).

2.5.2 Taxonomic and conservation considerations

Multiple data sources, including morphological, ecological, behavioural and now molecular, provide convincing scientific evidence that *P. r. robustus* is a distinct taxonomic unit separate from *P. r. fuscicollis* and *P. r. suahelicus*. This lineage fulfills the criteria for at least four methods of species delimitation including Biological (Mayr, 1942), Morphological (Mishler, 1985, Nelson and Plantick, 1981), Genotypic (Mallet, 1995) and Phylogenetic (Cracraft, 1983, Eldredge and Cracraft, 1980) species concepts. Reproductive isolation is a key criteria for the Biological Species Concept. Behavioural studies have reported that the two taxa whose distributions overlap in South Africa, *P. r. robustus* and *P. r. suahelicus,* breed at different times of the year, with *P. r. robustus* breeding from August to February (mainly utilizing *Afrocacarpus/Podocarpus* trees), while *P. r. suahelicus* breeds from April to August

(preferring *Adansonia* trees for nesting) (Symes and Perrin, 2004b, Wirminghaus et al., 2001). In addition, the genetic data presented in this study revealed no signs of introgression between these two taxa, providing additional evidence for reproductive isolation. Morphologically, *P. r. robustus* can be easily separated from the other subspecies, with distinctive colouration (Clancey, 1997, Forshaw, 1989, Wirminghaus et al., 2002a), small body size and much smaller, narrower bill (Wirminghaus et al., 2002a). These unique diagnostic morphological characters support the reclassification of this taxon using the Morphological Species Concept. The elevation of *P. r. robustus* to species is also supported by the genetic differentiation of this taxon from other *Poicephalus* species using both microsatellite and sequence data. The multilocus genotype data unambiguously assigned *P. r. robustus* individuals to a single genetic cluster separate from other *Poicephalus* species and subspecies. This finding is further strengthened by phylogenetic analysis of both mtDNA and nuclear sequences, with *P. r. robustus* recovered as monophyletic on both the maximum likelihood and Bayesian trees. The observed molecular divergence between *P. r. robustus* and other subspecies is congruent with that separating other well-established parrot species. The *P. r. robustus* lineage can also be diagnosed by fixed molecular characters (three synapomophic and eleven autapomorphic).

We propose that *P. r. robustus* should be elevated to species status, namely *P. robustus* sensu stricto following Gmelin (1788). Given that our molecular data support a close relationship between *P. r. fuscicollis* and *P. r. suahelicus*, we recommend that these two taxa remain as subspecies under *P. fuscicollis*, namely *P. f. fuscicollis* stat. nova. (Kuhl, 1820) and *P. f. suahelicus* stat. nova. (Reichenow, 1898) following previous authors (Clancey, 1997, Perrin, 2005, Wirminghaus et al., 2002a). The reclassification of *P. robustus* will have considerable implications for conservation management. Given that the South African Cape Parrot (*P. robustus* sensu stricto) has a population size of fewer than 10 000 mature individuals, with no subpopulation containing more than 1000 mature individuals (Downs et al., 2014, IUCN, 2012), *P. robustus* sensu stricto meets criterion C2a(i) for a Vulnerable listing in the IUCN Red List of Threatened Species. The Cape Parrot also meets all of the biological criteria for a CITES Appendix I listing (Perrin, 2005). The recognition of *P. robustus* sensu stricto will allow for the effective regulation and monitoring of any international trade under CITES.

2.6 Conclusion

Our study is the most comprehensive analysis of the taxonomic relationships within the *P. robustus* clade using molecular data. The clear genetic differentiation of *P. r. robustus* from *P.*

r. fuscicollis and *P. r. suahelicus* coupled with the differences in morphology, habitat and dietary needs provides strong scientific evidence for the elevation of *P. r. robustus* to *P. robustus* sensu stricto. Our results are sufficient to provide conservation authorities with strong evidence that the South African endemic Cape Parrot should be viewed as a Vulnerable species of conservation priority. This recognition will in turn assist the biodiversity conservation sector to prioritize, plan and implement conservation strategies.

2.7 Acknowledgements

The authors would like to thank a number of researchers, private breeders and organisations for providing samples; East London Museum (East London, South Africa), Ditsong National Museum of Natural History (Pretoria, South Africa), Durban Natural Sciences Museum (Durban, South Africa), the British Natural History Museum at Tring (United Kingdom), the National Zoological Gardens (Pretoria, South Africa), the Umgeni River Bird Park (Durban, South Africa), the Loro Parque Foundation (Canary Islands, Spain), Dr. Craig Symes, Mr. William Horsfield and Dr. Luke Arnot. I would also like to acknowledge the following individuals for their assistance during field sampling: Dr. Riddhika Kalle, Dr. T. Ramesh and Dr. Lindy Thompson. I would like to thank Cyril Laubscher for use of his photographs.

2.8 References

AKAIKE, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control,* 19**,** 716-723.

AMERICAN ORNITHOLOGISTS' UNION. 1998. *Checklist of North American Birds, 7th ed.,* Washington, D.C., American Ornithologists' Union.

BARROWCLOUGH, G. F. 1980. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. *Auk,* 97**,** 655-688.

BIRDLIFE INTERNATIONAL. 2012. *Poicephalus robustus* [Online]. Available: http://www.iucnredlist.org [Accessed 09 December 2014.

BOBE, R. & BEHRENSMEYER, A. 2003. The expansion of grassland exosystems in Africa in ralation to mammalian evolution and the origin of the genus *Homo*. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 207**,** 399-420.

BRANDSTRÖM, M. & ELLEGREN, H. 2008. Genome-wide analysis of microsatellite polymorphism in chicken circumventing the ascertainment bias. *Genome Research,* 18**,** 881-887.

CALDERÓN, L., QUINTANA, F., CABANNE, G. S., LOUGHEED, S. C. & TUBARO, P. L. 2014. Phylogeography and genetic structure of two Patagonian shag species (Aves: Phalacrocoracidae). *Molecular Phylogenetics and Evolution,* 72**,** 42-53.

CARLSSON, J. 2008. Effects of microsatellite null alleles on assignment testing. *Journal of Heredity,* 99**,** 616-623.

CHAPUIS, M.-P. & ESTOUP, A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution,* 24**,** 621-631.

CLANCEY, P. A. 1997. The Cape Parrot: an additional valid species. *Honeyguide,* 43**,** 61-62.

CRACRAFT, J. 1983. Species concepts and speciation analysis. *In:* JOHNSTON, R. F. (ed.) *Current ornithology.* New York: Plenum Press.

DAKIN, E. E. & AVISE, J. C. 2004. Microsatellite null alleles in parentage analysis. *Heredity,* 93**,** 504-509.

DARRIBA, D., TABOADA, G. L., DOALLO, R. & POSADA, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods,* 9**,** 772.

DEMPSTER, A. P., LAIRD, N. M. & RUBIN, D. B. 1977. Maximum likelihood from incomplete data via the EM algorithm. *J Roy. Stat. Soc. B,* 39**,** 1-38.

DOWNS, C. T., PFEIFFER, M. & HART, L. A. 2014. Fifteen years of annual Cape Parrot *Poicephalus robustus* censuses: current population trends and conservation contributions. *Ostrich,* 85**,** 273-280.

DRUMMOND, A. J., HO, S. Y. W., PHILLIPS, M. J. & RAMBAUT, A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology,* 4**,** e88.

DRUMMOND, A. J., SUCHARD, M. A., XIE, D. & RAMBAUT, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution,* 29**,** 1969-1973.

EARL, D. 2009. *STRUCTURE Harvester, Version 0.3* [Online]. Available: http://users.soe.ucsc.edu/~dearl/software/struct_harvest/ [Accessed 01 May 2013.

EBERHARD, J. R. & BERMINGHAM, E. 2005. Phylogeny and comparative biogeography of *Pionopsitta* parrots and *Pteroglossus* toucans. *Molecular Phylogenetics and Evolution,* 36**,** 288-304.

EBERHARD, J. R., BERMINGHAM, E. & ZINK, R. 2004. Phylogeny and biogeography of the *Amazona ochrocephala* (Aves: Psittacidae) complex. *The Auk,* 121**,** 318-332.

ELDREDGE, N. & CRACRAFT, J. 1980. *Phylogenetic patterns and the evolutionary process,* New York, Columbio University Press.

EVANNO, G., REGNAUT, S. & GOUDET, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* 14**,** 2611-2620.

EXCOFFIER, L., LAVAL, G. & SCHNEIDER, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online,* 1**,** 4750.

FARIA, P., GUEDES, N. R., YAMASHITA, C., MARTUSCELLI, P. & MIYAKI, C. 2008. Genetic variation and population structure of the endangered Hyacinth Macaw (*Anodorhynchus hyacinthinus*): implications for conservation. *Biodiversity and Conservation,* 17**,** 765-779.

FELSENSTEIN, J. 1989. PHYLIP–Phylogeny Inference Package, Version 3.2. *Cladistics,* 5**,** 164-166.

FELSENSTEIN, J. 2009. PHYLIP – phylogeny inference package, Version 3.69.

FORSHAW, J. M. 1978. *Parrots of the world,* Newton Abbot, London, David & Charles.

FORSHAW, J. M. 1989. *Parrots of the world,* Willoughby, Lansdowne Books.

FRY, C. H., KEITH, S. & URBAN, E. K. 1988. *The Birds of Africa, Vol. 3,* New York, London, Academic Press.

GIPPOLITI, S. & AMORI, G. 2007. The problem of subspecies and biased taxonomy in conservation lists: the case of mammals. *Folia Zoologica,* 56**,** 113-117.

GMELIN, J. F. 1788. Systema Naturae.

HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acids Symposium Series,* 41**,** 95-98.

HAZEVOET, C. 1996. Conservation and species lists: taxonomic neglect promotes the extinction of endemic birds, as exemplified by taxa from eastern Atlantic islands. *Bird Conservation International,* 6**,** 181-196.

HEBERT, P. D. N., STOECKLE, M. Y., ZEMLAK, T. S. & FRANCIS, C. M. 2004. Identification of birds through DNA barcodes. *PLoS Biology,* 2**,** e312.

HEWITT, G. 2000. The genetic legacy of the Quaternary ice ages. *Nature,* 405**,** 907-913.

IUCN 2012. *IUCN Red List Categories and Criteria: Version 3.1,* Gland, Switzerland and Cambridge, UK, IUCN.

JUNIPER, T. & PARR, M. 1998. *Parrots: A guide to parrots of the world,* New Haven, Connecticut, Yale University Press.

KALINOWSKI, S. T., TAPER, M. L. & MARSHALL, T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology,* 16**,** 1099 -1106.

KUHL 1820. Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum.

KUNDU, S., JONES, C. G., PRYS-JONES, R. P. & GROOMBRIDGE, J. J. 2012. The evolution of the Indian Ocean parrots (Psittaciformes): extinction, adaptive radiation and eustacy. *Molecular Phylogenetics and Evolution,* 62**,** 296-305.

KÜPPER, C., AUGUSTIN, J., KOSZTOLÁNYI, A., BURKE, T., FIGUEROLA, J. & SZÉKELY, T. 2009. Kentish versus Snowy Plover: Phenotypic and genetic analyses of *Charadrius alexandrinus* reveal divergence of Eurasian and American subspecies. *Auk,* 126**,** 839-852.

LAWES, M. 1990. The distribution of the samango monkey (*Cercopithecus mitis erythrarchus* Peters, 1852 and *Cercopithecus mitis labiatus* I. Geoffroy, 1843) and forest history in southern Africa. *Journal of Biogeography***,** 669-680.

MALLET, J. 1995. A species definition for the modern synthesis. *Trends in Ecology and Evolution,* 10**,** 294-299.

MANEGOLD, A. 2013. Two new parrot species (Psittaciformes) from the early Pliocene of Langebaanweg, South Africa, and their palaeoecological implications. *Ibis,* 155**,** 127-139.

MASELLO, J. F., QUILLFELDT, P., MUNIMANDA, G. K., KLAUKE, N., SEGELBACHER, G., SCHAEFER, H. M., FAILLA, M., CORTES, M. & MOODLEY, Y. 2011. The high Andes, gene flow and a stable hybrid zone shape the genetic structure of a wide-ranging South American parrot. *Frontiers in Zoology,* 8**,** 16.

MASSA, R., SARA, M., PIAZZA, M., DI GAETANO, C., RANDAZZO, M. & COGNETTI, G. 2000. A molecular approach to the taxonomy and biogeography of African parrots. *Italian Journal of Zoology,* 67**,** 313-317.

MAYR, E. 1942. *Systematics and the origin of species,* New York, NY, USA, Columbia University Press.

MAYR, E. 1982. Of what use are subspecies? *Auk,* 99**,** 593-595.

MINISTER OF ENVIRONMENTAL AFFAIRS AND TOURISM 2007. National Environmental Management: Biodiversity Act (ACT 10 of 2004): Publication of Lists of Critically Endangered, Endangered, Vulnerable and Protected Species. *In:* DEAT (ed.). Cape Town, South Africa.

MISHLER, B. D. 1985. The morphological, developmental, and phylogenetic basis of species concepts in bryophytes. *Bryologist,* 88**,** 207-217.

MOREAU, R. E. 1963. Vicissitudes of the African biomes in the late Pleistocene. *Proceedings of the Zoological Society of London,* 141**,** 395-421.

NELSON, G. & PLANTICK, N. 1981. *Systematics and biogeograph: cladistics and vicariance,* New York, Columbia Universiy Press.

PALUMBI, S., MARTIN, A., ROMANO, S., MCMILLAN, W., STICE, L. & GRABOWSKI, G. 1991. The Simple Fool's Guide to PCR, Version 2.0. *Honolulu: Department of Zoology and Kewalo Marine Laboratory, University of Hawaii*.

PEAKALL, R. & SMOUSE, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* 28**,** 2537-2539.

PERRIN, M. R. 2005. A review of the taxonomic status and biology of the Cape Parrot *Poicephalus robustus*, with reference to the Brown-necked Parrot *P. fuscicollis fuscicollis* and the Grey-headed Parrot *P. f. suahelicus*. *Ostrich,* 76**,** 195-205.

PERRIN, M. R. 2012. *Parrots of Africa, Madagascar and the Mascarene Islands,* Johannesburg, Wits University Press.

PHILLIMORE, A. B. & OWENS, I. P. F. 2006. Are subspecies useful in evolutionary and conservation biology? *Proceedings of the Royal Society B: Biological Sciences,* 273**,** 1049-1053.

PILLAY, K., DAWSON, D. A., HORSBURGH, G. J., PERRIN, M. R., BURKE, T. & TAYLOR, T. D. 2010. Twenty-two polymorphic microsatellite loci aimed at detecting illegal trade in the Cape parrot, *Poicephalus robustus* (Psittacidae, AVES). *Molecular Ecology Resources,* 10**,** 142-149.

PRITCHARD, J. K., WEN, X. & FALUSH, D. 2010. *Structure, version 2.3* [Online]. Available: http://pritch.bsd.uchicago.edu/software [Accessed 20 November 2013.

PRYCHITKO, T. M. & MOORE, W. S. 1997. The utility of DNA sequences of an intron from the β-fibrinogen gene in phylogenetic analysis of woodpeckers (Aves: Picidae). *Molecular Phylogenetics and Evolution,* 8**,** 193-204.

RAMBAUT, A. 2012. *FigTree v1.4.0* [Online]. Available: http://tree.bio.ed.ac.uk/software/figtree/ [Accessed 12 December 2014.

RAMBAUT, A. & DRUMMOND, A. J. 2007. *Molecular evolution, phylogenetics and epidemiology, Tracer v.1.5*. [Online]. Available: http://tree.bio.ed.ac.uk/software/tracer/ [Accessed 12 December 2014.

REICHENOW, A. N. T. 1898. Bericht uber die Februar-Sitzung. *Journal für Ornithologie,* 46**,** 314.

RHEINDT, F. E., CHRISTIDIS, L., KUHN, S., DE KLOET, S., NORMAN, J. A. & FIDLER, A. 2014. The timing of diversification within the most divergent parrot clade. *Journal of Avian Biology,* 45**,** 140-148.

RHEINDT, F. E., SZÉKELY, T., EDWARDS, S. V., LEE, P. L. M., BURKE, T., KENNERLEY, P. R., BAKEWELL, D. N., ALRASHIDI, M., KOSZTOLÁNYI, A., WESTON, M. A., LIU, W.-T., LEI, W.-P., SHIGETA, Y., JAVED, S., ZEFANIA, S. & KÜPPER, C. 2011. Conflict between genetic and phenotypic differentiation: the evolutionary history of a 'Lost and Rediscovered' shorebird. *PLoS ONE,* 6**,** e26995.

RONQUIST, F., TESLENKO, M., VAN DER MARK, P., AYRES, D. L., DARLING, A., HÖHNA, S., LARGET, B., LIU, L., SUCHARD, M. A. & HUELSENBECK, J. P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology,* 61**,** 539-542.

RUSSELLO, M. A., STAHALA, C., LALONDE, D., SCHMIDT, K. L. & AMATO, G. 2010. Cryptic diversity and conservation units in the Bahama parrot. *Conservation Genetics,* 11**,** 1809-1821.

SCHWEIZER, M., SEEHAUSEN, O. & HERTWIG, S. T. 2011. Macroevolutionary patterns in the diversification of parrots: effects of climate change, geological events and key innovations. *Journal of Biogeography* 38**,** 2176-2194.

SIBLEY, C. G. & MONROE, B. L. 1990. *Distribution and taxonomy of birds of the world,* New Haven, Connecticut, Yale University Press.

SILVESTRO, D. & MICHALAK, I. 2012. raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution,* 12**,** 335-337.

SNOW, D. W. 1978. *An Atlas of Speciation of African Non-passerine Birds,* London, British Museum (Natural History).

STOVER, B. & MULLER, K. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics,* 11**,** 7.

SYMES, C. T. & PERRIN, M. R. 2003. Feeding biology of the Grey-headed Parrot, *Poicephalus fuscicollis suahelicus* (Reichenow), in Northern Province, South Africa. *Emu,* 103**,** 49-58.

SYMES, C. T. & PERRIN, M. R. 2004a. Behaviour and some vocalisations of the Grey-headed Parrot *Poicephalus fuscicollis suahelicus*. *Durban Museum Novitates,* 29**,** 5-13.

SYMES, C. T. & PERRIN, M. R. 2004b. Breeding biology of the Grey-headed Parrot (*Poicephalus fuscicollis suahelicus*) in the wild. *Emu,* 104**,** 45-57.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution,* 30**,** 2725-2729.

TAVARES, E. S., BAKER, A. J., PEREIRA, S. L. & MIYAKI, C. Y. 2006. Phylogenetic relationships and historical biogeography of neotropical parrots (Psittaciformes: Psittacidae: Arini) inferred from mitochondrial and nuclear DNA sequences. *Systematic Biology,* 55**,** 454-470.

TAYLOR, M. 2014. Eskom Red Data Book of Birds of South Africa, Lesotho and Swaziland. Birdlife South Africa, Johannesburg.

TAYLOR, T. D. 2011. Cross-utility of microsatellite markers across species of the African *Poicephalus* parrots to encourage conservation research. *Ostrich,* 82**,** 65-70.

THOMPSON, J. D., HIGGINS, D. G. & GIBSON, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research,* 22**,** 4673-4680.

VILAÇA, S. T. & SANTOS, F. R. 2010. Biogeographic history of the species complex *Basileuterus culicivorus* (Aves, Parulidae) in the Neotropics. *Molecular Phylogenetics and Evolution,* 57**,** 585-597.

WENNER, T. J., RUSSELLO, M. A. & WRIGHT, T. F. 2012. Cryptic species in a Neotropical parrot: genetic variation within the *Amazona farinosa* species complex and its conservation implications. *Conservation Genetics,* 13**,** 1427-1432.

WHITE, N. E., PHILLIPS, M. J., GILBERT, M. T., ALFARO-NUNEZ, A., WILLERSLEV, E., MAWSON, P. R., SPENCER, P. B. & BUNCE, M. 2011. The evolutionary history of cockatoos (Aves: Psittaciformes: Cacatuidae). *Molecular Phylogenetics and Evolution,* 59**,** 615-622.

WIRMINGHAUS, J. O., DOWNS, C. T., PERRIN, M. R. & SYMES, C. T. 2001. Breeding biology of the Cape Parrot, *Poicephalus robustus*. *Ostrich,* 72**,** 159-164.

WIRMINGHAUS, J. O., DOWNS, C. T., PERRIN, M. R. & SYMES, C. T. 2002a. Taxonomic relationships of the subspecies of the Cape Parrot *Poicephalus robustus* (Gmelin). *Journal of Natural History,* 36**,** 361-378.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 1999. Conservation of the Cape parrot in southern Africa. *South African Journal of Wildlife Research,* 29**,** 118-129.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 2002b. Diet of the Cape Parrot, *Poicephalus robustus*, in Afromontane forests in KwaZulu-Natal, South Africa. *Ostrich,* 73**,** 20-25.

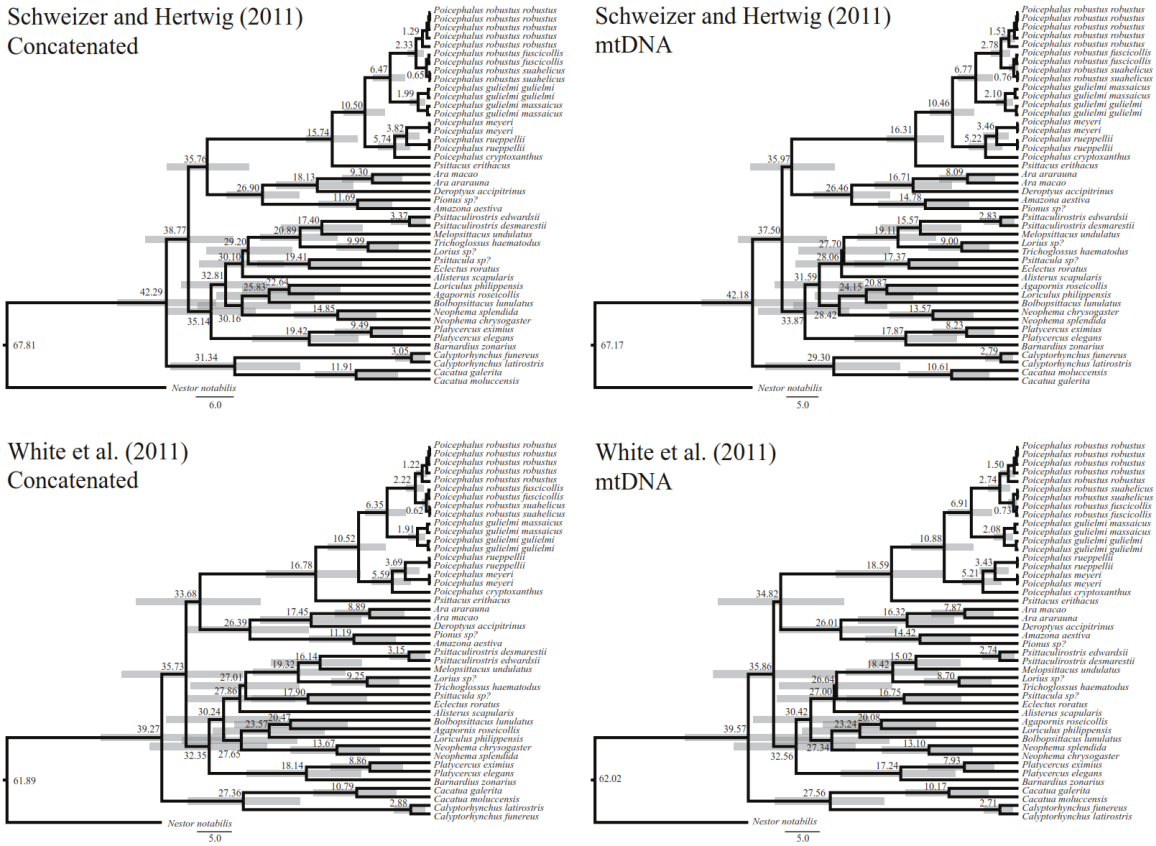
WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T., PERRIN, M. R. & DEMPSTER, E. R. 2000. Vocalisations, and some behaviours of the Cape Parrot *Poicephalus robustus*. *Durban Museum Novitates,* 25**,** 12-17.

XLSTAT 2014. XLSTAT Version 2014.1.01. Addinsoft 1995–2014.

ZINK, R. M. 2004. The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proceedings of the Royal Society of London, Series B: Biological sciences* 271**,** 561-564.

ZWICKL, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. *Ph.D. dissertation.* Austin, Texas: The University of Texas at Austin.

2.9 Supplementary Information



Supplementary Figure 2-1. Maximum clade probability trees generated using the concatenated data (16S rRNA, COI and β-fib) and mitochondrial DNA (16S rRNA and COI) data. Separate analyses were conducted using Schweizer et al. 2011 and White et al. 2011 calibration points. Values at nodes indicate the posterior mean divergence dates in millions of years before present. Shaded bars indicate the 95% highest posterior density (HPD) credibility intervals.

Supplementary Table 2-1. The *Poicephalus* specimens included in the present study. Collection numbers and locations are those assigned to specimens by museums or the collections of researchers. GPS coordinates are taken from the general area of sample collection. The institutions where samples can be accessed are listed. Specimens used for DNA sequencing are indicated by *.

Species/Subspecies:	Analysis code:	Collection numbers:	Sampling location:	GPS[s]:	Sample storage:	Sample type:
P. robustus robustus	Prob01	FH50*	Alice, Eastern Cape Province, RSA	-32.796097, 26.850024	University of KwaZulu-Natal (UKZN)	Whole blood
	Prob02	LG02	King Williams Town, Eastern Cape Province, RSA	-32.880202, 27.398856	UKZN	Whole blood
	Prob03	KMB638	Eastern Cape Province, RSA	Unknown	East London Museum (ELM), South Africa	Archival museum sample
	Prob04	7201	Cambridge district, East London, Eastern Cape Province, RSA	-33.008834, 27.802254	ELM	Archival museum sample
	Prob05	13276	Lusikisiki, Wild Coast, Eastern Cape Province, RSA	-31.366218, 29.570018	ELM	Archival museum sample
	Prob06	13277	Lusikisiki, Wild Coast, Eastern Cape Province, RSA	-31.366218, 29.570018	ELM	Archival museum sample
	Prob07	16100	Frankfort, Eastern Cape Province, RSA	-32.720507, 27.453272	ELM	Archival museum sample
	Prob08	16104	King William's Town, Eastern Cape Province, RSA	-32.880202, 27.398856	ELM	Archival museum sample
	Prob09	16105	Pirie hatchery, Eastern Cape Province, RSA	-32.791100, 27.247902	ELM	Archival museum sample
	Prob10	16106	King William's Town, Eastern Cape Province, RSA	-32.880202, 27.398856	ELM	Archival museum sample
	Prob11	KZNT01*	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Muscle tissue
	Prob12	KZNT02*	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Muscle tissue
	Prob13	KZNT03	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Muscle tissue
	Prob14	KZNT04	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Muscle tissue
	Prob15	KZNT05	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Muscle tissue
	Prob16	KZNT06	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Muscle tissue
	Prob17	CR01	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Whole blood
	Prob18	CR02	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Whole blood
	Prob19	CR03	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Whole blood
	Prob20	CR04	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Whole blood

Supplementary Table 2-1 (Continued)

Species/Subspecies:	Analysis code:	Collection numbers:	Sampling location:	GPS[s]:	Sample storage:	Sample type:
	Prob21	CR05	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Feather
P. robustus robustus	Prob22	CR06	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Feather
	Prob23	CR07	Creighton, KwaZulu-Natal, RSA	-30.027832, 29.838148	UKZN	Feather
	Prob24	RMO1*	Tzaneen, Limpopo, RSA	-23.859859, 30.006596	UKZN	Whole blood
	Prob25	RMO2*	Tzaneen, Limpopo, RSA	-23.859859, 30.006596	UKZN	Whole blood
	Prob26	Pool1	Tzaneen, Limpopo, RSA	-23.859859, 30.006596	UKZN	Feather
	Prob27	Pool2	Tzaneen, Limpopo, RSA	-23.859859, 30.006596	UKZN	Feather
	Prob28	TMIIa 2078	Limpopo, RSA	-23.822019, 30.131136	Ditsong National Museum of Natural History (DNM), South Africa	Archival museum sample
	Prob29	TM 16406	Limpopo, RSA	-23.822019, 30.131136	DNM	Archival museum sample
	Prob30	TM 16407	Limpopo, RSA	-23.822019, 30.131136	DNM	Archival museum sample
	Prob31	TM 25266	Limpopo, RSA	-23.822019, 30.131136	DNM	Archival museum sample
	Prob32	TM80817	Magoebaskloof, Limpopo, RSA	-23.822019, 30.131136	DNM	Archival museum sample
P. r. suahelicus	Prs01	Prs 1	Unknown	Unknown	UKZN	Whole blood
	Prs02	Prs 2	Unknown	Unknown	UKZN	Whole blood
	Prs03	Prs 3	Unknown	Unknown	UKZN	Whole blood
	Prs04	Prs 4	Unknown	Unknown	UKZN	Whole blood
	Prs05	Prs 24	Unknown	Unknown	UKZN	Whole blood
	Prs06	P.f.suah1	Unknown	Unknown	UKZN	Whole blood
	Prs07	P.f.suah2	Unknown	Unknown	UKZN	Whole blood
	Prs08	P.f.suah3	Unknown	Unknown	UKZN	Whole blood
	Prs09	P.f.suah4	Unknown	Unknown	UKZN	Whole blood
	Prs10	P.f.suah5	Unknown	Unknown	UKZN	Whole blood
	Prs11	P.f.suah6	Unknown	Unknown	UKZN	Whole blood
	Prs12	20814	Captive bred	CB	Loro Parque Foundation (LPF), Spain	Whole blood
	Prs13	20815	Captive bred	CB	LPF	Whole blood

Supplementary Table 2-1 (Continued)

Species/Subspecies:	Analysis code:	Collection numbers:	Sampling location:	GPS[s]:	Sample storage:	Sample type:
P. r. suahelicus	Prs14	8269*	Zambia	Unknown	National Zoological Gardens (NZG), South Africa	Whole blood
	Prs15	8270*	Zambia	Unknown	NZG	Whole blood
	Prs16	8271	Zambia	Unknown	NZG	Whole blood
	Prs17	8272	Zambia	Unknown	NZG	Whole blood
	Prs18	8273	Zambia	Unknown	NZG	Whole blood
	Prs19	8274	Zambia	Unknown	NZG	Whole blood
	Prs20	8275	Zambia	Unknown	NZG	Whole blood
	Prs21	CS01	Levubu, Limpopo, RSA	-23.109336, 30.323897	NZG	Feather
	Prs22	CS03	Levubu, Limpopo, RSA	-23.109336, 30.323897	NZG	Feather
	Prs23	CS04	Levubu, Limpopo, RSA	-23.109336, 30.323897	NZG	Feather
P. r. fuscicollis	Prf01	249 ♂	Unknown	Unknown	NZG	Whole blood
	Prf02	249 ♀	Unknown	Unknown	NZG	Whole blood
	Prf03	383 ♂	Unknown	Unknown	NZG	Whole blood
	Prf04	383 ♀*	Unknown	Unknown	NZG	Whole blood
	Prf05	386 ♂*	Unknown	Unknown	NZG	Whole blood
	Prf06	386 ♀	Unknown	Unknown	NZG	Whole blood
	Prf07	388 ♂	Unknown	Unknown	NZG	Whole blood
	Prf08	388 ♀	Unknown	Unknown	NZG	Whole blood
	Prf09	1423 ♂	Unknown	Unknown	NZG	Whole blood
	Prf10	1423 ♀	Unknown	Unknown	NZG	Whole blood
	Prf11	1440	Captive bred	CB	LPF	Whole blood
	Prf12	1441	Captive bred	CB	LPF	Whole blood
	Prf13	1514	Captive bred	CB	LPF	Whole blood
	Prf14	8639	Captive bred	CB	LPF	Whole blood
	Prf15	11065	Captive bred	CB	LPF	Whole blood
	Prf16	11068	Captive bred	CB	LPF	Whole blood

Supplementary Table 2-1 (Continued)

Species/Subspecies:	Analysis code:	Collection numbers:	Sampling location:	GPS[$]:	Sample storage:	Sample type:
P. r. fuscicollis	Prf17	17305	Captive bred	CB	LPF	Whole blood
	Prf18	24091	Captive bred	CB	LPF	Whole blood
	Prf19	25200	Captive bred	CB	LPF	Whole blood
	Prf20	25932	Captive bred	CB	LPF	Whole blood
	Prf21	25960	Captive bred	CB	LPF	Whole blood
	Prf22	BMNH 1929.2.18.110	Farafenni, N.B.Prov., Gambia	13.566788, -15.599320	British Museum of Natural History at Tring (BMNH), United Kingdom	Archival museum sample
	Prf23	BMNH 1929.2.18.112	Kerewan, N.B.Prov., Gambia	13.490150, -16.085272	BMNH	Archival museum sample
	Prf24	BMNH 1929.2.18.114	Farafenni, N.B.Prov., Gambia	13.566788, -15.599320	BMNH	Archival museum sample
	Prf25	BMNH 1929.2.18.109	Kerewan, N.B.Prov., Gambia	13.490150, -16.085272	BMNH	Archival museum sample
	Prf26	BMNH 1910.5.6.147	Guinea-Bissau	Unknown	BMNH	Archival museum sample
P. rueppellii	Prup01	8250	Swakop River, Namibia	-22.651557, 14.624207	Durban Natural Sciences Museum (DNSM), South Africa	Archival museum sample
	Prup02	8251	Swakop River, Namibia	-22.651557, 14.624207	DNSM	Archival museum sample
	Prup03	8252	Swakop River, Namibia	-22.651557, 14.624207	DNSM	Archival museum sample
	Prup04	8253	Swakop River, Namibia	-22.651557, 14.624207	DNSM	Archival museum sample
	Prup05	8254	Swakop River, Namibia	-22.651557, 14.624207	DNSM	Archival museum sample
	Prup06	8255	Swakop River, Namibia	-22.651557, 14.624207	DNSM	Archival museum sample
	Prup07	8256	Swakop River, Namibia	-22.651557, 14.624207	DNSM	Archival museum sample
	Prup08	8257	Okahandja, Namibia	-21.983572, 16.916715	DNSM	Archival museum sample
	Prup09	8258	Windhoek, Namibia	-22.566294, 17.059741	DNSM	Archival museum sample
	Prup10	8259	Klipkop Farm, South of Otjiwarongo, Namibia	-20.667061, 16.742880	DNSM	Archival museum sample

Supplementary Table 2-1 (Continued)

Species/Subspecies:	Analysis code:	Collection numbers:	Sampling location:	GPS[s]:	Sample storage:	Sample type:
P. rueppellii	Prup11	8260	Klipkop Farm, South of Otjiwarongo, Namibia	-20.667061, 16.742880	DNSM	Archival museum sample
	Prup12	8261	Okahandja, Namibia	-21.983572, 16.916715	DNSM	Archival museum sample
	Prup13	Pru01*	Captive bred	CB	UKZN	Whole blood
	Prup14	Pru02*	Captive bred	CB	UKZN	Whole blood
	Prup15	Pru03	Captive bred	CB	UKZN	Whole blood
	Prup16	Pru04	Captive bred	CB	UKZN	Whole blood
P. meyeri	Pm01	Pm01*	Captive bred	CB	UKZN	Whole blood
	Pm02	Pm02*	Captive bred	CB	UKZN	Whole blood
	Pm03	Pm03	Captive bred	CB	UKZN	Whole blood
	Pm04	Pm04	Captive bred	CB	UKZN	Whole blood
	Pm05	16406	Sentinel Ranch, Beit Bridge, Zimbabwe	-22.2167, 30	DNSM	Archival museum sample
	Pm06	29461	Humani Ranch, Sabi Valley, Zimbabwe	-20.491367, 32.242027	DNSM	Archival museum sample
	Pm07	32040	Okavango River, Andara, Namibia	-18.061680, 21.443950	DNSM	Archival museum sample
	Pm08	32041	Okavango River, Andara, Namibia	-18.061680, 21.443950	DNSM	Archival museum sample
	Pm09	8272	Victoria Falls, Zimbabwe	-17.907320, 25.821980	DNSM	Archival museum sample
	Pm10	25474	Northam, South Africa	-24.950103, 27.267488	DNSM	Archival museum sample
	Pm11	29462	Humani Ranch, Sabi Valley, Zimbabwe	-20.491367, 32.242027	DNSM	Archival museum sample
	Pm12	29463	Humani Ranch, Sabi Valley, Zimbabwe	-20.491367, 32.242027	DNSM	Archival museum sample
P. cryptoxanthus	Pcryp01	11920	N of Siteki, Swaziland	-26.449321, 31.949929	DNSM	Archival museum sample
	Pcryp02	20733	Chimonzo, Mozambique	-24.9483, 33.2917	DNSM	Archival museum sample
	Pcryp03	20734	Chimonzo, Mozambique	-24.9483, 33.2917	DNSM	Archival museum sample
	Pcryp04	20735	Massinga, Mozambique	-23.332600, 35.385160	DNSM	Archival museum sample

Supplementary Table 2-1 (Continued)

Species/Subspecies:	Analysis code:	Collection numbers:	Sampling location:	GPS[s]:	Sample storage:	Sample type:
P. cryptoxanthus	Pcryp05	23857	Inhaminga, Mozambique	-18.416012, 35.024962	DNSM	Archival museum sample
	Pcryp06	26840	Massinga, Mozambique	-23.332600, 35.385160	DNSM	Archival museum sample
	Pcryp07	26841	Massinga, Mozambique	-23.332600, 35.385160	DNSM	Archival museum sample
	Pcryp08	8238	Newington, Malamala, South Africa	-24.805498, 31.540776	DNSM	Archival museum sample
	Pcryp09	8239	Newington, Malamala, South Africa	-24.805498, 31.540776	DNSM	Archival museum sample
	Pcryp10	8240	Newington, Malamala, South Africa	-24.805498, 31.540776	DNSM	Archival museum sample
	Pcryp11	8241	Newington, Malamala, South Africa	-24.805498, 31.540776	DNSM	Archival museum sample
	Pcryp12	8242	Newington, Malamala, South Africa	-24.805498, 31.540776	DNSM	Archival museum sample
	Pcryp13	8243	Newington, Malamala, South Africa	-24.805498, 31.540776	DNSM	Archival museum sample
	Pcryp14	8248	Chimonzo, Mozambique	-24.9483, 33.2917	DNSM	Archival museum sample
		URB Pcryp1*	Captive bred	CB	UKZN	Whole blood
P.g.massaicus	Pgm01	8263	Naro Moru, Mt. Kenya, Kenya	-0.159133, 37.313424	DNSM	Archival museum sample
	Pgm02	8264	Naro Moru, Mt. Kenya, Kenya	-0.159133, 37.313424	DNSM	Archival museum sample
	Pgm03	8265	Molo, Kenya	-0.249101, 35.732303	DNSM	Archival museum sample
	Pgm04	38005	Captive bred	CB	DNSM	Archival museum sample
	Pgm05	38006	Captive bred	CB	DNSM	Archival museum sample
	Pgm06	Pgm01*	Captive bred	CB	UKZN	Whole blood
	Pgm07	Pgm02*	Captive bred	CB	UKZN	Whole blood
	Pgm08	Pgm03	Captive bred	CB	UKZN	Whole blood
	Pgm09	Pgm04	Captive bred	CB	UKZN	Whole blood
	Pgm10	Pgm05	Captive bred	CB	UKZN	Whole blood

Supplementary Table 2-1 (Continued)

Species/Subspecies:	Analysis code:	Collection numbers:	Sampling location:	GPS[$]:	Sample storage:	Sample type:
P.g.massaicus	Pgm11	Pgm06	Captive bred	CB	UKZN	Whole blood
P.g.gulielmi	Pgg01	Pgg01*	Captive bred	CB	UKZN	Whole blood
	Pgg02	Pgg02*	Captive bred	CB	UKZN	Whole blood
	Pgg03	Pgg03	Captive bred	CB	UKZN	Whole blood
	Pgg04	Pgg04	Captive bred	CB	UKZN	Whole blood

*Samples used for sequencing of COI, 16S rRNA and β-fibrinogen gene regions
[$] GPS coordinates for museum samples were estimated using the provided locality information
CB = Captive bred

Supplementary Table 2-2. GenBank accession numbers for *Poicephalus* sequences generated in the present study and accession numbers for the 27 additional parrot sequences used for molecular clock analysis.

Species:	**Accession numbers:**		
	16S rRNA	**β-fib**	**COI**
Poicephalus robustus robustus1	KP856844	KP856857	KP856872
P. r. robustus2	KP856845	-	KP856873
P. r. robustus3	KP856846	KP856858	KP856874
P. r. robustus4	KP856847	KP856859	KP856875
P. r. robustus5	KP856848	KP856860	KP856876
P. r. suahelicus1	KP856849	KP856861	KP856877
P. r. suahelicus2	KP856850	KP856862	KP856878
P. r. fuscicollis1	KP856842	KP856856	KP856870
P. r. fuscicollis2	KP856843	-	KP856871
P. rueppellii1	KP856851	-	KP856879
P. rueppellii2	KP856852	-	KP856880
P. meyeri1	KP856840	-	KP856868
P. meyeri2	KP856841	-	KP856869
P. cryptoxanthus	KP856835	-	KP856863
P. gulielmi gulielmi1	KP856836	-	KP856864
P. gulielmi2	KP856837	KP856853	KP856865
P.g.massaicus1	KP856838	KP856854	KP856866
P.g.massaicus2	KP856839	KP856855	KP856867
Agapornis roseicollis	EU410486.1	GQ395348.1	EU410486.1
Alisterus scapularis	EU197096.1	EU739363.1	JN801395.1
Amazona aestiva	EU197111.1	AY301472.1	FJ027055.1
Ara ararauna	-	AY301514.1	FJ808626.1
Ara macao	EF635432.1	-	JN801493.1
Barnardius zonarius	-	-	JN801399.1
Bolbopsittacus lunulatus	-	-	KC354896.1
Cacatua galerita	-	-	JN801403.1
Cacatua moluccensis	JF414239.1	-	JF414239.1
Calyptorhynchus funereus	-	AY695167.1	JF414279.1
Calyptorhynchus latirostris	EU197114.1	-	JF414274.1
Deroptyus accipitrinus	-	-	JQ174682.1
Eclectus roratus	EU197113.1	-	JN801439.1
Loriculus philippensis	-	-	KC354935.1
Lorius sp.	EU197097.1 (*Lorius lory*)	-	JQ175284.1 (*Lorius albidinucha*)
Melopsittacus undulatus	EF450826.1	-	F450826.1
Neophema chrysogaster	JX133087.1	-	JX133087.1
Neophema splendida	EU197100.1	-	JQ175546.1
Nestor notabilis	EU197116.1	-	HQ616639.1
Pionus sp.	EU197112.1 (*Pionus menstruus*)	AY301516.1 (*Pionus menstruus*)	JQ175856.1 (*Pionus fuscus*)
Platycercus elegans	-	EU739470.1	JQ175887.1
Platycercus eximius	EU197095.1	-	JQ175889.1
Psittacula sp.	EU197107.1 (*Psittacula cyanocephala*)	EU739474.1 (*Psittacula alexandri*)	KC439335.1 (*Psittacula eupatria*)
Psittaculirostris desmarestii	EU197121.1	-	-
Psittaculirostris edwardsii	EU197117.1	-	-
Psittacus erithacus	EU197109.1	AY301518.1	KF381364.1
Trichoglossus haematodus	-	-	JN801465.1

Supplementary Table 2-3. The genetic diversity, polymorphic information content and null allele frequencies of each locus over all *Poicephalus* specimens analysed.

All samples (n = 138)

Locus	Mean number of alleles:	Observed Heterozygosity (H_O):	Expected Heterozygosity (H_E):	Polymorphic information content (PIC):	Null allele frequency (Na):
Prob6	5.625	0.579	0.628	0.868	0.146
Prob15	7.125	0.498	0.759	0.858	0.198
Prob18	4.250	0.341	0.526	0.836	0.229
Prob30	6.625	0.634	0.649	0.847	0.074
Prob28	2.875	0.351	0.397	0.566	0.145
Prob29	3.250	0.391	0.453	0.796	0.264
Prob34	4.500	0.566	0.608	0.783	0.151
Prob35	2.250	0.309	0.306	0.513	0.189
Prob25	6.375	0.634	0.685	0.88	0.123
Prob26	6.875	0.611	0.723	0.891	0.15
Prob23	6.875	0.782	0.771	0.895	0.063

P. r. robustus (n = 32)

Locus	Number of alleles:	Observed Heterozygosity (H_O):	Expected Heterozygosity (H_E):	Polymorphic information content (PIC):	Null allele frequency (Na):
Prob6	8.000	0.633	0.679	0.631	0.038
Prob15	8.000	0.500	0.738	0.702	0.136
Prob18	4.000	0.767	0.681	0.618	0.000
Prob30	7.000	0.813	0.790	0.759	0.000
Prob28	3.000	0.625	0.505	0.393	0.000
Prob29	4.000	0.375	0.366	0.336	0.000
Prob34	4.000	0.433	0.481	0.431	0.011
Prob35	2.000	0.406	0.441	0.344	0.026
Prob25	6.000	0.844	0.744	0.702	0.000
Prob26	8.000	0.692	0.687	0.662	0.046
Prob23	6.000	0.750	0.698	0.658	0.000

P. r. suahelicus (n = 23)

Locus	Number of alleles:	Observed Heterozygosity (H_O):	Expected Heterozygosity (H_E):	Polymorphic information content (PIC):	Null allele frequency (Na):
Prob6	7.000	0.652	0.765	0.728	0.090
Prob15	8.000	0.565	0.834	0.813	0.146
Prob18	8.000	0.304	0.827	0.804	0.288
Prob30	10.000	0.696	0.818	0.794	0.042
Prob28	3.000	0.348	0.294	0.262	0.000
Prob29	4.000	0.391	0.474	0.432	0.083
Prob34	6.000	0.739	0.737	0.696	0.000
Prob35	3.000	0.478	0.381	0.334	0.000
Prob25	7.000	0.957	0.827	0.803	0.000
Prob26	10.000	0.913	0.857	0.841	0.000
Prob23	12.000	0.913	0.902	0.893	0.000

P. r. fuscicollis (n = 26)

Locus	Number of alleles:	Observed Heterozygosity (H_O):	Expected Heterozygosity (H_E):	Polymorphic information content (PIC):	Null allele frequency (Na):
Prob6	7.000	0.720	0.738	0.708	0.000
Prob15	7.000	0.462	0.752	0.711	0.161
Prob18	6.000	0.500	0.769	0.732	0.162
Prob30	5.000	0.885	0.643	0.593	0.000
Prob28	3.000	0.500	0.437	0.357	0.000
Prob29	3.000	0.280	0.306	0.278	0.000
Prob34	3.000	0.423	0.382	0.35	0.000
Prob35	2.000	0.154	0.260	0.226	0.112
Prob25	7.000	0.462	0.700	0.665	0.134
Prob26	5.000	0.333	0.402	0.383	0.000
Prob23	7.000	0.615	0.740	0.711	0.069

P. meyeri (n = 12)

Locus	Number of alleles:	Observed Heterozygosity (H_O):	Expected Heterozygosity (H_E):	Polymorphic information content (PIC):	Null allele frequency (Na):
Prob6	5.000	0.417	0.747	0.707	0.176
Prob15	5.000	0.250	0.729	0.684	0.276
Prob18	5.000	0.333	0.694	0.641	0.206
Prob30	11.000	0.917	0.885	0.875	0.000
Prob28	3.000	0.500	0.601	0.533	0.068
Prob29	2.000	0.000	0.375	0.305	0.290
Prob34	5.000	0.583	0.625	0.575	0.000
Prob35	4.000	0.583	0.580	0.523	0.000
Prob25	8.000	0.667	0.837	0.817	0.114
Prob26	7.000	0.667	0.844	0.824	0.112
Prob23	8.000	0.750	0.837	0.816	0.053

P. rueppellii (n = 16)

Locus	Number of alleles:	Observed Heterozygosity (H_O):	Expected Heterozygosity (H_E):	Polymorphic information content (PIC):	Null allele frequency (Na):
Prob6	2.000	0.250	0.219	0.195	0.000
Prob15	9.000	0.500	0.797	0.776	0.178
Prob18	5.000	0.333	0.747	0.709	0.233
Prob30	8.000	0.688	0.807	0.78	0.053
Prob28	2.000	0.313	0.482	0.366	0.116
Prob29	2.000	0.000	0.408	0.325	0.302
Prob34	2.000	0.067	0.420	0.332	0.260
Prob35	2.000	0.063	0.170	0.155	0.138
Prob25	7.000	0.625	0.809	0.782	0.095
Prob26	6.000	0.333	0.691	0.66	0.229
Prob23	8.000	0.875	0.836	0.815	0.000

P. cryptoxanthus (n = 14)

Locus	Number of alleles:	Observed Heterozygosity (H_O):	Expected Heterozygosity (H_E):	Polymorphic information content (PIC):	Null allele frequency (Na):
Prob6	4.000	0.462	0.618	0.561	0.118
Prob15	10.000	0.571	0.855	0.838	0.156
Prob18	3.000	0.308	0.322	0.29	0.000
Prob30	8.000	0.714	0.816	0.793	0.064
Prob28	2.000	0.000	0.133	0.124	0.176
Prob29	5.000	0.538	0.624	0.572	0.001
Prob34	6.000	0.714	0.694	0.651	0.045
Prob35	2.000	0.286	0.245	0.215	0.000
Prob25	11.000	0.769	0.873	0.86	0.065
Prob26	9.000	0.400	0.845	0.827	0.238
Prob23	5.000	0.786	0.709	0.673	0.000

P. gulielmi gulielmi (n = 4)

Locus	Number of alleles:	Observed Heterozygosity (H_O):	Expected Heterozygosity (H_E):	Polymorphic information content (PIC):	Null allele frequency (Na):
Prob6	3.000	0.500	0.406	0.371	0.000
Prob15	3.000	0.500	0.594	0.511	0.047
Prob18	1.000	0.000	0.000	0	0.001
Prob30	1.000	0.000	0.000	0	0.001
Prob28	2.000	0.250	0.219	0.195	0.000
Prob29	3.000	1.000	0.656	0.582	0.000
Prob34	4.000	0.750	0.719	0.667	0.000
Prob35	2.000	0.500	0.375	0.305	0.000
Prob25	4.000	0.750	0.688	0.63	0.000
Prob26	5.000	1.000	0.750	0.712	0.000
Prob23	3.000	0.750	0.625	0.555	0.000

P. g. massaicus (n = 11)

Locus	Number of alleles:	Observed Heterozygosity (H_O):	Expected Heterozygosity (H_E):	Polymorphic information content (PIC):	Null allele frequency (Na):
Prob6	9.000	1.000	0.855	0.839	0.000
Prob15	7.000	0.636	0.777	0.747	0.078
Prob18	2.000	0.182	0.165	0.152	0.000
Prob30	3.000	0.364	0.430	0.385	0.000
Prob28	5.000	0.273	0.508	0.483	0.188
Prob29	3.000	0.545	0.417	0.36	0.000
Prob34	6.000	0.818	0.806	0.777	0.007
Prob35	1.000	0.000	0.000	0	0.001
Prob25	1.000	0.000	0.000	0	0.001
Prob26	5.000	0.545	0.707	0.659	0.110
Prob23	6.000	0.818	0.818	0.792	0.000

Supplementary Table 2-4. The pairwise F_{ST} values for all *Poicephalus* specimens used in this study. The F_{ST} values are below the diagonal and probability values (*p*-values) above diagonal. All *p-values* are significant (*p*-values < 0.003).

	P. robustus robustus	*P. r. suahelicus*	*P. r. fuscicollis*	*P. rueppellii*	*P. meyeri*	*P. cryptoxanthus*	*P. gulielmi massaicus*	*P. g. gulielmi*
P. robustus robustus	*	0.001	0.001	0.001	0.001	0.001	0.001	0.001
P. r. suahelicus	0.14	*	0.001	0.001	0.001	0.001	0.001	0.001
P. r. fuscicollis	0.22	0.16	*	0.001	0.001	0.001	0.001	0.001
P. rueppellii	0.28	0.23	0.31	*	0.001	0.001	0.001	0.001
P. meyeri	0.18	0.13	0.24	0.13	*	0.001	0.001	0.001
P. cryptoxanthus	0.19	0.15	0.25	0.21	0.14	*	0.001	0.001
P. gulielmi massaicus	0.37	0.33	0.4	0.41	0.32	0.36	*	0.001
P. g. gulielmi	0.36	0.29	0.39	0.38	0.29	0.33	0.25	*

Supplementary Table 2-5. Summary statistics of the COI, 16S rRNA and β-fib sequences generated for the *Poicephalus* specimens analysed in the current study.

Marker name:	Number of sequences:	Base pair length:	Number of variable sites:	Number of transitions:	Number of transversions:	Number of Indels:	T%:	C%:	A%:	G%:
COI	18	592	66	54	14	1	24.6	35.2	24.1	16.2
16S rRNA	18	535	32	24	8	2	19.1	28.1	32.6	20.2
β-fibrinogen	10	707	4	3	1	0	33.1	22.3	26.8	17.8

Supplementary Table 2-6. The COI pairwise genetic distances calculated in RaxML for four *Calyptorhynchus* sp. sequences. (Downloaded from GenBank: JF414241.1, JN801424.1, JF414242.1, JF414279.1, JF414274.1).

	C. lathami	*C. banksii*	*C. baudinii*	*C. funereus*	*C. latirostris*
C. lathami	*				
C. banksii	0.151	*			
C. baudinii	0.632	0.499	*		
C. funereus	0.686	0.627	0.036	*	
C. latirostris	0.594	0.497	0.009	0.03	*

CHAPTER 3: HISTORICAL BIOGEOGRAPHY: THE INFLUENCE OF ANCIENT AND CONTEMPORARY HABITAT CHANGES ON THE ENDEMIC SOUTH AFRICAN PARROT *POICEPHALUS ROBUSTUS*

3.1 Abstract

The effect of anthropogenic habitat fragmentation on species, which live in naturally patchy habitats, is rarely examined. The extent of habitat fragmentation can have an effect on the genetic structure of a species. The Cape Parrot (*Poicephalus robustus*) is the only endemic parrot species in South Africa. Recent census estimates suggest that there are less than a 1600 Cape Parrots left in the wild. The Cape Parrot is a habitat specialist, restricted to forest patches in the Eastern Cape, KwaZulu-Natal and Limpopo Provinces of South Africa. Although current overexploitation of forests in southern Africa is certainly an important driver of fragmentation, this is not solely responsible for the relictual nature of South African forests. In the Pliocene, periods of climate change driven aridity and increased fire frequency, contributed towards 'natural' fragmentation of the forests in southern Africa. In this study, 29 historical samples, collected from 1870 to 1946, and 85 modern samples, collected from 1951 to 2014, were used to investigate the historical and contemporary genetic structure of Cape Parrots using 16 microsatellite loci. Bayesian clustering analysis identified three geographically correlated genetic clusters. A southern group restricted to forest patches in the Eastern Cape, a central group including birds from KwaZulu-Natal and a genetically distinct northern Limpopo cluster. Results suggest that Cape Parrots have experienced at least two population bottlenecks. An ancient decline during the mid-Holocene (~1800-3000 years before present) linked to climate change, and a more recent bottleneck, associated with logging of forests during the early 1900's. This study highlights the effects of climate change and human activities on an endangered species associated with the naturally fragmented forests of eastern South Africa. These results will aid conservation authorities with the planning and implementation of future conservation endeavours.

Keywords: Cape Parrot, *Poicephalus robustus*, habitat fragmentation, contemporary, historic, population decline

3.2 Introduction

Examining spatial patterns of genetic diversity is an important component of conservation planning and implementation (Arif and Khan, 2009, DeSalle and Amato, 2004, McNeely et al., 1990). Interpreting the processes underlying the spatial patterns observed in genetic data are difficult, as the genetic diversity present in a population is a product of both long-term historical processes such as global climate change, as well as more recent anthropogenic factors such as habitat loss, pollution, introduced species and diseases, poaching and over exploitation of natural resources (Bickham et al., 2000, Bouzat, 2010, Bouzat et al., 1998, Daszak et al., 2000, Dussex et al., 2015). Both long-term and more recent human-associated factors can fragment habitat and reduced population size which can lead to populations with lowered genetic variation, evolutionary potential and population fitness (Bruggeman et al., 2010, Couvet, 2002, dos Anjos et al., 2011, Shaffer, 1981, Spielman et al., 2004). The roles played by these processes in shaping extant populations is an important consideration in conservation as interventions that target single threats often have limited success (Brook et al., 2008). But linking reduction in genetic variation to a specific cause is often difficult. Many studies use genetic data collected from contemporary populations to infer historical phylogeographic patterns (Callens et al., 2011, Kotlík et al., 2014, Taberlet et al., 1998). The use of genotypes from historical samples, however, allows for a more direct measure of the change in demographic and/or phylogeographic patterns in populations over time. Differences in the genetic diversity over time provides valuable information on whether modern genetic patterns are more heavily influenced by long-term processes such as climate change, or if the decline in genetic diversity is more recent, caused primarily by population fragmentation and population decline driven by human-associated factors (Campos et al., 2010, Dussex et al., 2015, Gottelli et al., 2013, Lorenzen et al., 2011, Welch et al., 2012). Some studies have shown no change in phylogeographic patterns across temporal scales (Teacher et al., 2011, Thomas et al., 1990), while others show significant shifts in the distribution of genetic diversity over relatively short time-scales (Goldstein and Desalle, 2003, Leonard et al., 2000, Mende and Hundsdoerfer, 2013, Pergams et al., 2003).

The South African endemic Cape Parrot (*Poicephalus robustus*; Coetzer et al., 2015) provides a suitable model system for studying the relative impact of ancient climate change versus the more recent impact of anthropogenic factors on genetic variation. The species is listed as endangered in the Red Data Book of Birds of South Africa, Lesotho and Swaziland (Taylor, 2014) and is protected under South African legislation (Minister of Environmental

Affairs and Tourism, 2007). A recent study based on morphological, ecological, behavioural and molecular data, called for the reclassification of the Cape Parrot and following criterion C2a(i) called for a CITES Appendix I listing and Vulnerable listing in the IUCN Red List of Threatened Species (Coetzer et al., 2015).

Cape Parrots are habitat specialists feeding and nesting predominantly in yellowwood (*Afrocarpus spp.* and *Podocarpus spp.*) forests (Downs and Symes, 2004, Perrin, 2009, Wirminghaus et al., 2001a, Wirminghaus et al., 2002, Wirminghaus et al., 2001b). The current distribution of the Cape Parrot is fragmented, and the species is restricted to forest patches in the Eastern Cape (EC), KwaZulu-Natal (KZN) (Figure 3-1), with a relic population in the Limpopo Province (Wirminghaus, 1997). Historical records show that populations of these parrots have drastically contracted over the last century, especially in the northern parts of KZN and along the escarpment of Mpumalanga (Downs, 2005a, Downs et al., 2014, Symes et al., 2004, Wirminghaus et al., 2000). Large flocks of Cape Parrots were frequently observed during the early 1950's (Wirminghaus et al., 1999), these numbers declined sharply after 1950, which in part could be linked to eradication of "pest' parrots during the early 1900's (Symes, 2010). In recent times large flocks are rarely seen except when parrots congregate at agricultural pecan orchards during periods of food shortages (Downs et al., 2014). Although the illegal harvesting of individuals for the pet trade and Psittacine beak and feather disease (Wirminghaus et al., 2000, Wirminghaus et al., 1999) are considered to play a role in the decline of Cape Parrots, one of the greatest threats to this species is habitat destruction. Habitat loss is one of the main extinction drivers in wild parrots globally (Collar, 2000, Pain et al., 2006). For example, the now extinct in the wild Spix's macaw (*Cyanopsitta spixii*) (Juniper and Yamashita, 1991, Pain et al., 2006), the Mauritian endemic Echo parakeet (*Psittacula eques*) and the Puerto Rican Parrot (*Amazona vittata*) have all suffered drastic population declines directly linked to anthropogenic habitat destruction (BirdLife International, 2013a, b, Greenwood, 1996, Jones, 1987, Snyder et al., 1987, Swinnerton, 1998).

As the smallest biome in southern Africa, forests have a long history of human habitation and utilization. The principal threat in recent times has been exploitation for timber, with deforestation in South Africa reaching a peak during the colonial era (1850-1910) (Lawes et al., 2007), with less rigorous removal of *Podocarpus* ssp. during the mid-1920's to 1930's (King, 1941) and some forest areas being logged up until 1940 (McCracken, 1986, Rycroft, 1942). The availability of food and nesting sites for Cape Parrots is directly affected by forest fragmentation, as patch size has an influence on *Afrocarpus*/*Podocarpus* fruit availability, with

the larger patches having more trees with longer fruiting (Hart et al., 2013). Cape Parrots are secondary cavity nesters (Downs, 2005b, Wirminghaus et al., 2001a) and prefer to nest in pre-existing cavities in tall, dead *Afrocarpus*/*Podocarpus* trees (Downs and Symes, 2004, Wirminghaus et al., 2001a). A limiting factor to secondary nester species is the availability of pre-existing cavities in mature trees (Newton, 1994). Potential nest sites are often destroyed during logging operations which target the old, large trees (Brightsmith, 2005, Downs, 2005a, Wirminghaus et al., 2001a, Wirminghaus et al., 1999). The impact of earlier commercial harvesting of *Afrocarpus*/*Podocarpus* trees, as well as current subsistence harvesting of pole-sized trees (Lawes et al., 2007) threatens the South African mistbelt forests. Currently, the forest biome is highly fragmented with few large blocks of intact habitat existing outside of conservation areas. Forest fragments are found in small isolated patches interspersed by large areas of non-forest patches (Eeley et al., 1999)

Human-mediated processes are not always the main driver of species extinction. For example, population decline in the New Zealand Kea (*Nestor notabilis*) pre-dates the arrival of humans on these islands (Dussex et al., 2015). Using molecular data it was estimated that the Kea suffered a population contraction during the Holocene, in response ancient climatic and habitat changes (Dussex et al., 2015). Although overexploitation of forests in southern Africa is certainly an important driver of fragmentation, this is not solely responsible for the relictual nature of South African forests. The distribution of this biome is also strongly linked to prevailing climate (Eeley et al., 1999). Beginning in the Pliocene, periods of climate change driven aridity and increased fire frequency, contributed towards 'natural' fragmentation of the forest biome in southern Africa (Geldenhuys, 1989, Scott et al., 1997). The Quaternary global interglacial-glacial cycles prompted the contraction and expansion of the forest biome in Africa (Miller and Gosling, 2014). Analysis of pollen taken in cores from Lake Eteza on the South African east coast have shown an increase in *Podocarpus* containing forests between ca. 6500 – 3700 calibrated years before present (cal yrs BP). In contrast, ca. 3600 cal yrs BP to 3500 cal yrs BP pollen data indicate a rapid decrease in *Podocarpus*, with cores dominated by Poaceae and Ateraceae pollen, indicative of a drier grassy environment at ca. 3500 – 2000 cal yrs BP (Neumann et al., 2010). Climate changes during the Pliocene and Pleistocene have been suggested as the major (although not exclusive) determinate of the faunal biogeographical pattern within other biomes in South Africa (for example, Cape Floristic Region: Makokha et al., 2007, Swart et al., 2009, Tolley et al., 2006, Tolley et al., 2008, Tolley et al., 2009).

In this study I compared the historical and contemporary genetic structure of the South African endemic Cape Parrot populations. I tested for evidence of partitioning of haplotypes on a temporal scale using historical (1870 – 1946) and contemporary (1951 – 2014) samples. Cape Parrots are long-lived birds reaching an age of ~25 years in captivity (C.T. Downs pers. comm.), the effects of habitat loss might therefore only be observed in the gene pool years later. Commercial logging of indigenous timber reached a peak in the 1910's – 1940's (Lawes et al., 2007, Lawes et al., 2004). The date of 1950 was chosen for the separation of historical and contemporary samples, following the observed population decline around that time (Downs et al., 2014, Wirminghaus et al., 1999). Multilocus microsatellite data are used to estimate changes in population structure over time. These results will provide insight into past and more recent, natural and anthropogenic, events that might have affected Cape Parrot population dynamics. This study also aimed to provide a better understanding of the subpopulation structure and connectivity between the contemporary populations. Given the current distribution of suitable habitat the populations from the three provincial regions (EC, KZN and Limpopo) could be considered as three management units (MUs; Moritz, 1994). Estimating connectivity of disjunct populations, however, will provide conservation authorities with the necessary information needed to make and implement the appropriate conservation measures to protect the Cape Parrot.

3.3 Materials and Methods

3.3.1 Ethics

Ethical approval for this study was obtained from the University of KwaZulu-Natal Animal Ethics sub-committee (Ref numbers: 074/13/Animal, 017/14/Animal). The KwaZulu-Natal sampling permit was obtained through Ezemvelo KZN Wildlife (Permit number: OP 1546/2014). Import permits were obtained through Ezemvelo KZN Wildlife (Permit numbers: OP 1230/2014 and OP 878/2014).

3.3.2 Specimen sampling and DNA extraction

3.3.2.1 Historical samples

Historical Cape Parrot samples were sourced from four natural history collections (Supplementary Table 3-1) covering the museums most relevant to the study area. Only specimens with collection date and some locality information (at least a provincial allocation)

were selected for inclusion in the study. To minimize damage to specimens, toe pad samples were taken. In total 29 archival Cape Parrot samples collected between 1870 and 1946 from six localities were selected for inclusion in the historical data set (Supplementary Table 3-1). Samples collected from Ermelo (Mpumalanga) and Bela Bela (Limpopo), fall outside the current Cape Parrot distribution (Figure 3-1). These records suggest that these specimens represent migrants or that during the late 1800's and early 1900's, when these specimens were collected, forests and hence Cape Parrots were more widely distributed. Eight samples had only provincial allocations (EC = 4; KZN = 4). These samples were grouped as EC unknown (n = 4) and KZN unknown (n = 4). Although these are indicated on the map, the exact sampling locality is unknown.

Standard precautions (Pääbo et al. 2006, Hofreiter et al. 2001, Römpler et al. 2006) were followed when working with archival DNA to ensure no contamination occurred. Three wash steps (with 95% ethanol, 70% ethanol and ultrapure water) were performed prior to DNA extraction to minimize surface contamination. Wash steps were followed by a hydration step where samples were soaked in 1 ml ultrapure water for 60 min. The NucleoSpin® Tissue kit was used for DNA extraction following a modified protocol. The lysis step was extended overnight to ensure complete lysis of the samples. The final elution step was also modified. Initially 40 µl of preheated elution buffer was added to the spin column and samples were incubated for 10 min at 70 °C. After centrifuging of the samples at 11000 x g, the buffer was placed back into the same spin column. An additional 40 µl preheated elution buffer was added to each spin column and centrifuged at 11000 x g to obtain the final DNA product. All DNA samples were stored at -20 °C.

3.3.2.2 Contemporary samples

Whole blood, muscle tissue, archival toe pads and feathers were acquired from a number of sources (Supplementary Table 3-1). These specimens were sampled between 1951 and 2014 from wild caught Cape Parrots from across their distribution range (n = 75; Figure 3-1). Sixty samples were collected from four localities in the Eastern Cape Province of South Africa (Alice, n = 41; King William's Town, n = 11; East London, n = 3; Lusikisiki, n = 3; EC unknown, n = 2), 19 samples from Creighton (KZN), one unknown KZN sample and five samples from the relic population in the Tzaneen area (Limpopo). Although the sample size of the Limpopo population is small, this sample does represent a significant portion of the census population (n = 80-100; Downs et al., 2014).

The whole blood samples collected from wild parrots trapped by mist-netting were stored on Whatman™ FTA™ Elute cards. Small moulted feathers were collected from under feeding trees and muscle tissue biopsies were taken from Cape Parrot carcasses sent to the University of KwaZulu-Natal for analysis. These muscle biopsies were stored in absolute ethanol. DNA extraction from the whole blood stored on the Whatman™ FTA™ Elute cards was performed following the manufacturer's protocols. The NucleoSpin® Tissue kit (Macherey-Nagel) was used for DNA extraction from the muscle tissue samples, following the manufacturer's suggested protocol. The modified protocol (described above for the archival DNA extraction) was followed for both toe pad and feather samples. In feather samples the whole tip of the quill was used for DNA extraction, making sure to include the small blood clot found in the calamus.

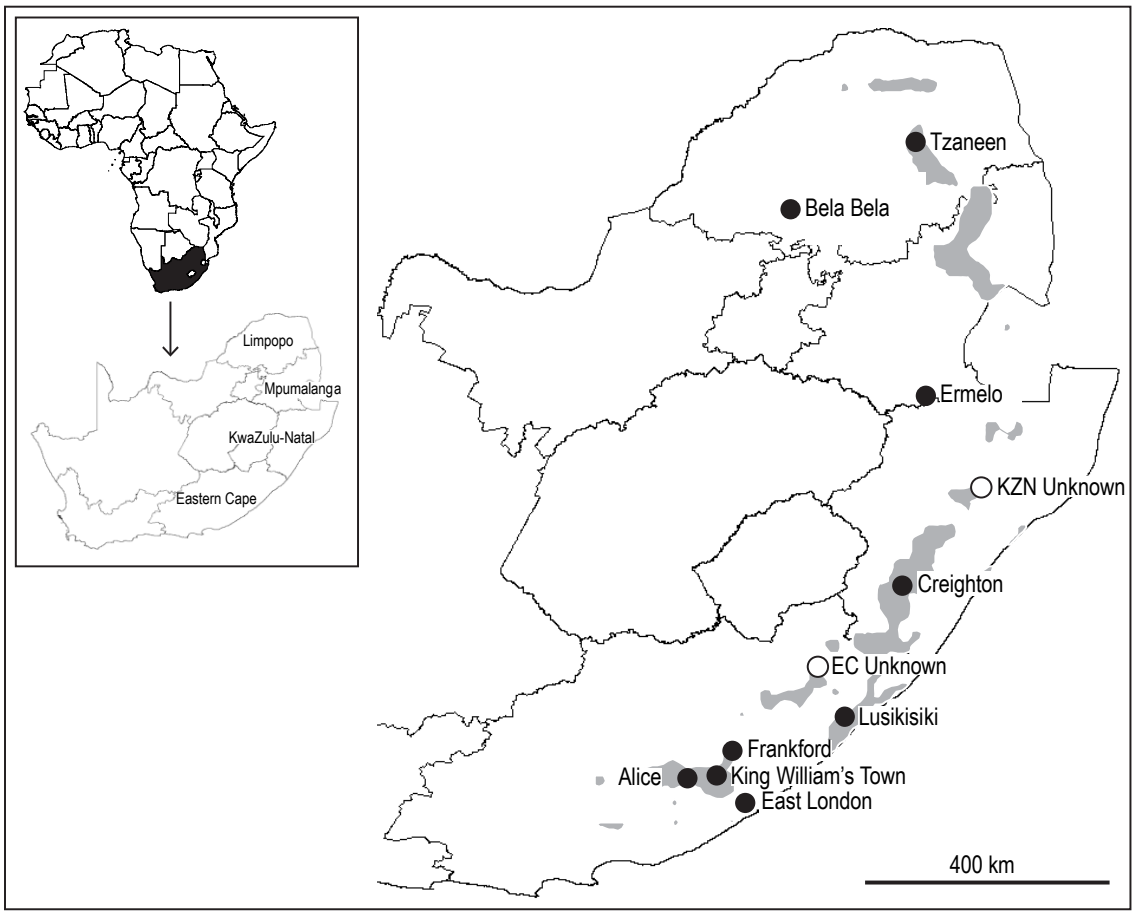


Figure 3-1. Sampling sites for Cape Parrot (*Poicephalus robustus*) in South Africa. Grey shading indicates the extent of current forest cover in the region (Mucina and Rutherford, 2006). The black circles indicate sampling sites with detailed locality information, with the white circles representing samples from the Eastern Cape (EC Unknown) and KwaZulu-Natal (KZN Unknown) that do not have precise locality information.

3.3.3 Microsatellite amplification

A panel of 16 microsatellite markers designed specifically for use in *P. robustus* were used - *Prob01*, *Prob06*, *Prob09*, *Prob15*, *Prob17*, *Prob18*, *Prob23*, *Prob25*, *Prob26*, *Prob28*, *Prob29*, *Prob30*, *Prob31*, *Prob34*, *Prob35* and *Prob36* (Pillay et al., 2010). In each primer pair the 5' end of forward primer was labelled with fluorescent dye. The microsatellite panel was divided into six multiplex sets (Multiplex 1: *Prob06*, *Prob15* and *Prob26*; Multiplex 2: *Prob30* and *Prob36*; Multiplex 3: *Prob18, Prob25* and *Prob31*; Multiplex 4: *Prob01*, *Prob09* and *Prob17*; Multiplex 5: *Prob23* and *Prob28*; Multiplex 6: *Prob29*, *Prob34* and *Prob35*). The PCR reactions for the contemporary samples (blood and muscle tissue samples) consisted of: ~2-30 ng template DNA, 5 µl KAPA2G Fast Multiplex mix (KAPA Biosystems), 0.2 µM of each primer and dH_2O to give a final reaction volume of 10 μl. Each 10 μl PCR reaction for feather and archival toe pad samples consisted of: ~20-200 ng template DNA, 5 µl KAPA2G Fast Multiplex mix, 0.2 µM of each primer, 0.3 µl of 1 mg/ml BSA and dH_2O to final volume of 10 μl. The same PCR cycle parameters were used for all multiplex reactions and consisted of an initial denaturation step for 3 min at 94 °C followed by 30 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and a final extension step for 5 min at 72 °C. The PCR cycles for the feather and archival samples were extended to 40 cycles to increase amplification success. All steps of the PCR setup, except the DNA addition step, was performed at a DNA free area to avoid contamination.

All amplified products were sent to the Central Analytical Facility at Stellenbosch University, South Africa for fragment analysis. The software program Gene Marker® v2.4.0 (Soft Genetics) was used for subsequent genotype scoring. To check for genotyping consistency, I reamplified 20% of the fresh samples (whole blood, muscle tissue and feathers). All archival samples were re-extracted and reamplified at least three times to ensure that genotypes were consistently scored and to check for contamination.

3.3.4 Analysis of genetic variation

To better elucidate temporal changes in populations of Cape Parrots, historical (1870-1946) and contemporary (1951-2014) samples were analysed separately. Analyses were also conducted on the combined data set (1870-2014). Null allele frequencies were estimated for each of the three data sets using the maximum likelihood (ML) method as implemented in ML-NullFreq (Kalinowski and Taper, 2006). Null allele frequencies for each marker were also estimated using the software program FreeNA (Chapuis and Estoup, 2007) using the

Expectation Maximization algorithm (EM; Dempster et al., 1977). The null allele corrected and uncorrected global F_{ST} values were compared using the excluding null alleles (ENA) method (Chapuis and Estoup, 2007).

The software program GenAlEx v6.5 (Peakall and Smouse, 2012) was used to assess genetic diversity by calculating the number of alleles (N_A), observed heterozygosity (H_O), unbiased expected heterozygosity (uH_E) and fixation index (F). Allelic richness (Ar) and inbreeding coefficient (F_{IS}) was calculated in FSTAT (Goudet, 2001). Allelic richness was estimated using the rarefaction method as implemented in FSTAT, to account for differences in sample sizes within the data. To assess the genetic differences between the contemporary and historical data, genetic diversity values were compared per locus using the Wilcoxon signed-ranked test applying a Bonferroni correction for multiple comparisons (Rice, 1986).

Polymorphic information content (PIC) estimates for each locus were calculated using Cervus v3.0.7 (Kalinowski et al., 2007). PIC values ranging from 0-0.29 are considered as uninformative, 0.3-0.59 are moderately informative and above 0.6 are highly informative (Mateescu et al., 2005). Tests for deviation from Hardy-Weinberg equilibrium (HWD) and estimates of genotypic disequilibrium (GD) between pairs of loci within sampling regions were performed using GENEPOP v4.2 (Rousset, 2008) using the default settings for Markov chain parameters.

3.3.5 Population structure analysis

To test for correlation between geographic distance and genetic distance, a Mantel test (Mantel, 1967) was performed for the historical, contemporary and combined data sets using GenAlEx. Bayesian clustering analysis was performed on contemporary and historical data independently. Analyses were also conducted on the combined data set, with the rationale that if the contemporary structure corresponded to the historical one, the historical and contemporary samples from the same geographical region should cluster together. On the other hand, if the contemporary and historical structures differ, samples from the same region should not cluster well or have more admixed membership. Bayesian clustering analysis was performed using the program STRUCTURE v2.3 (Pritchard et al., 2010). To estimate the number of genetic clusters (K), simulations were conducted with K ranging from 1 to 10 for the contemporary and historical data sets and K ranging from 1 to 12 for the combined data set. Twenty independent runs were performed for each K value, using 500,000 Markov chain Monte Carlo (MCMC) replicates per run and a burn-in of 50,000 iterations. In each run the

correlated allele frequencies and the admixture ancestry model were selected. The LOCPRIOR was used, incorporating population locality information. STRUCTURE harvester (Earl, 2009) was used to estimate the optimal number of genetic clusters using the method implemented by Evanno et al. (2005). ClumpAK (available from http://clumpak.tau.ac.il) was used to estimate the probability of membership (Q-values) for each individual and summarized for each genetic cluster.

To estimate the level of genetic differentiation among localities, pairwise F_{ST} values and analysis of molecular variance (AMOVA) were conducted using GenAlEx. The pairwise F_{ST} and AMOVA analyses were performed for two grouping schemes. First, samples were grouped according to sampling locality. For the histrorical data, individuals were assigned to eight groups (Alice, King Williams's Town, Frankfort, EC unknown, KZN unknown, Bela Bela, Ermelo and Tzaneen). Seven groups (Alice, King Williams's Town, East London, Lusikisiki, EC unknown, Creighton and Tzaneen) were analysed for the contemporary data set, with the one unknown contemporary KwaZulu-Natal sample included in the Creighton sample set for this analysis. The samples from the combined data set were grouped into 11 groups (Alice, King Williams's Town, Frankfort, East London, Lusikisiki, EC unknown, Creighton, KZN unknown, Bela Bela, Ermelo and Tzaneen). Geographically close populations were then combined into three regional groupings for the historical, contemporary and combined analyses (historical: South (Alice, King Williams's Town, Frankfort, EC unknown), Central (KZN unknown) and North (Bela Bela, Ermelo and Tzaneen); contemporary: South (Alice, King Williams's Town, East London, Lusikisiki, EC unknown), Central (Creighton) and North (Tzaneen); combined: South (Alice, King Williams's Town, Frankfort, East London, Lusikisiki, EC unknown), Central (Creighton, KZN unknown) and North (Bela Bela, Ermelo and Tzaneen).

To examine microsatellite variation temporally, a genotype network was constructed using TempNet (Prost and Andersong, 2011). Collection dates were used to assign individual genotypes to either historical (1870-1946) or contemporary (1951-2014) layers. By construction of a temporal statistical parsimony genotype network, 'ghost' genotypes and alleles lost through bottlenecks can be identified. The TempNet software is written to analyse sequence data, so the microsatellite data for each individual was converted by assigning a unique four nucleotide sequence to each allele. This produced 32, four nucleotide segments per individual which was combined to produce a 128 bp segment per individual.

3.3.6 Demographic history

The sample planning optimization tool for conservation and population genetics (SPOTG; Hoban et al., 2013) was used to assess if the historical and contemporary data sets used had enough statistical power to identify bottleneck events (Hoban et al., 2013). Three bottleneck simulations and one temporal simulation were performed, using sample sizes of 29, 85 and 114 (Supplementary Table 3-2). The first bottleneck simulation was performed with recovery of the simulated population after the bottleneck ended. Pre-bottleneck *Ne* was set at 1000 or 2000, with *Ne* at the end of the bottleneck set at 100, 200 or 300. The start of the population decline was set at 1000 years before present (YBP) and recovered *Ne* size of the population after a 100 years since the bottleneck event was set at 400 individuals. The second and third bottleneck simulations were performed with the pre-bottleneck *Ne* set at 1000 or 2000 in each simulation, with *Ne* at the lowest point during the population decline set at 100, 200, 300 or 400. Only the start time for each of the second and third bottleneck simulations differed, with the one simulation set at 100 YBP and the following simulation set at 1000 YBP. The temporal simulation accounts for the inclusion of archival samples in the analyses. The temporal (historical individuals) samples were set at 29, with the age of these samples set at 100 years. The *Ne* of the ancient population was set at 1000, 2000 or 20,000, with the current *Ne* set at 300 or 400 individuals. The time since the population decline began was set at 100, 1000 or 2000 YBP. A generation time for Cape Parrots was set at 5 years, with the loci mutation rate set at 0.0005 and 90% stepwise mutations for all simulations. A 1000 replicates were performed per simulation (Supplementary Table 3-2).

Three methods were used to detect the genetic signature of population bottlenecks - the heterozygosity excess method, mode-shift method and M-ratio test. The heterozygosity excess method as implemented in the program Bottleneck v 1.2.02 (Piry et al., 1999) was used to assess the possible occurrence of a bottleneck event within the historical, contemporary and combined data sets, using the Wilcoxon sigh-rank test method of Luikart and Cornuet (1998). This method is effective in detecting recent declines in *Ne* and assumes that populations that recently suffered a decline in *Ne* will have a higher level of heterozygosity as opposed to a population at mutation-drift equilibrium (Cornuet and Luikart, 1996). Given the limited sample size of the historical samples (1870-1946) analysis was performed placing all individuals in a single group. Two analyses were performed for the contemporary data (1951-2014). One analysis grouping individuals according to geographical origin of the individuals (South, Central and North; see results), and a second analysis with all individuals grouped together.

Two mutation models were chosen, the TPM model for microsatellites (Di Rienzo et al., 1994) with 90% and 95% stepwise mutations following Garza and Williamson (2001) and Dussex et al. (2015) with a variance of 12 as suggested by Piry et al. (1999), to encompass the observed range of multistep mutations in natural populations (Di Rienzo et al., 1994); and the stepwise mutation model (SMM). The mode-shift test was used as implemented in Bottleneck v1.2.02 to assist in discriminating between recent bottlenecked and stable populations (Luikart et al., 1998). The mode-shift distortion caused by a population decline can only be detected within a few dozen generation (Tucker et al., 2012), therefore only very recent bottleneck events will be detected by this method. The *M*-ratio test is more appropriate for detecting bottlenecks that occurred at a more distant time (<100 generations) than the heterozygote excess method (Peery et al., 2012, Williamson-Natesan, 2005). The *M*-ratio analysis was performed using *M-P-Val* and *Critical_M* (Garza and Williamson, 2001). Pre-bottleneck N_e values were set at 100, 1000 and 2000 individuals, with a constant mutation rate of 5×10^{-4}. Two TPM mutation models were followed. The first parameter set is more widely used with single step mutations of $p_s = 0.88$ and the number of steps for multi-step mutations at $\Delta g = 2.8$ and a second parameter set, described to be more conservative, with $p_s = 0.9$ and $\Delta g = 3.5$ (Garza and Williamson, 2001).

A coalescent-based Bayesian method was used to estimate the change in effective population size through time, using the program MSVAR 1.3 (Beaumont, 1999, Storz and Beaumont, 2002). Eight simulations were run for each heterochronous data set (historical, contemporary and combined). The coalescent-based method used in MSVAR is known to be affected by strong population structure (Radespiel and Bruford, 2014). For this reason analyses were also performed on the three regional groupings (South, Central and North) independently. A generation time of five years was chosen (Wirminghaus et al., 2001a). Five simulations for each contemporary regional data set (South, Central and North) was performed. Each simulation consisted of 2×10^8 iterations with output values recorded every 10000 step per simulation. Prior distribution settings used are provided in Supplementary Table 3-3. The first 50% of the output from each chain was removed as burn-in. Convergence of MCMC chains was assessed using Tracer v1.5 (Rambaut and Drummond, 2007). The simulations for each data set were then combined, giving 80000 sampling points for the heterochronous data sets and 50000 for each contemporary subpopulation. The mean and 95% highest posterior densities (HPD) were estimated in Tracer.

3.3.7 Contemporary vs historical gene flow

The program 2MOD v0.2 (Ciofi et al., 1999) was used to estimate the relative effect of migration-drift versus genetic-drift alone on the population structure of the historical and contemporary data. The program compares a migration-drift equilibrium model (gene flow model) versus a non-equilibrium model with drift and no gene flow among subpopulations (drift model; Ciofi et al., 1999). Each simulation consisted of 500000 MCMC iterations with the first 10% discarded as burn-in. Convergence of the MCMC run were assessed using Tracer, when the effective sample size (ESS) values of all parameters exceeded 200. The probability that two genes share a common ancestor within a particular population (F) and the number of migrants per population ($M = (1-F)/2F$) was used to assess the strength of interaction between gene flow and genetic-drift. Small F values indicate that the population is strongly influence by gene flow, with large F values indicating a larger influence of genetic drift.

Gene flow among Cape Parrot subpopulations in the historical and contemporary data sets were estimated using the program BayesAss v1.3 (Wilson and Rannala, 2003). Analyses were performed to test for gene flow among the three regional groupings (South, Central and North) for the historical and contemporary data sets. A second gene flow analysis was performed among four groupings for the contemporary data set, using the three regional groupings as suggested by STRUCTURE and placing Lusikisiki and EC unknown in a separate group as these specimens showed signs of admixture from the South and Central clusters (Group 1: Alice, King William's Town and East London; Group 2: Lusikisiki and EC unknown; Group 3: Creighton and KZN unknown; Group 4: Tzaneen). Three independent runs were conducted each consisting of 1×10^7 iterations, with a burn-in of 4×10^5 and a sampling frequency of 2000. The delta values for each parameter were adjusted to achieve a 40% – 60% acceptance rate as recommended in the BayesAss manual. The final delta values used were: delta allele frequency = 0.4, delta migration rate = 0.1 and delta inbreeding coefficient = 0.5. Migration rates below 0.10 indicate that the two populations are demographically independent (Hastings, 1993).

3.4 Results

3.4.1 Genetic diversity

For this study a total of 114 individuals (historical = 29; contemporary = 85) were genotyped using 16 microsatellite loci. Given that DNA quality of archival samples is expected to be lower

than fresh samples (Wandeler et al., 2007), it is unsurprising that the historical data set contained the highest amount of missing data, with 15.517% missing data across all loci. In contrast, a minimal amount of missing data were included in the contemporary data set, with only 0.74% missing data across all loci. The mean null allele frequency over all loci and samples was 4.4% (Na; Supplementary Table 3-4). As expected, the historical data set had the highest level of null alleles (Na = 11.6%), with a null allele frequency of only 2.6% observed for the contemporary data set. These observed null allele values are, however, well below values reported in other studies (Dakin and Avise, 2004) and there was no significant difference between ENA corrected and uncorrected F_{ST} values (p-value > 0.003) and all loci were used for the subsequent analyses. Hardy-Weinberg equilibrium estimates showed that within the historical data seven loci (*Prob01, Prob15, Prob17, Prob23, Prob26. Prob34* and *Prob36, p*-value < 0.05) deviating from equilibrium. Only two loci (*Prob15* and *Prob17*; p-value < 0.05) deviated significantly from Hardy-Weinberg equilibrium within the contemporary data. No signs of linkage disequilibrium was detected in either the historical and contemporary data sets.

Private alleles were detected in the historical (n = 9) and contemporary data sets (n = 39; Table 3-1). The detection of private alleles within the historical data set could be an indication of 'ghost alleles' suggesting the possible loss of alleles from the contemporary Cape Parrot populations. All genetic diversity estimates, except PIC and uH_E, showed significant differences between the historical and contemporary data sets (p-value > 0.003). The PIC values of loci across all samples ranged from 0.172 (*Prob36*) to 0.936 (*Prob17*) with a mean PIC of 0.598 (SE = 0.053; Supplementary Table 3-4). For the combined data set 10 of the 16 loci were highly informative with PIC values ranging from 0.625 to 0.936, five loci were moderately informative with PIC values ranging from 0.302 – 0.569 and one locus (*Prob36*) was uninformative with a PIC of 0.172 (Supplementary Table 3-4). The average number of alleles, allelic richness and observed heterozygosity were highest for the contemporary data set (Table 3-1). The inbreeding coefficient indicated significantly lower levels of heterozygotes within the historical samples (F_{IS} = 0.314), with low levels of inbreeding observed within the contemporary samples (F_{IS} = 0.039). The level of genetic diversity was not significantly different between historical and contemporary samples, and only slightly higher for the contemporary samples (Table 3-1).

The genetic diversity for each regional grouping using contemporary data were also assessed. The average number of alleles and allelic richness was the highest for the South group and Central group (Table 3-1). The Central group had the highest observed heterozygosity (H_O

= 0.647, SE = 0.058). Low levels of inbreeding was observed in the South group (F_{IS} = 0.042), with slightly negative values for the Central and North groups (Table 3-1). The South and Central groups contain the highest level of genetic diversity (Table 3-1). The heterozygosity estimates observed for the contemporary Cape Parrot population are within range of genetic diversity estimates observed in three other old world parrot species.

Table 3-1. Sampling details and genetic diversity for each *Poicephalus robustus* population analysed in the current study. Standard error values for the mean number of alleles, observed heterozygosity, unbiased expected heterozygosity and allelic richness are provided in parentheses.

Region/Time period:	**Number of samples:**	**Average number of alleles (N_A)**	**Observed Heterozygosity (H_O):**	**Unbiased Expected Heterozygosity (uH_E):**	**Allelic richness (Ar):**	**Inbreeding coefficient (F_{IS}):**	**Private alleles (P_A):**
Historical (1870-1946)	29	3.688 (0292)	0.445 (0.041)	0.604 (0.035)	4.302 (0.314)	0.314	9
South	15	4.188 (0.579)	0.382 (0.058)	0.604 (0.051)	2.792 (0.222)	0.384	15
Central	4	2.688 (0.373)	0.443 (0.093)	0.563 (0.084)	NC**	0.258	2
North	10	4.188 (0.476)	0.509 (0.057)	0.645 (0.044)	2.985 (0.224)	0.224	14
Contemporary (1951-2014)	85	4.917 (0.561)	0.617 (0.033)	0.613 (0.030)	7.239 (0.039)	0.039	39
South	60	6.563 (1.252)	0.605 (0.055)	0.632 (0.053)	3.791 (0.400)	0.042	23
Central	20	5.313 (0.898)	0.647 (0.058)	0.635 (0.050)	3.708 (0.386)	-0.02	6
North	5	2.875 (0.340)	0.600 (0.063)	0.572 (0.052)	2.875 (0.340)	-0.055	5
All specimens:	114	5.854 (0.599)	0.581 (0.030)	0.633 (0.028)	7.680 (0.095)	0.095	
South	75	6.563 (1.252)	0.574 (0.053)	0.632 (0.053)	4.792 (0.651)	0.093	22
Central	24	5.313 (0.898)	0.625 (0.059)	0.632 (0.053)	4.718 (0.667)	0.011	6
North	15	2.875 (0.340)	0.544 (0.044)	0.634 (0.043)	4.725 (0.638)	0.146	8

* Negative values were converted to zero

** Value could not be calculated due to missing data

3.4.2 Historical vs contemporary population structure

Mantel test estimates showed no correlation between pairwise geographic and pairwise genetic distances for any of the data sets. Using the historical data the Bayesian clustering analysis recovered two clusters as the most probable number of genetic clusters (K = 2, mean LnP(K) = -985.140; Supplementary Figure 3-1), following the methods of Evanno et al. (2005). These two genetic clusters were geographically correlated (Figure 3-2). The results of this analysis group together samples from the Eastern Cape distribution of Cape Parrots (Alice, Q = 0.869; Frankfort, Q = 83; King William's Town, Q = 0.551; Figure 3-2). The remaining samples (EC Unknown, Q = 0.97; KZN unknown, Q = 0.856; Ermelo, Q = 0.603; Bela Bela, Q = 0.957; Tzaneen, Q = 0.94) belong to the second genetic cluster. There is some evidence of admixture as one of the Ermelo samples (LH 10) clustered strongly with the southern Eastern Cape cluster (Q = 0.833). In this analysis of historical samples there is no evidence of genetic isolation of the northern Limpopo population. In contrast, Bayesian analysis of the contemporary data recovered three genetic clusters (K = 3, mean LnP(K) = -3683.365; Supplementary Figure 3-1). The Eastern Cape and KZN Cape Parrot populations cluster separately in this analysis, although individuals collected from Lusikisiki (Q = 0.51 for the Southern cluster) and EC Unknown (Q = 0.81 for the Central cluster) do show admixture. Lusikisiki is located on the EC and KZN boundary and this population could represent the transitional zone separating the South lineage from the Central lineage. A striking result to emerge from the analysis of the contemporary data is the genetic distinctiveness of Limpopo population (Q = 0.99).

In general historical samples clustered together with contemporary samples from the same geographic region in the combined analysis (Figure 3-2). For example the historical KZN unknown samples clustered with the Creighton and contemporary KZN unknown samples and the historical Alice and King William's Town samples clustered with their contemporary counterparts, with the Frankford samples strongly clustered with the South lineage (Q = 0.94). Two of the historical Tzaneen samples (*LH03* and *LH04*) also clustered with their contemporary counterparts. In contrast, the two remaining historical Tzaneen samples (*LH01* and *LH02*) showed signs of admixture between the South and Central lineages. The four historical and two contemporary EC unknown samples and the four Bela Bela and one of the Ermelo samples clustered with the Creighton and KZN unknown samples. The remaining Ermelo sample showed signs of admixture between the South and Central lineages.

The STRUCTURE analyses were supported by pairwise F_{ST} values. The historical data set showed significant genetic partitioning between the South and Central groupings (F_{ST} = 0.067; *P*-value = 0.001). There was no support for the differentiation between isolated northern populations of Bela Bela, Ermelo and Tzaneen from the central Cape Parrot population (F_{ST} = 0.025; *P*-value = 0.148) or between the southern and central populations (F_{ST} = 0.014; *P*-value = 0.225). The contemporary and combined data sets, however, showed significant genetic differentiation between the three regional groupings (South, Central and North; Table 3-2). The global F_{ST} values significantly differed from zero for all three data sets (historical, F_{ST} = 0.049; *p*-value = 0.001; contemporary, F_{ST} = 0.034, *p*-value = 0.001; combined, F_{ST} =0.026, p-value = 0.001). The analysis of molecular variance (AMOVA) conducted using the historical data indicate that 5% of the variation is observed between sampling regions, 39% among individuals and 56% within individuals. A similar trend was seen with the contemporary data, with 3% of the genetic variation observed among sampling regions, 4% between individuals and 93% within individuals. Similar results were recovered when the samples were grouped according to sampling locality (Supplementary Table 3-5).

The temporal statistical parsimony network (Figure 3-3) showed that no genotypes were shared between historical and contemporary samples. This analysis showed how historical samples are ancestral to many contemporary lineages. Five historical genotypes form the central position within contemporary clades that exhibit a starburst pattern. This pattern is usually seen in taxa that have undergone a recent range expansion or population expansion from a small number of founders. Two historical EC specimens, in particular, have given rise to multiple contemporary individuals which are found in contemporary populations in EC and KZN. Specimen *ECH10* (Frankfort, EC) is linked to 20 contemporary Cape Parrots (EC, n = 5; KZN, n = 15) and *ECH22* (unknown EC) to 16 contemporary individuals (EC, n = 13; KZN, n = 3;). This could be an indication that the historical Cape Parrot lineages might have been more widespread than their contemporary counterparts.

Table 3-2. The pairwise F_{ST} estimates for the combined data set and the historical and contemporary data sets. Comparisons were made between the three sampling regions; South, Central and North. The pairwise F_{ST} values are below the diagonal, with *p-values* above the diagonal. The significance threshold was adjusted for multiple tests: *p*-value = 0.003.

Historical data set:				**Contemporary data set:**				**Combined data set:**			
	Southern	Central	Northern		Southern	Central	Northern		Southern	Central	Northern
Southern	*	0.225	**0.001**	Southern	*	0.007	**0.001**	Southern	*	0.004	**0.001**
Central	0.014	*	0.148	Central	0.014	*	**0.001**	Central	0.013	*	0.003
Northern	**0.067**	0.025	*	Northern	**0.091**	**0.078**	*	Northern	**0.043**	0.031	*

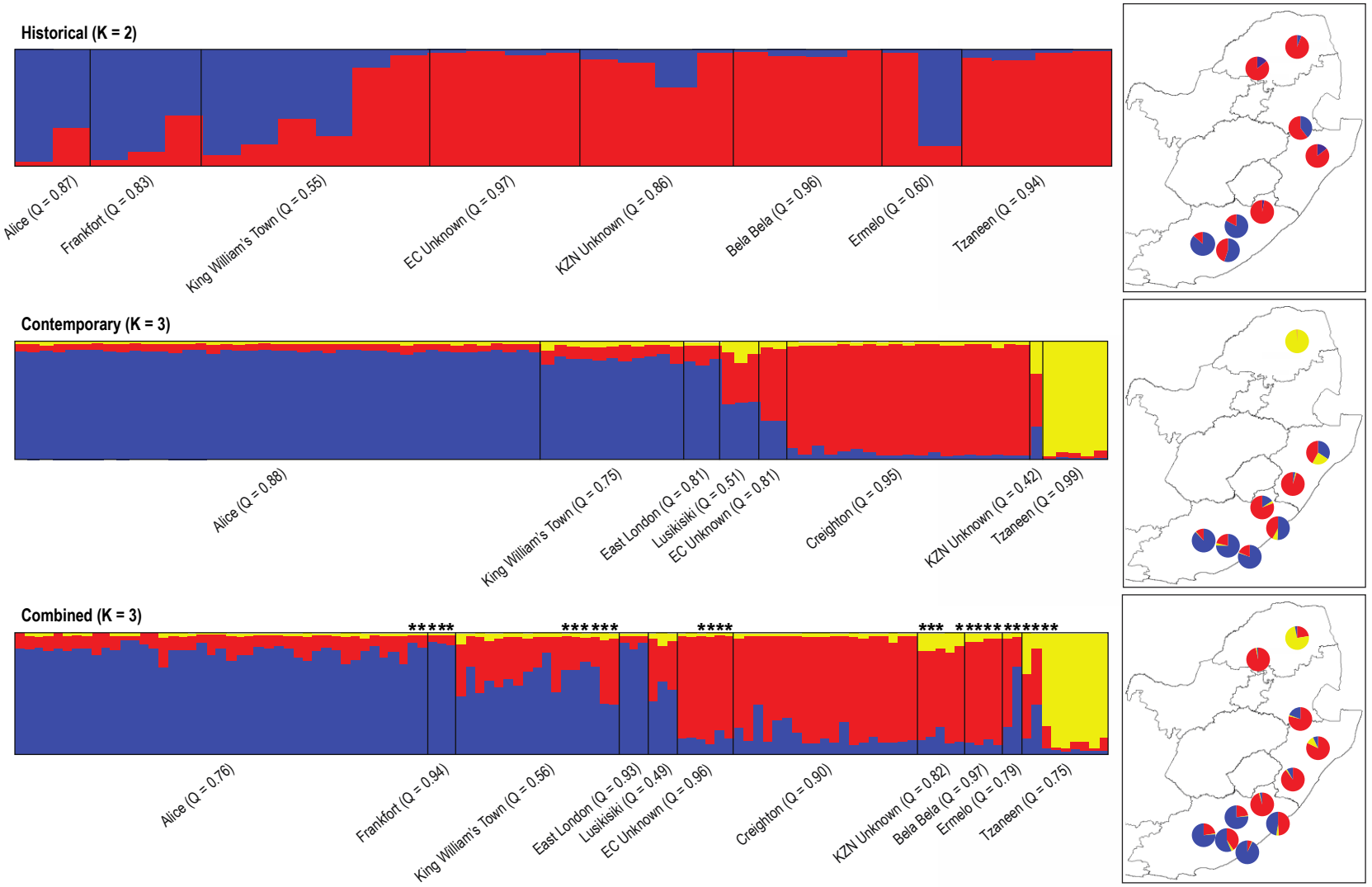


Figure 3-2. The estimated population genetic structure of *Poicephalus robustus* in South Africa using historical, contemporary and combined data sets (left). Each vertical line in the barplot represents an individual and is coloured according to every individual's estimated membership coefficient (Q) values. Asterisks indicates historical samples. The mean Q-values of each cluster is provided. Maps showing the Mean Bayesian assignment probabilities per locality for historical, contemporary and combined data sets (right). Each colour indicates the mean proportion of individual's genotypes assigned to a particular lineage in each locality.

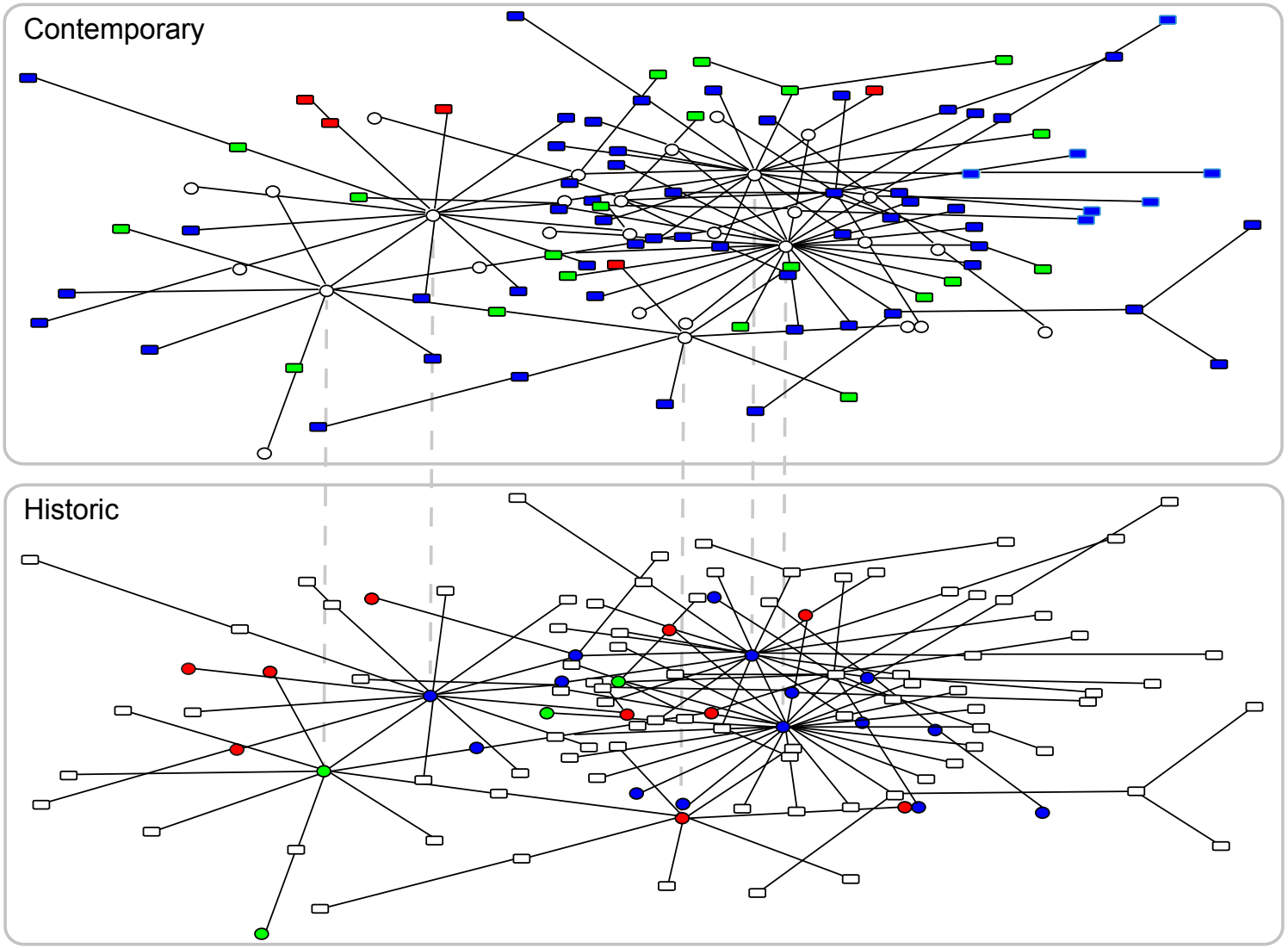


Figure 3-3. A temporal statistical parsimony network displaying the relationships between historical and contemporary genotypes. The network is based on individual 16 loci genotypes. Genotypes are represented by circles (historical) or rectangles (contemporary) colour codes to represent sampling region (blue = South; Green = Central; Red = North), with empty circles and rectangles representing genotypes absent from the given time period. Stippled lines indicate ancestral genotypes.

3.4.3 Demographic history

The SPOTG analyses indicated that the temporal model had the strongest power, with a 100% statistical power for scenarios with a high ancient *Ne* (*Ne* = 20 000) and current *Ne* values of 300 or 400 individuals using sample sizes of 114 and 85. Additionally two of the bottleneck scenarios also showed high statistical power. A bottleneck event at 1000 years (yrs), with an ancient *Ne* of 2000 and current *Ne* of 100, showed the strongest power at 82.2%. The bottleneck simulation with recovery showed strong power using an ancient *Ne* of 2000, with the lowest *Ne* during the bottleneck at 100 and a current 'recovered' *Ne* at 400, with 79.1% statistical power for a sample size of 114 and 80.2% for a sample size of 85 (Supplementary Data 3-1).

Bottleneck analysis using the heterozygote excess method, implementing TPM models with 90% and 95% stepwise mutations, only showed signs of a recent (early 1900's) bottleneck for the South and North regional groupings of contemporary Cape Parrot populations (Table 3-3). The more conservative SSM model (Luikart and Cornuet, 1998), however, showed no signs of a bottleneck event. Only the North group showed significant deviation from the normal L-shaped distribution of allele frequencies expected from a population in mutation-drift equilibrium, providing strong evidence of a recent population decline in this population. The *M*-ratio analyses showed no signs of bottlenecks in any of the data sets, with *M*-ratio values ranging from 0.645 to 0.826.

A severe decline in effective population size (*Ne*) was observed for all data sets following change in effective population size analyses in MSVAR. The ratio of the contemporary and historical population sizes ($r = N_C/N_H$) where used to assess the direction of demographic change, with $r < 1$ indicating a population decline and $r > 1$ a population expansion. All r values were smaller than 0.0021 (Table 3-4). This indicates that the contemporary effective populations size (N_C) is less than 1% of the historical effective population size (N_H). The historical effective population sizes for the three main data sets were large (historical N_H = 236047.823, contemporary N_H = 269153.48 and combined N_H = 278612.117), with much smaller contemporary effective population sizes (historical N_C = 104.713, contemporary N_C = 305.492 and combined N_C = 495.45), indicating a severe bottleneck. None of the 95% HPD intervals overlapped between N_C and N_H. There is clear support for a population decline occurring long before European settlement of South Africa in the 17th century, with the average time since the decline estimated at 2649.289 YBP (historical: 2437.811 YBP; contemporary: 2355.049 YBP; combined: 3155.005 YBP). Estimates for the

three contemporary regional clusters also supported a drastic population decline (r_{south} = 0.0008 – 0.0014; $r_{central}$ = 0.0003 – 0.0004; r_{north} = 0.00023 – 0.00024) with the time since decline ranging from 1807.174 – 3026.913 YBP (Table 3-4). The N_C value for the contemporary data (N_C = 305.492) was three times larger than a century ago, as the N_C recovered from the historical data is three times lower (N_C = 104.713). This suggests that although the Cape Parrot population experienced a drastic population decline circa 2600 YBP, contemporary populations have increased during the last century.

Table 3-3. Bottleneck results (*p-values*) obtained from a signed rank Wilcoxon test for heterozygous excess (one tail) using two mutation models, the Mode-shift test and the *M*-ratio method for bottleneck detection. The two-phase mutation (TPM) and single-step mutation (SSM) models were used for the heterozygous excess tests. The Bonferroni correction was applied to all *p*-values (*p*-value = 0.003).

Test	Parameter		Historical (n = 29)	South (n = 60)	Central (n = 20)	North (n = 5)	Contemporary (n = 85)	All samples (n = 114)
He excess	TPM (90% SMM)		0.391	0.003	0.004	**0.0001**	0.334	0.57
	TPM (95% SMM)		0.53	0.003	0.005	**0.0001**	0.353	0.812
	SMM		0.666	0.59	0.334	0.008	0.666	0.884
Mode shift			NO	NO	NO	YES	NO	NO
M-Ratio	p_s = 0.88	Δg = 2.8						
	θ = 0.2; *Ne* = 100	Mc	0.532	0.532	0.531	0.528	0.534	0.533
	θ = 4; *Ne* = 2000	Mc	0.542	0.586	0.517	0.415	0.605	0.618
	θ = 10; *Ne* = 5000	Mc	0.543	0.517	0.507	0.281	0.632	0.652
		M-Ratio	0.78	0.826	0.82	0.645	0.817	0.772
	p_s = 0.9	Δg = 3.5						
	θ = 0.2; *Ne* = 100	Mc	0.456	0.457	0.457	0.455	0.458	0.456
	θ = 4; *Ne* = 2000	Mc	0.453	0.493	0.429	0.34	0.511	0.527
	θ = 10; *Ne* = 5000	Mc	0.455	0.606	0.42	0.349	0.544	0.563
		M-Ratio	0.78	0.826	0.82	0.645	0.817	0.772

Table 3-4. Estimates of effective population size changes over time as calculated in MSVAR using the exponential model. The 95% highest posterior density for each estimate is provided in parentheses.

	Mean ancestral effective population size (N_H):	**Mean current effective population size (N_C):**	**Time (in years) since decline:**	**r = N_C/N_H**
Historical (n = 29)	236047.823 (67142.885 - 824138.115)	104.713 (25.351 - 436.516)	2437.811 (645.654 - 8933.055)	0.0004 (0.0004 - 0.0005)
Contemporary (n = 85)	269153.48 (83176.377 - 847227.414)	305.492 (69.984 -1462.177)	2355.049 (568.853 - 8810.489)	0.0011 (0.0008 - 0.0017)
South (n = 60)	272270.131 (86696.188 - 843334.758)	275.423 (69.823 - 1150.800)	3026.913 (874.984 - 9931.160)	0.001 (0.0008 - 0.0014)
Central (n = 20)	198609.492 (56234.133 - 685488.226)	69.502 (17.458 - 286.418)	1807.174 (454.988 - 7311.391)	0.0003 (0.0003 - 0.0004)
North (n = 5)	169433.78 (44771.330 -656145.266)	40.272 (10.447 - 154.882)	1815.516 (473.151 - 7379.042)	0.0002 (0.0002 - 0.0002)
Combined (n = 114)	278612.117 (85506.671 - 897428.795)	495.45 (127.938 - 1896.706	3155.005 (824.138 - 12941.958)	0.0018 (0.0015 - 0.0021)

3.4.4 Gene flow

The 2MOD estimates indicated that the migration-drift model best fits both the historical and contemporary data (historical $P = 0.558$, contemporary $P = 0.502$; Supplementary Table 3-6). There were regional differences in the contribution of migration and genetic drift among the contemporary Cape Parrot populations. Migration had a larger effect on the contemporary South ($F = 0.011$, $M = 44.183$) and Central ($F = 0.028$, $M = 17.44$) regions; with drift playing a bigger role in the North ($F = 0.214$, $M = 1.836$). In contrast, migration played an important role for all three regions in the historical data set (South $F = 0.037$, $M = 13.069$; Central $F = 0.045$, $M = 10.673$; North $F = 0.035$, $M = 13.721$), indicating that when the historical samples were collected (1870 -1946) there was still high levels of gene flow between the now isolated Limpopo population and the rest of the distribution.

Migration rates estimated by BayesAss were largely unidirectional. Migration analysis of the historical data indicates that there was gene flow between populations in South and Central geographical regions ($m = 0.12$, % confidence interval (CI) = 0.012 - 0.282) and between the Central to North regions ($m = 0.119$, 95% CI = 0.002 - 0.258). This was unsurprising given the STRUCTURE results, but what is interesting is that BayesAss analysis suggests that gene flow occurred in strictly South to North direction with very limited

bidirectionality. A similar pattern of gene flow was observed in the contemporary data. Analysis indicated elevated migration rates between individuals when the data were grouped by region (South-Central, m = 0.278, 95% CI = 0.182 - 0.327; Central-North, m = 0.199, 95% CI = 0.001 - 0.286) or by sampling locality (Alice/King William's Town-Lusikisiki, m = 0.222, 95% CI = 0.097 - 0.311; Alice/King William's Town-Creighton, m = 0.267, 95% CI = 0.176 - 0.325; Creighton-Tzaneen, m = 0.181, 95% CI = 0.001 - 0.307) (Figure 3-4). This analysis suggests that the South population may act as source for northern Cape Parrot populations as the highest migration rates were observed from South to Central regions (m = 0.278, 95% CI = 0.182 - 0.327). In contrast to STRUCTURE results which indicated that the contemporary Limpopo population is isolated from the rest of Cape Parrot distribution, the migration analysis indicates some gene flow between the contemporary and Central and North regions (m = 0.199, 95% CI = 0.001 - 0.286). This value may be due to the retention of ancestral alleles in individuals belonging to the North population, as these populations are separated by large areas of unsuitable habitat for Cape Parrot and it is unlikely that contemporary migration occurs.

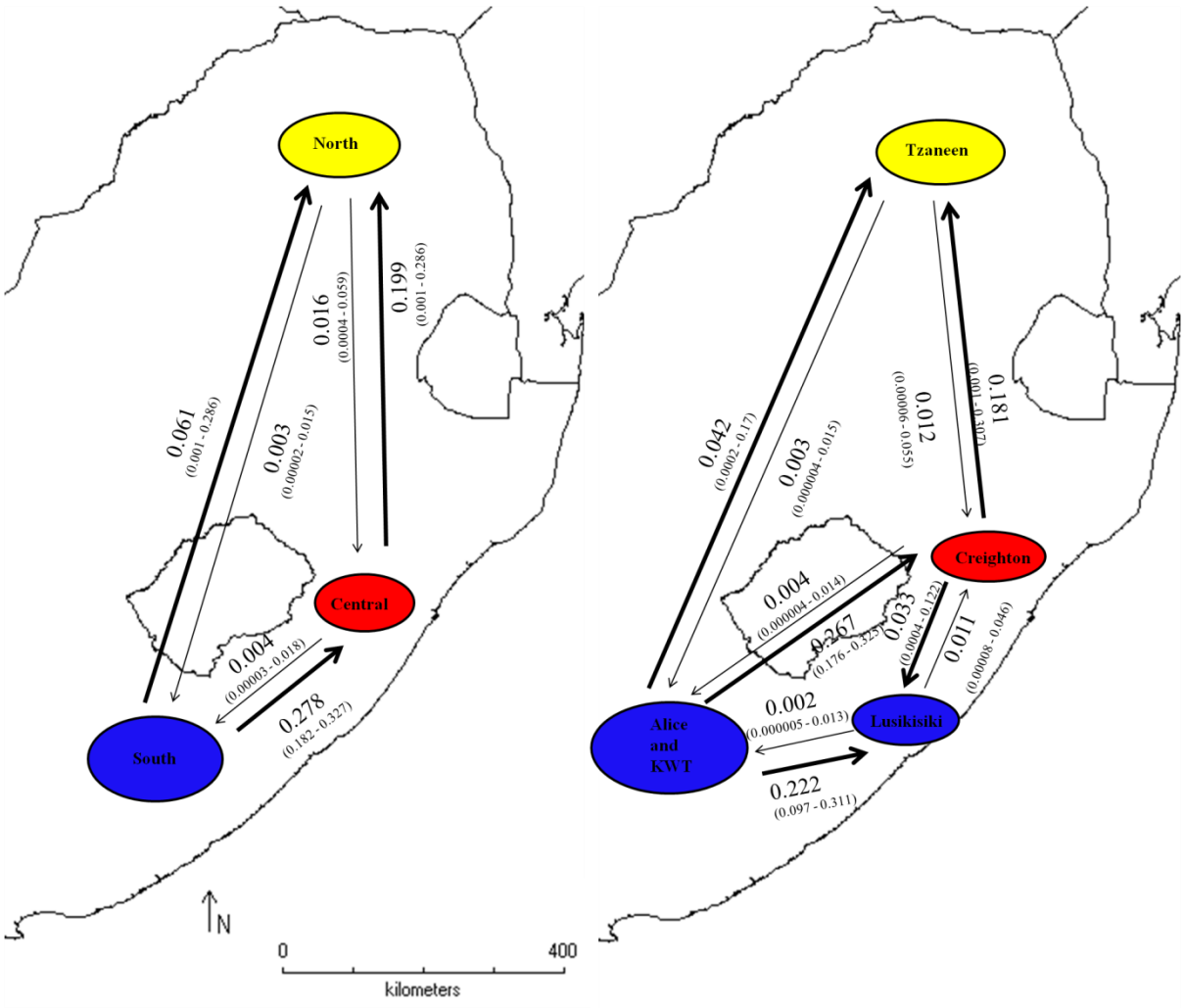


Figure 3-4. Migration rates (*m*), estimated using BayesAss from the contemporary data. Samples were grouped according to (a) the three sampling regions and b) individual sampling locality sites. Higher migration rates are indicated by thicker lines. The 95% confidence interval is provided in parentheses.

3.5 Discussion

Many studies have used DNA from contemporary populations to infer past demographic changes in populations of South African species (Eick et al., 2005, Herron et al., 2005, Tolley et al., 2006). This indirect approach, although useful, may not accurately reconstruct changes in population structure over time. For example, low levels of genetic variation in contemporary populations could be the result of recent anthropogenic-driven changes, or could represent an ancestral state (Wandeler et al., 2007). Advances in molecular biology now allows historical and even ancient genotypes to be added to phylogeographic studies (Bouzat et al., 1998, Dussex et al., 2015, Edwards et al., 2012, Groombridge et al., 2000, Welch et al., 2012) allowing for temporal changes in allele frequencies and effective population size to be directly measured. This is the first study in South Africa to use archival genotypes to directly measure changes in the phylogeographic pattern of a range-restricted species over time. Using microsatellite data, this study recovered high levels of spatial genetic structuring within this southern African endemic parrot species. The results showed clear differences between the historical and contemporary genetic structure of Cape Parrot populations. Analysis of microsatellite data provides evidence in support of at least three independently evolving lineages within the species. The South group is distributed within the Eastern Cape Province mistbelt forests, the Central group is found within the KwaZulu-Natal mistbelt forests and the relictual Northern group is found in the Limpopo Province mistbelt forests in the vicinity of Tzaneen and the Magoebaskloof area.

3.5.1 Historical vs contemporary population structure

Bayesian structure analysis of historical and contemporary data recovered strikingly different patterns of phylogeographic structure (Figure 3-2). In particular, the historical and contemporary data do not agree on the genetic affinity of the Tzaneen lineage. This lineage represents the most northern distribution boundary of this species. In the historical data there is no evidence to suggest the genetic distinctiveness of the Tzaneen lineage with individuals genotyped from this region clustering together with populations in Mpumalanga, and KZN. In contrast, genotypes from Tzaneen collected after 1950 form a separate well-supported cluster (Q = 0.99) with analyses providing evidence that this population is genetically isolated from all other South African parrots. The temporal sampling used in this study suggests that the genetic isolation of the Tzaneen lineage has occurred rapidly (within the last century), which

highlights anthropogenic influence as the main driver of genetic differentiation of this population. The distribution of historical records provide additional evidence of severe habitat loss during the last century with six historical specimens included in the study collected in areas of South Africa (Bela Bela, Limpopo and Ermelo, Mpumalanga) were neither Cape Parrots or *Podocarpus*/*Afrocarpus* species are currently found (Figure 3-1; Supplementary Table 3-1).

The role gene flow and genetic drift have played on the demographic history of these parrots have also changed over time. It is clear from the historical data that migration played a larger role than drift alone in all three regional groups of Cape Parrots, probably because forest fragmentation was likely less extensive a century ago. This higher level of connectivity observed in the historical data set is further supported by the STRUCTURE results (Figure 3-2). The contemporary data set on the other hand showed larger effects from genetic drift within the northern region. Current vegetation records clearly indicate a large area, unsuitable to Cape Parrots, between the northern and southern mistbelt forests (Mucina and Rutherford, 2006), isolating the northern Cape Parrot population from the other populations. Modern-day gene flow between populations from these regions is unlikely. The moderate levels of gene flow that was detected between the northern and central regions can be attributed to the retention of ancestral alleles within the northern Cape Parrot population. It should be noted that higher migration rates were observed from the southern populations into the central region. There is clearly a higher level of connectivity between the southern and central populations, leading to higher migration rates between these regions. Conservation of these mistbelt forests is therefore crucial to allow natural gene flow to occur between the southern and central populations.

3.5.2 Recent population decline

There is no doubt that the extensive logging of indigenous forests during the colonial period (1850 to 1940; McCracken, 1986, Rycroft, 1942) has had a detrimental effect on populations of Cape Parrots. The genetic signatures of population decline are often subtle and difficult to detect in very recent bottleneck events (Cornuet and Luikart, 1996, Luikart et al., 1998). In the current study a number of private alleles were identified within the historical data set, suggesting the possible recent loss of allelic diversity ('ghost alleles') in the contemporary Cape Parrot population. The occurrence of a recent bottleneck was supported by the detection of a bottleneck event in the southern and northern groups using the heterozygote excess method in BayesAss, although none was detected with the *M*-ratio analysis (Table 3-3). The detection of a more recent population bottleneck can be hampered if the genetic diversity at the time of

the bottleneck is already low due to the occurrence of a more ancient population decline (Cornuet and Luikart, 1996) as is the case here (see below) as *M*-ratio estimate change slowly immediately after a bottleneck, whereas heterozygosity excess tests are more sensitive (Peery et al., 2012, Williamson-Natesan, 2005).

3.5.3 Ancient population decline

Although human-mediated habitat loss has certainly had a profound effect on indigenous Cape Parrot populations, the role of long-term ancient climatic change cannot be discounted. The molecular data provides strong support for a drastic population decline dated at ~1815 yrs BP (North population), ~1807 yrs BP (Central population) and up to ~3026 yrs BP (South population). These dates predate European settlement of South Africa which occurred during the early 17th century. Southern African climate changes are known to have underwent significant regional fluctuations during the Holocene (Chase et al., 2009, Lee-Thorp et al., 2001, Metwally et al., 2014, Scott and Vogel, 2000). The rapid decrease in *Podocarpus* containing forests in eastern South Africa noted by Neumann et al. (2010) from ca. 3600 cal yrs BP to ca. 3500 cal yrs BP, falls within the 95% HPD of the time since population decline detected for the Central Cape Parrot lineage in the current study (Central; Time since decline = 1807.174 yrs BP, 95% HPD: 454.988 - 7311.391). A rapid climate change (RCC) event was also noted to cause extensive arid conditions in Eastern Africa from ca. 3500 yrs BP to ca. 2500 yrs BP (Mayewski et al., 2004). It was suggested that this RCC event could be linked to the dry period experienced along the South African eastern coast as noted by Neumann et al. (2010). Mazus (2000) reported a similar decrease in *Podocarpus* containing forests ca. 3100 yrs BP in KwaZulu-Natal. This decline in *Podocarpus* densities was a regional phenomenon across KwaZulu-Natal from ca. 3100 yrs BP, which is strongly linked to the dry period during the late Holocene (Neumann et al., 2010). This decline in *Podocarpus* densities occurring within the same time period as the decline estimated for the Central Cape Parrot lineage. This decrease in suitable habitat, could be seen as the cause to the Cape Parrot decline.

A shift from C3 to C4 plants were observed in the Pondoland, Eastern Cape area during the middle Holocene (Fisher et al., 2013). This points to drier conditions in this region during the middle Holocene (~3000 BP), since C4 plants are generally more drought resistant than C3 plants (Tilman and Downing, 1994). It can therefore be assumed that a reduction of *Podocarpus* forests (C3 plants) might also have occurred in this region during the estimated

Cape Parrot population decline observed for the South lineage (Time since decline = 3026.913 yrs BP, 95% HPD: 874.984 – 9931.160).

Cool, dry conditions were recorded at Wonderkrater, Limpopo and Tswaing Crater, Gauteng (Metwally et al., 2014, Scott, 1999, Scott et al., 2003) at around 2000 cal yrs BP. It was also noted that *Podocarpus* forests in the Zoutpansberg region, Limpopo declined at ca. 1500 yr BP (Scott, 1987), coinciding with the arrival of early Iron Age people in Limpopo at around 1700 yr BP (Evers, 1975, Klapwijk, 1974, Maggs, 1984). These early settlers might have cleared forest for settlements and pastures (Bruton et al., 1980, Neumann et al., 2008). Drier conditions and early anthropogenic factors could have led to declines in *Podocarpus* containing forests, and thus adversely influencing local Cape Parrot populations. This time period overlaps with the 95% HPD for the time since the Cape Parrot population decline estimate for the northern region (Time since decline = 1815.516 yrs BP, 95% HPD: 473.151 - 7379.042).

These decreases in *Podocarpus* forests would have deprived ancient Cape Parrot populations from food and nesting sources. This, in turn, would have placed these birds under tremendous pressure to survive, which might have led to the population decline observed in Cape Parrots during the mid-Holocene. Both the ancient climate driven habitat fragmentation and the more recent anthropogenic habitat contraction have most definitely influenced the current population structure observed for the Cape Parrot in South Africa. In addition to the decrease in *Podocarpus sp*., Neumann et al. (2010) also observed declines in other forest plant genera namely *Isoglossa, Minusops, Manilkara* and *Acacia* ca. 3600 cal yrs BP to ca. 700 cal yrs BP.

3.5.4 Population recovery

There were no significant differences in uHE between the historical and contemporary data sets, but a significant difference was observed in allelic richness levels. Allelic richness tend to be affected by population fluctuations at a faster rate than levels of heterozygosity (Kalinowski, 2004, Maruyama and Fuerst, 1985). The lower allelic richness and higher inbreeding values observed for the historical data set, suggest that the population had started to recover from the ancient climate-change driven population contractions. One mechanism responsible for an increase in allelic richness following a bottleneck is gene flow (Greenbaum et al., 2014, Lacy, 1987) as new migrants can introduce new alleles to the recipient population. Recovery of the Cape Parrot populations can also be observed in the increase in *Ne* over the

last century. The classic starburst pattern observed in the statistical parsimony genotype network is also indicative of a population which has recently undergone a rapid range/population expansion. The end of extensive commercial logging practices in South Africa during the early 1900's might have allowed the Cape Parrots to adjust to their altered habitat, allowing the population to recover. An increase in genetic variation, over a relatively short time (<30 years), has been recorded in a great reed warbler (*Acrocephalus arundinaceus*) population founded by only a few individuals in 1978 (Hansson et al., 2000). It has been suggested that the way bird species will react to large-scale population declines is largely associated with resource specialization and brain size (Shultz et al., 2005). Parrots are known to be highly intelligent animals, demonstrating ape-like intelligence (Emery, 2006). The Cape Parrot's ability to utilize food sources other than *Podocarpus*/*Afrocarpus* kernels (Wirminghaus et al., 2002) and their high level of intelligence can certainly assist these birds to recover from a population decline following habitat loss.

The ability of avian species to recover from catastrophic population declines has been well recorded. The Echo parakeet, for instance, was able to recover from only 20 birds following an intense management program (Duffy, 1993, Lovegrove et al., 1995, Raisin et al., 2012). Raisin et al. (2012) highlighted the effectiveness of intense conservation management on the genetic structure of a recovering population and reducing the risk of inbreeding effects. The Mauritius kestrel is another well-known success story. These birds recovered from only four wild birds, including one breeding pair (Jones, 1987, Jones et al., 1995), to an estimated 200 birds in 1997 (Safford and Jones, 1997). This species underwent a reduction in fitness, but an improvement has been observed since the bottleneck (Groombridge et al., 2000, Jones et al., 1995).

3.5.5 Conservation implications

The mid-Holocene population contraction led to a severe decrease in the effective population size of the Cape Parrot, which in turn led to decreased levels of allelic diversity. This reduced population suffered an additional decrease in population size through anthropogenic habitat destruction. The genetic data does suggest a tentative signs of a more recent bottleneck, but it was observed that the Cape Parrot populations are either in the process of recovering or have already recovered from the population decline. It is, however, important to monitor the Cape Parrot populations in spite of the observed signs of population recovery. The more recent population contraction event led to the isolation of the northern Cape Parrot population from

the remaining populations. The northern Cape Parrot population is clearly a distinct isolated population, and it is therefore important to monitor the genetic health of this population to prevent possible inbreeding.

The two populations at the southern end of the distribution (Alice and King William's Town, Eastern Cape) clearly play an important role as source populations for migrats to the intermediate Lusikisiki (Eastern Cape) and the central Creighton (KwaZulu-Natal) populations. The highest levels of genetic diversity were also observed within the southern populations, highlighting the significance of these populations for future conservation management planning. These populations can be used as source populations for future translocations. Birds from the central Cape Parrot populations can for instance be used to supplement the northern Cape Parrot gene pool, if needed, to create an artificial migration event.

Further habitat contraction would be highly detrimental to contemporary Cape Parrot populations. It is therefore crucial to monitor climatic trends in South Africa and to preserve contemporary mistbelt forest patches for these, and other, taxa. The Cape Parrot populations should be regularly monitored to ensure the survival of these birds.

3.6 Acknowledgements

I would like to thank all the researcher and organisations who assisted by providing me with specimens for use in this study; the curators of the East London Museum (East London, South Africa), Ditsong National Museum of Natural History (Pretoria, South Africa), Durban Natural Sciences Museum (Durban, South Africa), the British Natural History Museum at Tring (United Kingdom), the Loro Parque Foundation (Canary Islands, Spain); Dr Craig Symes, University of the Witwatersrand, and Dr Steve Boyes, University of Cape Town, for supplying additional field samples.

3.7 References

ARIF, I. & KHAN, H. 2009. Molecular markers for biodiversity analysis of wildlife animals: a brief review. *Animal Biodiversity and Conservation,* 32**,** 9-17.

BEAUMONT, M. A. 1999. Detecting expansion and decline using microsatellites. *Genetics,* 153**,** 2013-2029.

BICKHAM, J. W., SANDHU, S., HEBERT, P. D. N., CHIKHI, L. & ATHWAL, R. 2000. Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. *Mutation Research/Reviews in Mutation Research,* 463**,** 33-51.

BIRDLIFE INTERNATIONAL. 2013a. *Amazona vittata* [Online]. BirdLife International. Available: http://www.iucnredlist.org [Accessed 31 March 2015].

BIRDLIFE INTERNATIONAL. 2013b. *Psittacula eques* [Online]. BirdLife International. Available: http://www.iucnredlist.org [Accessed 31 March 2015].

BOUZAT, J. L. 2010. Conservation genetics of population bottlenecks: the role of chance, selection, and history. *Conservation Genetics,* 11**,** 463-478.

BOUZAT, J. L., PAIGE, K. N. & LEWIN, H. A. 1998. The ghost of genetic diversity past: historical DNA analysis of the greater prairie chicken. *The American Naturalist,* 152**,** 1-6.

BRIGHTSMITH, D. J. 2005. Parrot nesting in southeastern Peru: seasonal patterns and keystone trees. *Wilson Bulletin,* 117**,** 296-305.

BROOK, B. W., SODHI, N. S. & BRADSHAW, C. J. 2008. Synergies among extinction drivers under global change. *Trends in Ecology and Evolution,* 23**,** 453-460.

BRUGGEMAN, D. J., WIEGAND, T. & FERNANDEZ, N. 2010. The relative effects of habitat loss and fragmentation on population genetic variation in the red-cockaded woodpecker (*Picoides borealis*). *Molecular Ecology,* 19**,** 3679-3691.

BRUTON, M. N., SMITH, M. & TAYLOR, R. H. 1980. A brief history of human evolvement in Maputaland. *In:* BRUTON, M. N. & COOPER, K. H. (eds.) *Studies on the Ecology of Maputaland.* Durban: Rhodes University and the Natal Branch of the Wildlife Society of Southern Africa.

CALLENS, T., GALBUSERA, P., MATTHYSEN, E., DURAND, E. Y., GITHIRU, M., HUYGHE, J. R. & LENS, L. 2011. Genetic signature of population fragmentation varies with mobility in seven bird species of a fragmented Kenyan cloud forest. *Molecular Ecology,* 20**,** 1829-1844.

CAMPOS, P. F., WILLERSLEV, E., SHER, S., ORLANDO, L., AXELSSON, E., AARIS-SØRENSEN, K., GREENWOOD, A. D., KAHLKE, R.-D., KOSINTSEV, P., TIKHONOV, A., KRAKHMALNAYA, T., KUZNETSOVA, T., LEMEY, P., MACPHEE, R., NORRIS, C. A., SHEPHERD, K., ZAZULA, G. D., SHAPIRO, B. & GILBERT, M. T. P. 2010. Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. *Proceedings of the National Academy of Sciences,* 107**,** 5675-5680.

CHAPUIS, M.-P. & ESTOUP, A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution,* 24**,** 621-631.

CHASE, B. M., MEADOWS, M. E., SCOTT, L., THOMAS, D. S., MARAIS, E., SEALY, J. & REIMER, P. J. 2009. A record of rapid Holocene climate change preserved in hyrax middens from southwestern Africa. *Geology,* 37**,** 703-706.

CIOFI, C., BEAUMONT, M. A., SWINGLAND, I. R. & BRUFORD, M. W. 1999. enetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. *Proceedings of the Royal Society of London, Series B: Biological Sciences,* 266**,** 2269 – 2274.

COETZER, W. G., DOWNS, C. T., PERRIN, M. R. & WILLOWS-MUNRO, S. 2015. Molecular Systematics of the Cape Parrot (*Poicephalus robustus*): Implications for Taxonomy and Conservation. *PLoS ONE,* 10**,** e0133376.

COLLAR, N. 2000. Globally threatened parrots: criteria, characteristics and cures. *International Zoo Yearbook,* 37**,** 21-35.

CORNUET, J. M. & LUIKART, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics,* 144**,** 2001-2014.

COUVET, D. 2002. Deleterious effects of restricted gene flow in fragmented populations. *Conservation Biology,* 16**,** 369-376.

DAKIN, E. E. & AVISE, J. C. 2004. Microsatellite null alleles in parentage analysis. *Heredity,* 93**,** 504-509.

DASZAK, P., CUNNINGHAM, A. A. & HYATT, A. D. 2000. Emerging Infectious Diseases of Wildlife-- Threats to Biodiversity and Human Health. *Science,* 287**,** 443-449.

DEMPSTER, A. P., LAIRD, N. M. & RUBIN, D. B. 1977. Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society. Series B (Methodological)***,** 1-38.

DESALLE, R. & AMATO, G. 2004. The expansion of conservation genetics. *Nature Reviews: Genetics,* 5**,** 702-712.

DI RIENZO, A., PETERSON, A., GARZA, J., VALDES, A., SLATKIN, M. & FREIMER, N. 1994. Mutational processes of simple-sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences,* 91**,** 3166-3170.

DOS ANJOS, L., COLLINS, C. D., HOLT, R. D., VOLPATO, G. H., MENDONÇA, L. B., LOPES, E. V., BOÇON, R., BISHEIMER, M. V., SERAFINI, P. P. & CARVALHO, J. 2011. Bird species abundance–occupancy patterns and sensitivity to forest fragmentation: Implications for conservation in the Brazilian Atlantic forest. *Biological Conservation,* 144**,** 2213-2222.

DOWNS, C. & SYMES, C. 2004. Snag dynamics and forest structure in Afromontane forests in KwaZulu-Natal, South Africa: implications for the conservation of cavity-nesting avifauna. *South African Journal of Botany,* 70**,** 265-276.

DOWNS, C. T. 2005a. Abundance of the endangered Cape parrot, *Poicephalus robustus*, in South Africa: implications for its survival. *African Zoology,* 40**,** 15-24.

DOWNS, C. T. 2005b. Artificial nest boxes and wild Cape Parrots *Poicephalus robustus*: persistence pays off. *Ostrich,* 76**,** 222-224.

DOWNS, C. T., PFEIFFER, M. & HART, L. A. 2014. Fifteen years of annual Cape Parrot *Poicephalus robustus* censuses: current population trends and conservation contributions. *Ostrich,* 85**,** 273-280.

DUFFY, K. 1993. Echo parakeet project-Progress report August 1992-April 1993. Vacoas: Mauritian Wildlife Foundation.

DUSSEX, N., RAWLENCE, N. J. & ROBERTSON, B. C. 2015. Ancient and Contemporary DNA Reveal a Pre-Human Decline but No Population Bottleneck Associated with Recent Human Persecution in the Kea (*Nestor notabilis*). *PLoS ONE,* 10**,** e0118522.

EARL, D. 2009. *STRUCTURE Harvester, Version 0.3* [Online]. Available: http://users.soe.ucsc.edu/~dearl/software/struct_harvest/ [Accessed 01 May 2013.

EDWARDS, C. J., SOULSBURY, C. D., STATHAM, M. J., HO, S. Y., WALL, D., DOLF, G., IOSSA, G., BAKER, P. J., HARRIS, S. & SACKS, B. N. 2012. Temporal genetic variation of the red fox, *Vulpes vulpes*, across western Europe and the British Isles. *Quaternary Science Reviews,* 57**,** 95-104.

EELEY, H. A., LAWES, M. J. & PIPER, S. E. 1999. The influence of climate change on the distribution of indigenous forest in KwaZulu-Natal, South Africa. *Journal of Biogeography,* 26**,** 595-617.

EICK, G. N., JACOBS, D. S. & MATTHEE, C. A. 2005. A Nuclear DNA Phylogenetic Perspective on the Evolution of Echolocation and Historical Biogeography of Extant Bats (Chiroptera). *Molecular Biology and Evolution,* 22**,** 1869-1886.

EMERY, N. 2006. Cognitive ornighology: the evolution of avian intelligenec. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 361**,** 23-43.

EVANNO, G., REGNAUT, S. & GOUDET, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* 14**,** 2611-2620.

EVERS, T. M. 1975. Recent Iron Age research in the eastern Transvaal, South Africa. *S. Afr. Archaeol. Bull.,* 30**,** 71-83.

FISHER, E. C., ALBERT, R.-M., BOTHA, G., CAWTHRA, H. C., ESTEBAN, I., HARRIS, J., JACOBS, Z., JERARDINO, A., MAREAN, C. W., NEUMANN, F. H., PARGETER, J., POUPART, M. & VENTER, J. 2013. Archaeological reconnaissance for middle Stone Age sites along the Pondoland coast, South Africa. *PaleoAnthropology,* 1**,** 104-137.

GARZA, J. & WILLIAMSON, E. 2001. Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology,* 10**,** 305-318.

GELDENHUYS, C. J. 1989. Biogeography of the mixed evergreen forests of southern Africa. *Ecosystems Programmes Occasional Report no. 45.* Pretoria: Foundation for Research Development.

GOLDSTEIN, P. Z. & DESALLE, R. 2003. Calibrating phylogenetic species formation in a threatened insect using DNA from historical specimens. *Molecular Ecology,* 12**,** 1993-1998.

GOTTELLI, D., SILLERO-ZUBIRI, C., MARINO, J., FUNK, S. & WANG, J. 2013. Genetic structure and patterns of gene flow among populations of the endangered Ethiopian wolf. *Animal Conservation,* 16**,** 234-247.

GOUDET, J. 2001. *FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3)* [Online]. Available: http://www.unil.ch/zea/softwares/fstat.html.

GREENBAUM, G., TEMPLETON, A. R., ZARMI, Y. & BAR-DAVID, S. 2014. Allelic Richness following Population Founding Events–A Stochastic Modeling Framework Incorporating Gene Flow and Genetic Drift. *PLoS ONE,* 9**,** e115203.

GREENWOOD, A. 1996. The Echo responds - a partnership between conservation biology, aviculture and veterinary science. *PsittaScene.*

GROOMBRIDGE, J. J., JONES, C. G., BRUFORD, M. W. & NICHOLS, R. A. 2000. Conservation biology: 'Ghost' alleles of the Mauritius kestrel. *Nature,* 403**,** 616.

HANSSON, B., BENSCH, S., HASSELQUIST, D., LILLANDT, B. G., WENNERBERG, L. & VON SCHANTZ, T. 2000. Increase of genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNA fingerprinting. *Molecular Ecology,* 9**,** 1529-1538.

HART, L., GRIEVE, G. & DOWNS, C. 2013. Fruiting phenology and implications of fruit availability in the fragmented Ngele Forest Complex, KwaZulu-Natal, South Africa. *South African Journal of Botany,* 88**,** 296-305.

HASTINGS, A. 1993. Complex interactions between dispersal and dynamics - lessons from coupled logistic equations. *Ecology,* 74**,** 1362-1372.

HERRON, M. D., WATERMAN, J. M. & PARKINSON, C. L. 2005. Phylogeny and historical biogeography of African ground squirrels: the role of climate change in the evolution of Xerus. *Molecular Ecology,* 14**,** 2773-2788.

HOBAN, S. M., GAGGIOTTI, O. E., CONSORTIUM, C. & BERTORELLE, G. 2013. Sample Planning Optimization Tool for conservation and population Genetics (SPOTG): a software for choosing the appropriate number of markers and samples. *Methods in Ecology and Evolution,* 4**,** 299-303.

JONES, C. G. 1987. The larger land-birds of Mauritius. *In:* DIAMOND, A. W. (ed.) *Studies of Mascarene Island birds.* Cambridge, U.K.: Cambridge University Press.

JONES, C. G., HECK, W., LEWIS, R. E., MUNGROO, Y., SLADE, G. & CADE, T. 1995. The restoration of the Maritius Ksetrel *Falco punctatus* population. *Ibis***,** S173-S180.

JUNIPER, A. & YAMASHITA, C. 1991. The habitat and status of Spix's Macaw Cyanopsitta spixii. *Bird conservation international,* 1**,** 1-9.

KALINOWSKI, S. T. 2004. Counting alleles with rarefaction: Private alleles and hierarchical sampling designs. *Conservation Genetics,* 5**,** 539-543.

KALINOWSKI, S. T. & TAPER, M. L. 2006. Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. *Conservation Genetics,* 7**,** 991-995.

KALINOWSKI, S. T., TAPER, M. L. & MARSHALL, T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology,* 16**,** 1099-1106.

KING, N. L. 1941. The exploitation of the indigenous forests of South Africa. *Journal of the South African Forestry Association,* 6**,** 26-48.

KLAPWIJK, M. 1974. A prliminary report on pottery from the north-eastern Transvaal, South Africa. *The South African Archaeological Bulletin,* 29**,** 19-23.

KOTLÍK, P., MARKOVÁ, S., VOJTEK, L., STRATIL, A., ŠLECHTA, V., HYRŠL, P. & SEARLE, J. B. 2014. Adaptive phylogeography: functional divergence between haemoglobins derived from different glacial refugia in the bank vole. *Proceedings of the Royal Society of London B: Biological Sciences,* 281**,** 20140021.

LACY, R. C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology***,** 143-158.

LAWES, M. J., GRIFFITHS, M. E. & BOUDREAU, S. 2007. Colonial logging and recent subsistence harvesting affect the composition and physiognomy of a podocarp dominated Afrotemperate forest. *Forest Ecology and Management,* 247**,** 48-60.

LAWES, M. J., MACFARLANE, D. M. & EELEY, H. A. C. 2004. Forest landscape pattern in the KwaZulu–Natal midlands, South Africa: 50 years of change or stasis? *Austral Ecology,* 29**,** 613-623.

LEE-THORP, J. A., HOLMGREN, K., LAURITZEN, S. E., LINGE, H., MOBERG, A., PARTRIDGE, T. C., STEVENSON, C. & TYSON, P. 2001. Rapid climate shifts in the southern African interior throughout the mid to late Holocene. *Geophysical Research Letters,* 28**,** 4507-4510.

LEONARD, J. A., WAYNE, R. K. & COOPER, A. 2000. Population genetics of Ice Age brown bears. *Proceedings of the National Academy of Sciences,* 97**,** 1651-1654.

LORENZEN, E. D., NOGUÉS-BRAVO, D., OLRLANDO, L., WEINSTOCK, J., BINLADEN, J., MARSKE, K. A., UGAN, A., BORREGAARD, M. K., THOMAS, M., GILBERT, T. P., NIELSEN, R., HO, S. Y. W., GOEBEL, T., GRAF, K. E., BYERS, D., STENDERUP, J. T., RASMUSSEN, M., CAMPOS, P. F., LEONARD, J. A., KOEPFLI, K.-P., FROESE, D., ZAZULA, G., STAFFORD, T. W., AARIS-SØRENSEN, K., BATRA, P., HAYWOOD, A. M., SINGARAYER, J. S., VALDES, P. J., BOESKOROV, G., BURNS, J. A., DAVYDOV, S. P., HAILE, J., , D. L., KOSINTSEV, P., KUZNETSOVA, T., LAI, X., MARTIN, L. D., MCDONALD, H. G., MOL, D., MELDGAARD, M., MUNCH, K., STEPHAN, E., SABLIN, M., SOMMER, R. S., SIPKO, T., SCOTT, E., SUCHARD, M. A., TIKHONOV, A., WILLERSLEV, R., WAYNE, R. K., COOPER, A., HOFREITER, M., SHER, A., SHAPIRO, B., RAHBEK, C. & WILLERSLEV, E. 2011. Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature,* 479**,** 359-364.

LOVEGROVE, T. G., NIEUWLAND, A. B. & GREEN, S. 1995. Interim report on the echo parakeet conservation project, February 1995. Vacoas: Mauritian Wildlife Foundation.

LUIKART, G., ALLENDORF, F. W., CORNUET, J. M. & SHERWIN, W. B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Jounal of Heredity,* 89**,** 238-247.

LUIKART, G. & CORNUET, J. M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology,* 12**,** 228-237.

MAGGS, T. 1984. The Iron Age south of the Zambezi. *In:* KLEIN, R. G. (ed.) *Southern African Prehistory and Paleoenvironments.* Balkema, Rotterdam.

MAKOKHA, J. S., BAUER, A. M., MAYER, W. & MATTHEE, C. A. 2007. Nuclear and mtDNA-based phylogeny of southern African sand lizards, Pedioplanis (Sauria: Lacertidae). *Molecular Phylogenetics and Evolution,* 44**,** 622-633.

MANTEL, N. A. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.,* 27**,** 209-220.

MARUYAMA, T. & FUERST, P. A. 1985. Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics,* 111**,** 675-689.

MATEESCU, R., ZHANG, Z., TSAI, K., PHAVAPHUTANON, J., BURTON-WURSTER, N., LUST, G., QUAAS, R., MURPHY, K., ACLAND, G. M. & TODHUNTER, R. J. 2005. Analysis of allele fidelity, polymorphic information content, and density of microsatellites in a genome-wide screening for hip dysplasia in a crossbreed pedigree. *Journal of Heredity,* 96**,** 847-853.

MAYEWSKI, P. A., ROHLING, E. E., CURT STAGER, J., KARLÉN, W., MAASCH, K. A., DAVID MEEKER, L., MEYERSON, E. A., GASSE, F., VAN KREVELD, S., HOLMGREN, K., LEE-THORP, J., ROSQVIST, G., RACK, F., STAUBWASSER, M., SCHNEIDER, R. R. & STEIG, E. J. 2004. Holocene climate variability. *Quaternary Research,* 62**,** 243-255.

MAZUS, H. 2000. Clues on the history of Podocarpus forest in Maputaland, South Africa, during the Quaternary, based on pollen analysis. *Africa Geosciences Review,* 7**,** 75-82.

MCCRACKEN, D. P. 1986. The indigenous forests of Colonial Natal and Zululand. *Natalia,* 16**,** 19-38.

MCNEELY, J. A., MILLER, K. R., REID, W. V., MITTERMEIER, R. A. & WERNER, T. B. 1990. *Conserving the world's biological diversity*, International Union for conservation of nature and natural resources.

MENDE, M. B. & HUNDSDOERFER, A. K. 2013. Mitochondrial lineage sorting in action–historical biogeography of the Hyles euphorbiae complex (Sphingidae, Lepidoptera) in Italy. *BMC Evolutionary Biology,* 13**,** 83.

METWALLY, A. A., SCOTT, L., NEUMANN, F. H., BAMFORD, M. K. & OBERHÄNSLI, H. 2014. Holocene palynology and palaeoenvironments in the Savanna Biome at Tswaing Crater, central South Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 402**,** 125-135.

MILLER, C. S. & GOSLING, W. D. 2014. Quaternary forest associations in lowland tropical West Africa. *Quaternary Science Reviews,* 84**,** 7-25.

MINISTER OF ENVIRONMENTAL AFFAIRS AND TOURISM 2007. National Environmental Management: Biodiversity Act (ACT 10 of 2004): Publication of Lists of Critically Endangered, Endangered, Vulnerable and Protected Species. *In:* DEAT (ed.). Cape Town, South Africa.

MORITZ, C. 1994. Defining 'evolutionary significant units' for conservation. *Trends in Ecology and Evolution,* 9**,** 373-375.

MUCINA, L. & RUTHERFORD, M. C. 2006. *The vegetation of South Africa, Lesotho and Swaziland*, South African National Biodiversity Institute.

NEUMANN, F. H., SCOTT, L., BOUSMAN, C. & VAN AS, L. 2010. A Holocene sequence of vegetation change at Lake Eteza, coastal KwaZulu-Natal, South Africa. *Review of Palaeobotany and Palynology,* 162**,** 39-53.

NEUMANN, F. H., STAGER, J. C., SCOTT, L., VENTER, H. J. T. & WEYHENMEYER, C. 2008. Holocene vegetation adn climate records from Lake sibaya, KwaZulu-Natal (South Africa). *Review of Palaeobotany and Palynology,* 152**,** 113-128.

NEWTON, I. 1994. The role of nest sites in limiting the numbers of hole-nesting birds: A review. *Biological Conservation,* 70**,** 265-276.

PAIN, D., MARTINS, T., BOUSSEKEY, M., DIAZ, S., DOWNS, C., EKSTROM, J., GARNETT, S., GILARDI, J., MCNIVEN, D. & PRIMOT, P. 2006. Impact of protection on nest take and nesting success of parrots in Africa, Asia and Australasia. *Animal Conservation,* 9**,** 322-330.

PEAKALL, R. & SMOUSE, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* 28**,** 2537-2539.

PEERY, M. Z., KIRBY, R., REID, B. N., STOELTING, R., DOUCET-BËER, E., ROBINSON, S., VÁSQUEZ-CARRILLO, C., PAULI, J. N. & PALSBØLL, P. J. 2012. Reliability of genetic bottleneck tests for detecting recent population declines. *Molecular Ecology,* 21**,** 3403-3418.

PERGAMS, O. R., BARNES, W. M. & NYBERG, D. 2003. Mammalian microevolution: rapid change in mouse mitochondrial DNA. *Nature,* 423**,** 397-397.

PERRIN, M. R. Niche separation in African parrots. *In:* HAREBOTTLE, D. M., CRAIG, A. J. F. K., ANDERSON, M. D., RAKOTOMANANA, H. & MUCHAI, M., eds. Proceedings of the 12th Pan-African Ornithological Congress, 2008 September 07-12 2009 Rawsonville, South Africa. Cape Town: Animal Demography Unit, 29-37.

PILLAY, K., DAWSON, D. A., HORSBURGH, G. J., PERRIN, M. R., BURKE, T. & TAYLOR, T. D. 2010. Twenty-two polymorphic microsatellite loci aimed at detecting illegal trade in the Cape parrot, *Poicephalus robustus* (Psittacidae, AVES). *Molecular Ecology Resources,* 10**,** 142-149.

PIRY, S., LUIKART, G. & CORNUET, J. M. 1999. Bottleneck: a computer program for detecting recent reductions in the effective population size using allele frequency data. *The Journal of heredity,* 90**,** 502-503.

PRITCHARD, J. K., WEN, X. & FALUSH, D. 2010. *Structure, version 2.3* [Online]. Available: http://pritch.bsd.uchicago.edu/software [Accessed 20 November 2013.

PROST, S. & ANDERSONG, C. N. K. 2011. TempNet: a method to display statistical parsimony networks for heterochronous DNA sequence data. *Methods in Ecology and Evolution,* 2**,** 663 - 667.

RADESPIEL, U. & BRUFORD, M. 2014. Fragmentation genetics of rainforest animals: insights from recent studies. *Conservation Genetics,* 15**,** 245-260.

RAISIN, C., FRANTZ, A. C., KUNDU, S., GREENWOOD, A. G., JONES, C. G., ZUEL, N. & GROOMBRIDGE, J. J. 2012. Genetic consequences of intensive conservation management for the Mauritius parakeet. *Conservation Genetics,* 13**,** 707-715.

RAMBAUT, A. & DRUMMOND, A. J. 2007. *Molecular evolution, phylogenetics and epidemiology, Tracer v.1.5.* [Online]. Available: http://tree.bio.ed.ac.uk/software/tracer/ [Accessed 12 December 2014.

RICE, W. 1986. Analyzing tables of statistical tests. *Evolution,* 1**,** 223-225.

ROUSSET, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources,* 8**,** 103-106.

RYCROFT, H. B. 1942. *The plant ecology of the Karkloof forest, Natal.* MSc., University of Natal.

SAFFORD, R. J. & JONES, C. G. 1997. Did organochlorine pesticide use cause declines in Mauritian forest birds? *Biodiversity and Conservation,* 6**,** 1445-1451.

SCOTT, L. 1987. Late Quaternary forest history in Venda, southern Africa. *Review of Palaeobotany and Palynology,* 53**,** 1-10.

SCOTT, L. 1999. Vegetation history and climate in the savanna biome South Africa since 190,000 ka: a comparison of pollen data from the Tswaing crater (The Pretoria

Saltpan) and Wonderkrater. *Quat. Int.,* 57**,** 215-223.

SCOTT, L., ANDERSON, H., ANDERSON, J., COWLING, R., RICHARDSON, D. & PIERCE, S. 1997. Vegetation history. *Vegetation of southern Africa***,** 62-84.

SCOTT, L., HOLMGREN, K., TALMA, A. S., WOODBORNE, S. & VOGEL, J. 2003. Age interpretation of the Wonderkrater spring sediments and vegetation change in the Savanna Biome, Limpopo Province. *South African Journal of Science,* 99**,** 484-488.

SCOTT, L. & VOGEL, J. 2000. Evidence for environmental conditions during the last 20 000 years in Southern Africa from ^{13}C in fossil hyrax dung. *Global and Planetary Change,* 26**,** 207-215.

SHAFFER, M. L. 1981. Minimum population sizes for species conservation. *Bioscience,* 31**,** 131-134.

SHULTZ, S., BRADBURY, R. B., EVANS, K. L., GREYGORY, R. D. & BLACKBURN, T. M. 2005. Brain size and resource specialization predict long-term population trends in British birds. *Proceedings of the Royal Society B: Biological Sciences,* 272**,** 2305-2311.

SNYDER, N. F. R., WILEY, J. W. & KEPLER, C. B. 1987. *The parrots of Luquillo: natural history and conservation of the Puerto Rican parrot.,* Los Angeles, California, USA, Western Foundation of Vertebrate Zoology.

SPIELMAN, D., BROOK, B. W. & FRANKHAM, R. 2004. Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences,* 101**,** 15261-15264.

STORZ, J. & BEAUMONT, M. 2002. Testing for genetic evidence of population expansion and contraction: An empirical analysis of microsatellite DNA variation using a hierarchical Bayesian model. *Evolution,* 56**,** 156-166.

SWART, B. L., TOLLEY, K. A. & MATTHEE, C. A. 2009. Climate change drives speciation in the southern rock agama (Agama atra) in the Cape Floristic Region, South Africa. *Journal of Biogeography,* 36**,** 78-87.

SWINNERTON, K. 1998. *Echo parakeet (Psittacula eques) management report 1998,* Port Louis, Mauritius Wildlife Foundation.

SYMES, C., BROWN, M., WARBURTON, L., PERRIN, M. & DOWNS, C. 2004. Observations of Cape Parrot, *Poicephalus robustus*, nesting in the wild. *Ostrich,* 75**,** 106-109.

SYMES, C. T. 2010. Growing up with Cape Parrots. *Papousči.* PIPress.

TABERLET, P., FUMAGALLI, L., WUST-SAUCY, A.-G. & COSSON, J.-F. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology,* 7**,** 453-464.

TAYLOR, M. 2014. Eskom Red Data Book of Birds of South Africa, Lesotho and Swaziland. Birdlife South Africa, Johannesburg.

TEACHER, A. G., THOMAS, J. A. & BARNES, I. 2011. Modern and ancient red fox (Vulpes vulpes) in Europe show an unusual lack of geographical and temporal structuring, and differing responses within the carnivores to historical climatic change. *BMC Evolutionary Biology,* 11**,** 214.

THOMAS, W. K., PÄÄBO, S., VILLABLANCA, F. X. & WILSON, A. C. 1990. Spatial and temporal continuity of kangaroo rat populations shown by sequencing mitochondrial DNA from museum specimens. *Journal of Molecular Evolution,* 31**,** 101-112.

TILMAN, D. & DOWNING, J. A. 1994. Biodiversity and stability in grasslands. *Nature,* 367**,** 363-365.

TOLLEY, K. A., BURGER, M., TURNER, A. A. & MATTHEE, C. A. 2006. Biogeographic patterns and phylogeography of dwarf chameleons (*Bradypodion*) in an African biodiversity hotspot. *Molecular Ecology,* 15**,** 781-793.

TOLLEY, K. A., CHASE, B. M. & FOREST, F. 2008. Speciation and radiations track climate transitions since the Miocene Climatic Optimum: a case study of southern African chameleons. *Journal of Biogeography,* 35**,** 1402-1414.

TOLLEY, K. A., MAKOKHA, J. S., HOUNIET, D. T., SWART, B. L. & MATTHEE, C. A. 2009. The potential for predicted climate shifts to impact genetic landscapes of lizards in the South African Cape Floristic Region. *Molecular Phylogenetics and Evolution,* 51**,** 120-130.

TUCKER, J. M., SCHWARTZ, M. K., TRUEX, R. L., PILGRIM, K. L. & ALLENDORF, F. W. 2012. Historical and Contemporary DNA Indicate Fisher Decline and Isolation Occurred Prior to the European Settlement of California. *PLoS ONE,* 7**,** e52803.

WANDELER, P., HOECK, P. E. & KELLER, L. F. 2007. Back to the future: museum specimens in population genetics. *Trends in Ecology and Evolution,* 22**,** 634-42.

WELCH, A. J., WILEY, A. E., JAMES, H. F., OSTROM, P. H., STAFFORD, T. W. & FLEISCHER, R. C. 2012. Ancient DNA reveals genetic stability despite demographic decline: 3,000 years of population history in the endemic Hawaiian petrel. *Molecular Biology and Evolution,* 29**,** 3729-3740.

WILLIAMSON-NATESAN, E. G. 2005. Comparison of methods for detecting bottlenecks from microsatellite loci. *Conservation Genetics,* 6**,** 551-562.

WILSON, G. A. & RANNALA, B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics and Molecular Biology,* 163**,** 1177 - 1191.

WIRMINGHAUS, J. O. 1997. Cape Parrot *Poicephalus robustus*. *In:* HARRISON, J. A., ALAN, D. G., UNDERHILL, L. L., HERREMANS, M., TREE, A. J., PARKER, V. & BROWN, C. J. (eds.) *The Atlas of Southern African Birds.* Johannesburg: BirdLife South Africa.

WIRMINGHAUS, J. O., DOWNS, C. T., PERRIN, M. R. & SYMES, C. T. 2001a. Breeding biology of the Cape Parrot, *Poicephalus robustus*. *Ostrich,* 72**,** 159-164.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 1999. Conservation of the Cape parrot in southern Africa. *South African Journal of Wildlife Research,* 29**,** 118-129.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 2000. Abundance of the Cape Parrot in South Africa. *South African Journal of Wildlife Research,* 30**,** 43-52.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 2001b. Fruiting in two afromontane forests in KwaZulu-Natal, South Africa: the habitat type of the endangered Cape Parrot *Poicephalus robustus*. *South African Journal of Botany,* 67**,** 325-332.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 2002. Diet of the Cape Parrot, *Poicephalus robustus*, in Afromontane forests in KwaZulu-Natal, South Africa. *Ostrich,* 73**,** 20-25.

3.8 Supplementary information

Supplementary data 3-1. Results from the Sample planning tool (SPOTG) simulations for three bottleneck and one temporal simulations performed in Chapter 3. The bargraph below each set of results represents the power of each bottleneck scenario.

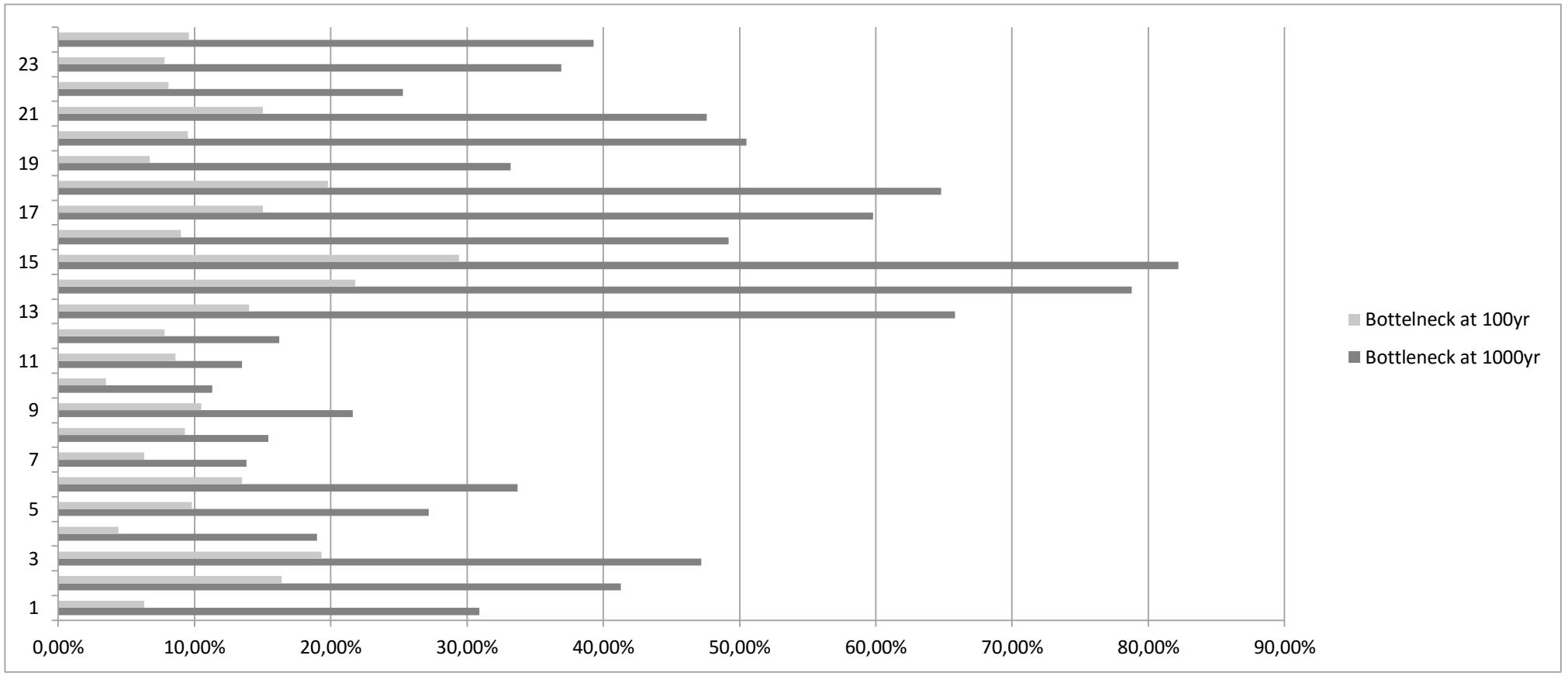
Bottleneck at 1000yr

Scenario	Starting populations size	Size of population at lowest point of bottleneck	Current population size	Constant population size for the null hypothesis, e.g., a hypothetical population similar to the one of interest, but assuming no bottleneck	Number of markers genotyped at	Number of sampled individuals	Power = probability of success of the study, distinguishing a bottlenecked population
1	1000	100	100	1000	16	29	30.90%
2	1000	100	100	1000	16	85	41.30%
3	1000	100	100	1000	16	114	47.20%
4	1000	200	200	1000	16	29	19.00%
5	1000	200	200	1000	16	85	27.20%
6	1000	200	200	1000	16	114	33.70%
7	1000	300	300	1000	16	29	13.80%
8	1000	300	300	1000	16	85	15.40%
9	1000	300	300	1000	16	114	21.60%
10	1000	400	400	1000	16	29	11.30%
11	1000	400	400	1000	16	85	13.50%
12	1000	400	400	1000	16	114	16.20%
13	2000	100	100	2000	16	29	65.80%
14	2000	100	100	2000	16	85	78.80%
15	2000	100	100	2000	16	114	82.20%
16	2000	200	200	2000	16	29	49.20%
17	2000	200	200	2000	16	85	59.80%
18	2000	200	200	2000	16	114	64.80%
19	2000	300	300	2000	16	29	33.20%
20	2000	300	300	2000	16	85	50.50%
21	2000	300	300	2000	16	114	47.60%
22	2000	400	400	2000	16	29	25.30%
23	2000	400	400	2000	16	85	36.90%
24	2000	400	400	2000	16	114	39.30%

Bottelneck at 100yr

Scenario	Starting populations size	Size of population at lowest point of bottleneck	Current population size	Constant population size for the null hypothesis, e.g., a hypothetical population similar to the one of interest, but assuming no bottleneck	Number of markers genotyped at	Number of sampled individuals	Power = probability of success of the study, distinguishing a bottlenecked population
1	1000	100	100	1000	16	29	6.30%
2	1000	100	100	1000	16	85	16.40%
3	1000	100	100	1000	16	114	19.30%
4	1000	200	200	1000	16	29	4.40%
5	1000	200	200	1000	16	85	9.80%
6	1000	200	200	1000	16	114	13.50%
7	1000	300	300	1000	16	29	6.30%
8	1000	300	300	1000	16	85	9.30%
9	1000	300	300	1000	16	114	10.50%
10	1000	400	400	1000	16	29	3.50%
11	1000	400	400	1000	16	85	8.60%
12	1000	400	400	1000	16	114	7.80%
13	2000	100	100	2000	16	29	14.00%
14	2000	100	100	2000	16	85	21.80%
15	2000	100	100	2000	16	114	29.40%
16	2000	200	200	2000	16	29	9.00%
17	2000	200	200	2000	16	85	15.00%
18	2000	200	200	2000	16	114	19.80%
19	2000	300	300	2000	16	29	6.70%
20	2000	300	300	2000	16	85	9.50%
21	2000	300	300	2000	16	114	15.00%
22	2000	400	400	2000	16	29	8.10%
23	2000	400	400	2000	16	85	7.80%
24	2000	400	400	2000	16	114	9.60%

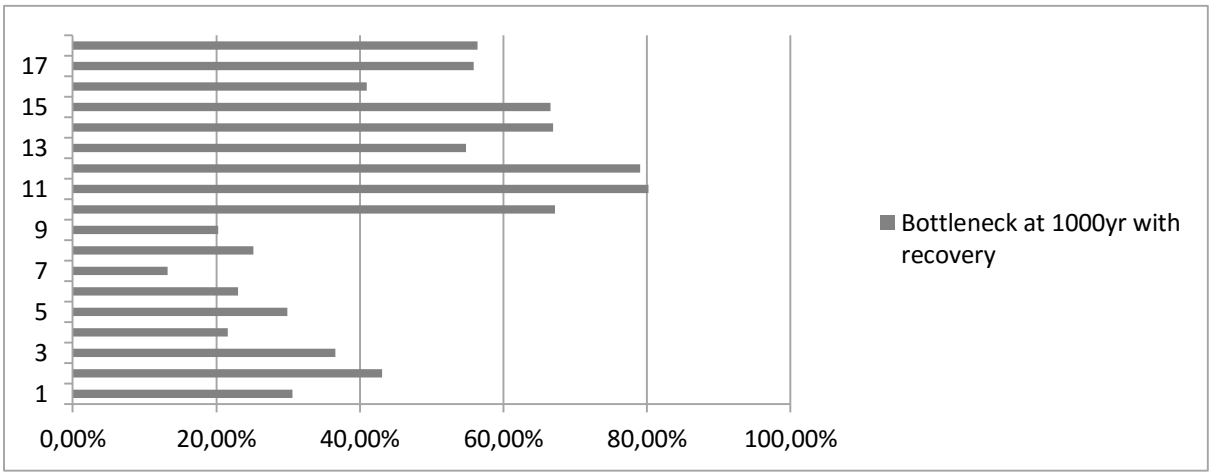
Supplementary data 3-1 (Cont.)



Supplementary data 3-1 (Cont.)

Bottleneck at 1000yr with recovery

Scenario	Starting populations size	Size of population at lowest point of bottleneck	Current population size	Constant population size for the null hypothesis, e.g., a hypothetical population similar to the one of interest, but assuming no bottleneck	Number of markers genotyped at	Number of sampled individuals	Power = probability of success of the study, distinguishing a bottlenecked population
1	1000	100	400	1000	16	29	30.60%
2	1000	100	400	1000	16	85	43.10%
3	1000	100	400	1000	16	114	36.60%
4	1000	200	400	1000	16	29	21.60%
5	1000	200	400	1000	16	85	29.90%
6	1000	200	400	1000	16	114	23.00%
7	1000	300	400	1000	16	29	13.20%
8	1000	300	400	1000	16	85	25.20%
9	1000	300	400	1000	16	114	20.30%
10	2000	100	400	2000	16	29	67.20%
11	2000	100	400	2000	16	85	80.20%
12	2000	100	400	2000	16	114	79.10%
13	2000	200	400	2000	16	29	54.80%
14	2000	200	400	2000	16	85	66.90%
15	2000	200	400	2000	16	114	66.60%
16	2000	300	400	2000	16	29	41.00%
17	2000	300	400	2000	16	85	55.90%
18	2000	300	400	2000	16	114	56.40%

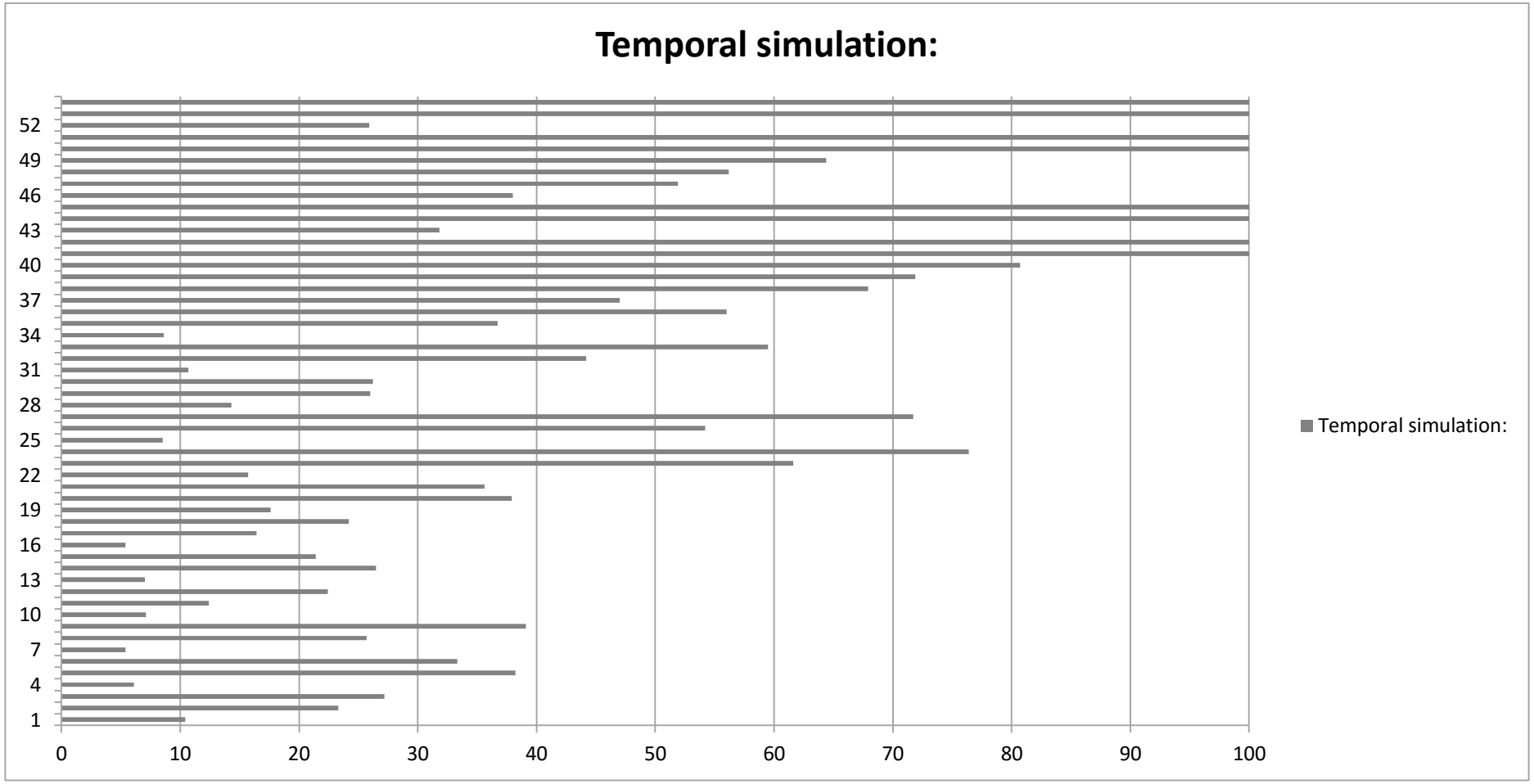


Supplementary data 3-1 (Cont.)

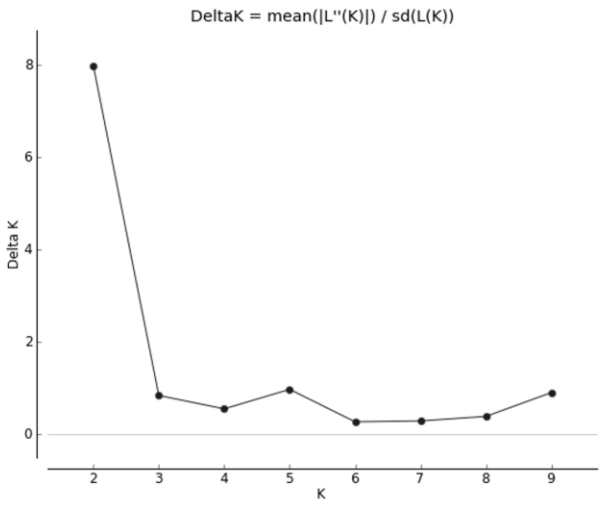
Temporal simulation:

Scenario	Ancient sample size (Nh)	Contemporary sample size (Nc)	Time of beginning of decline	Time of historical sampleing	Historic sample Sample size	size	Number of markers	Power
1	1000	300	100	100	29	29	16	1040.00%
2	1000	300	100	100	85	29	16	2330.00%
3	1000	300	100	100	114	29	16	2720.00%
4	1000	300	1000	100	29	29	16	610.00%
5	1000	300	1000	100	85	29	16	3820.00%
6	1000	300	1000	100	114	29	16	3330.00%
7	1000	300	2000	100	29	29	16	540.00%
8	1000	300	2000	100	85	29	16	2570.00%
9	1000	300	2000	100	114	29	16	3910.00%
10	1000	400	100	100	29	29	16	710.00%
11	1000	400	100	100	85	29	16	1240.00%
12	1000	400	100	100	114	29	16	2240.00%
13	1000	400	1000	100	29	29	16	700.00%
14	1000	400	1000	100	85	29	16	2650.00%
15	1000	400	1000	100	114	29	16	2140.00%
16	1000	400	2000	100	29	29	16	540.00%
17	1000	400	2000	100	85	29	16	1640.00%
18	1000	400	2000	100	114	29	16	2420.00%
19	2000	300	100	100	29	29	16	1760.00%
20	2000	300	100	100	85	29	16	3790.00%
21	2000	300	100	100	114	29	16	3560.00%
22	2000	300	1000	100	29	29	16	1570.00%
23	2000	300	1000	100	85	29	16	6160.00%
24	2000	300	1000	100	114	29	16	7640.00%
25	2000	300	2000	100	29	29	16	850.00%
26	2000	300	2000	100	85	29	16	5420.00%
27	2000	300	2000	100	114	29	16	7170.00%
28	2000	400	100	100	29	29	16	1430.00%
29	2000	400	100	100	85	29	16	2600.00%
30	2000	400	100	100	114	29	16	2620.00%
31	2000	400	1000	100	29	29	16	1070.00%
32	2000	400	1000	100	85	29	16	4420.00%
33	2000	400	1000	100	114	29	16	5950.00%
34	2000	400	2000	100	29	29	16	860.00%
35	2000	400	2000	100	85	29	16	3670.00%
36	2000	400	2000	100	114	29	16	5600.00%
37	20000	300	100	100	29	29	16	4700.00%
38	20000	300	100	100	85	29	16	6790.00%
39	20000	300	100	100	114	29	16	7190.00%
40	20000	300	1000	100	29	29	16	8070.00%
41	20000	300	1000	100	85	29	16	10000.00%
42	20000	300	1000	100	114	29	16	10000.00%
43	20000	300	2000	100	29	29	16	3180.00%
44	20000	300	2000	100	85	29	16	10000.00%
45	20000	300	2000	100	114	29	16	10000.00%
46	20000	400	100	100	29	29	16	3800.00%
47	20000	400	100	100	85	29	16	5190.00%
48	20000	400	100	100	114	29	16	5620.00%
49	20000	400	1000	100	29	29	16	6440.00%
50	20000	400	1000	100	85	29	16	10000.00%
51	20000	400	1000	100	114	29	16	10000.00%
52	20000	400	2000	100	29	29	16	2590.00%
53	20000	400	2000	100	85	29	16	10000.00%
54	20000	400	2000	100	114	29	16	10000.00%

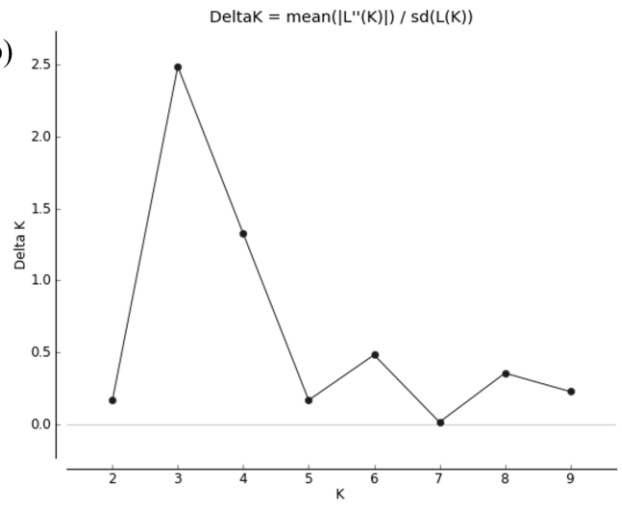
Supplementary data 3-1 (Cont.)



a)



b)



K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	20	-1009.695000	2.009838	—	—	—
2	20	-985.140000	11.287926	24.555000	90.130000	7.984638
3	20	-1050.715000	37.188033	-65.575000	30.845000	0.829434
4	20	-1085.445000	70.138378	-34.730000	37.845000	0.539576
5	20	-1158.020000	114.637748	-72.575000	110.320000	0.962336
6	20	-1120.275000	88.841846	37.745000	22.905000	0.257818
7	20	-1105.435000	172.678717	14.840000	47.865000	0.277191
8	20	-1042.730000	103.800579	62.705000	38.920000	0.374950
9	20	-1018.945000	17.923594	23.785000	15.995000	0.892399
10	20	-1011.155000	3.054501	7.790000	—	—

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	20	-3737.065000	2.138439	—	—	—
2	20	-3710.815000	7.294863	26.250000	1.200000	0.164499
3	20	-3683.365000	11.701789	27.450000	29.090000	2.485945
4	20	-3685.005000	48.596225	-1.640000	64.355000	1.324280
5	20	-3751.000000	54.560985	-65.995000	9.050000	0.165869
6	20	-3807.945000	102.354603	-56.945000	49.200000	0.480682
7	20	-3815.690000	132.538848	-7.745000	1.720000	0.012977
8	20	-3825.155000	135.331291	-9.465000	47.890000	0.353872
9	20	-3786.730000	58.337395	38.425000	13.155000	0.225499
10	20	-3761.460000	34.839140	25.270000	—	—

Supplementary Figure 3-1. The delta K and Evanno table output for a) the historical and b) the contemporary STRUCTURE analyses.

Supplementary Table 3-1. Sample information for all samples used in Chapter 3.

Sample code:	Museum ID:	Location:	GPS coordinates:	Date sampled:	Sourced:
FH01	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH02	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH03	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH04	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH05	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH06	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH07	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH08	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH09	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH10	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH11	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH12	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH13	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH14	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH15	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH16	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH17	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH18	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH19	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH20	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH21	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH22	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH23	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH24	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH25	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH26	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH27	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH28	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH29	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH30	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH31	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH32	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH33	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH34	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH35	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH36	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH37	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH38	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH39	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH40	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town
FH41	NA	Alice, Eastern Cape	-32.796097, 26.850024	2013	This study (University of KwaZulu-Natal)
ECH01	16102	Pirie Mission, Alice area	-32.791100, 27.247902	1928	East London Museum

Supplementary Table 3-1. (Cont.)

ECH02	16105	Pirie hatchery, Alice area	-32.791100, 27.247902	1944	East London Museum
KWT01	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT02	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT03	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT04	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT05	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT06	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT07	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT08	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT09	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT10	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town
KWT11	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2013	This study (University of KwaZulu-Natal)
ECH03	16103	King William's Town, Eastern Cape	-32.880202, 27.398856	1929	East London Museum
ECH04	16104	King William's Town, Eastern Cape	-32.880202, 27.398856	1934	East London Museum
ECH05	16106	King William's Town, Eastern Cape	-32.880202, 27.398856	1946	East London Museum
ECH06	16107	King William's Town, Eastern Cape	-32.880202, 27.398856	1935	East London Museum
ECH07	16108	King William's Town, Eastern Cape	-32.880202, 27.398856	1935	East London Museum
ECH08	BMNH 1877.8.1.30	King William's Town, Eastern Cape	-32.880202, 27.398856	1877	British Natural History Museum at Tring
ECH09	17599	Frankfort, Eastern Cape	-32.720507, 27.453272	1944	East London Museum
ECH10	16100	Frankfort, Eastern Cape	-32.720507, 27.453272	1943	East London Museum
ECH11	16101	Frankfort, Eastern Cape	-32.720507, 27.453272	1943	East London Museum
ECH12	6162	Needs Camp, Amathole, East London area, Eastern Cape	-32.995257, 27.647519	1958	East London Museum
ECH13	6163	Needs Camp, Amathole, East London area, Eastern Cape	-32.995257, 27.647519	1958	East London Museum
ECH14	7201	Cambridge district, East London, Eastern Cape	-33.008834, 27.802254	1959	East London Museum
ECH15	13276	Lusikisiki, Eastern Cape	-31.366218, 29.570018	1968	East London Museum
ECH16	13277	Lusikisiki, Eastern Cape	-31.366218, 29.570018	1968	East London Museum
ECH17	P.r.r 8266	Eastern Cape	Unknown	1960	Durban Natural Sciences Museum
ECH18	P.r.r 8267	Eastern Cape	Unknown	1954	Durban Natural Sciences Museum
ECH19	TM 7994	Eastern Cape	Unknown	1910	Ditsong National Museum of Natural History
ECH20	TM 40931	Eastern Cape	Unknown	1931	Ditsong National Museum of Natural History
ECH21	TM 40942	Eastern Cape	-31.366218, 29.570018	1951	Ditsong National Museum of Natural History
ECH22	KMB635	Eastern Cape	Unknown	1929	East London Museum
ECH23	KMB638	Eastern Cape	Unknown	1929	East London Museum
KZN01	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal
KZN02	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal
KZN03	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal
KZN04	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal
KZN05	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal
KZN06	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal
KZN07	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2013	This study (University of KwaZulu-Natal)
KZN08	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN09	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN10	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN11	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)

Supplementary Table 3-1. (Cont.)

KZN12	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN13	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN14	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN15	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN16	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN17	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN18	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KZN19	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)
KH01	TMIIa 886	KwaZulu-Natal	Unknown	1897	Ditsong National Museum of Natural History
KH02	TM 2296	KwaZulu-Natal	Unknown	1908	Ditsong National Museum of Natural History
KH03	TM 2297	KwaZulu-Natal	Unknown	1908	Ditsong National Museum of Natural History
KH04	TM 40930	KwaZulu-Natal	Unknown	1957	Ditsong National Museum of Natural History
KH05	BMNH 1890.10.10.40	Burg Mount, KwaZulu-NatalNatal	Unknown	1878	British Natural History Museum at Tring
Lim01	NA	Tzaneen, Limpopo	-23.859859, 30.006596	1999	Craig Symes (Wits University, RSA)
Lim02	NA	Tzaneen, Limpopo	-23.859859, 30.006596	1999	Craig Symes (Wits University, RSA)
Lim03	NA	Tzaneen, Limpopo	-23.859859, 30.006596	1999	Craig Symes (Wits University, RSA)
Lim04	NA	Tzaneen, Limpopo	-23.859859, 30.006596	1999	Craig Symes (Wits University, RSA)
Lim05	TM80817	Tzaneen, Limpopo	-23.822019, 30.131136	2014	Ditsong National Museum of Natural History
LH01	TMIIa 2078	Tzaneen, Limpopo	-23.822019, 30.131136	1887	Ditsong National Museum of Natural History
LH02	TM 16406	Tzaneen, Limpopo	-23.822019, 30.131136	1930	Ditsong National Museum of Natural History
LH03	TM 16407	Tzaneen, Limpopo	-23.822019, 30.131136	1930	Ditsong National Museum of Natural History
LH04	TM 25266	Tzaneen, Limpopo	-23.822019, 30.131136	1942	Ditsong National Museum of Natural History
LH05	BMNH 1878.12.31.503	Elands Poort, Limpopo	-24.68333, 28.38333	1870	British Natural History Museum at Tring
LH06	BMNH 1878.12.31.378	Elands Poort, Limpopo	-24.68333, 28.38333	1878	British Natural History Museum at Tring
LH07	BMNH 1878.12.31.666	Elands Poort, Limpopo	-24.68333, 28.38333	1870	British Natural History Museum at Tring
LH08	BMNH 1890.10.10.47	Elands Poort, Limpopo	-24.68333, 28.38333	1872	British Natural History Museum at Tring
LH09	BMNH 1905.12.29.532	Zuurbron, Mpumalanga	-27.282423, 30.449024	1904	British Natural History Museum at Tring
LH10	BMNH 1905.12.29.531	Zuurbron, Mpumalanga	-27.282423, 30.449024	1904	British Natural History Museum at Tring

Supplementary Table 3-2. SPOTG parameters used in Chapter 3.

Bottleneck simulation 1:		Bottleneck simulation 2:	
Number of loci:	16	Number of loci:	16
Number on samples:	29,85,114	Number on samples:	29,85,114
Pre-bottleneck:	1000, 2000	Pre-bottleneck:	1000, 2000
During bottleneck:	100, 200, 300, 400	During bottleneck:	100, 200, 300, 400
Bottleneck began:	100	Bottleneck began:	1000
Generation time:	5	Generation time:	5
mutation rate:	0.0005	mutation rate:	0.0005
Percent stepwise:	0.9	Percent stepwise:	0.9
Iterations:	1000	Iterations:	1000

Bottleneck simulation with recovery:		Temporal simulation:	
Number of loci:	16	Number of Individuals Samples:	29,85,114
Number on samples:	29,85,114	Number of Genetic Markers:	16
Pre-bottleneck:	1000, 2000	Number of Temporal/Past Individuals Samples:	29
During bottleneck:	100, 200, 300	Population Size Ancient:	1000,2000,20000
Post-bottleneck:	400	Population Size Current:	300,400
Bottleneck began:	1000	Timing of past samples (historic):	100
Bottleneck ended:	100	Decline began:	100,1000,2000
Generation tiime:	5	Generation time:	5
mutation rate:	0.0005	Mutation rate:	0.0005
Percent stepwise:	0.9	Number of runs:	1000
Iterations:	1000		

Supplementary Table 3-3. Prior settings used for the MSVAR analyses (init_v_file) in Chapter 3.

12345678																number of 'turns' of the random number generator (changed for each run)
2																ploidy number
5																generation time
1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	starting values for current size for all loci
1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	starting values for ancestral size for all loci
1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	1E-04	starting values for mutation rate for all loci
1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	1E+03	starting values for time since decline/expansion for all loci
0	0	0	0													indicators (0,1) whether to update values of these parameters.
3	2															Starting values for prior mean and variance for current size
3	2															..... ancestral size
-3.5	1															…..mutation rate
3	1															..... time
3	2	0	0.5													hyperprior mean and variance for means and variances for
3	2	0	0.5													ancestral size
-3.5	0.25	0	0.5													…..mutation rate
3	2	0	0.5													…..time since decline/expansion
1																0=linear growth; 1=exponential
20000																number of lines of output
10000																number of iterations between lines of output

Supplementary Table 3-4. Per locus genetic diversity estimates over all samples, contemporary samples only and historical samples only.

Locus:	All specimens:				Contemporary (1950-2014):				Historical (1870-1950):			
	Polimorphic information content (PIC):	Null allele frequency as % (Na):	Allelic richness (Ar):	Deviation from Hardy-Weinberg equlibrium (HWD):	Polimorphic information content (PIC):	Null allele frequency as % (Na):	Allelic richness (Ar):	Deviation from Hardy-Weinberg equlibrium (HWD):	Polimorphic information content (PIC):	Null allele frequency as % (Na):	Allelic richness (Ar):	Deviation from Hardy-Weinberg equlibrium (HWD):
Prob01	0.569	8.4	3	0.03	0.568	6.7	3	0.627	0.564	13.5	2.991	0.017
Prob06	0.63	3.3	9.87	0.012	0.66	08	8.988	0.258	0.454	12	3.51	0.251
Prob09	0.667	1.8	7.788	0.17	0.659	0.1	7.988	0.153	0.653	7.5	4.304	0.217
Prob15	0.807	20.8	9.997	HS	0.798	20.9	9	HS	0.798	18.9	6.623	0.0004
Prob17	0.936	4	28.558	HS	0.94	0.8	27.964	0.048	0.861	19.4	8.943	HS
Prob18	0.625	3.3	4.966	0.486	0.619	0	4.976	0.869	0.583	16.3	3.562	0.292
Prob23	0.691	2.3	7.608	0.054	0.694	0.8	6.988	0.281	0.669	7.1	4.502	0.006
Prob25	0.712	1.5	5.96	0.97	0.736	0.3	6	0.933	0.589	2.4	3.465	0.842
Prob26	0.802	5.8	12	0.079	0.79	1.7	9	0.657	0.744	36.7	6	0.004
Prob28	0.378	0	2.827	0.452	0.375	0	2.976	0.241	0.371	6.3	2	0.799
Prob29	0.302	1.7	5.643	0.199	0.325	2.6	5.953	0.34	0.212	0	2.375	NC
Prob30	0.765	2.4	7.843	0.039	0.739	1.4	7	0.181	0.819	4.8	6.542	0.347
Prob31	0.705	0	6	0.028	0.692	0	6	0.212	0.732	2	5.482	0.147
Prob34	0.484	4.8	5.847	0.122	0.469	0	5.988	0.764	0.526	14.3	4.053	0.004
Prob35	0.32	0.4	2	0.966	0.31	0	2	0.562	0.344	4.6	2	0.691
Prob36	0.172	10.4	2.978	0.001	0.143	5.7	2	0.182	0.264	19.8	2.486	0.008
Mean:	0.5978	4.4	7.6803125	-	0.5948	2.6	7.238813	-	0.5739	11.6	4.302375	-
SE:	0.053	1.3	1.562		0.054	1.3	1.503		0.050	2.3	0.490	

Supplementary Table 3-5. The analysis of molecular variance (AMOVA) results from the full, contemporary and historical datasets. Samples were grouped according to region of origin and then according to sampling locality.

Full dataset with regional grouping of samples:			**Full dataset with sampling locality grouping of samples:**		
Source	**Est. Var.**	**%**	**Source**	**Est. Var.**	**%**
Among Pops	0.139	3%	**Among Pops**	0.233	4%
Among Indiv	0.833	15%	**Among Indiv**	0.721	13%
Within Indiv	4.430	82%	**Within Indiv**	4.430	82%
Total	5.401	100%	**Total**	5.384	100%
Contemporary dataset with regional grouping of samples:			**Contemporary dataset with sampling locality grouping of samples:**		
Source	**Est. Var.**	**%**	**Source**	**Est. Var.**	**%**
Among Pops	0.177	3%	**Among Pops**	0.155	3%
Among Indiv	0.198	4%	**Among Indiv**	0.170	3%
Within Indiv	4.882	93%	**Within Indiv**	4.882	94%
Total	5.257	100%	**Total**	5.208	100%
Historical dataset with regional grouping of samples:			**Historical dataset with sampling locality grouping of samples:**		
Source	**Est. Var.**	**%**	**Source**	**Est. Var.**	**%**
Among Pops	0.268	5%	**Among Pops**	0.295	5%
Among Indiv	2.159	39%	**Among Indiv**	2.074	38%
Within Indiv	3.103	56%	**Within Indiv**	3.103	57%
Total	5.530	100%	**Total**	5.472	100%

Supplementary Table 3-6. Results from the migration-drift vs drift only model analysis performed in 2MOD for both the contemporary and historical datasets. *F* - the probability that two genes share a common ancestor within a population; *M* - the number of migrants per generation as calculated from *F*.

Model posterior probability (*P*)		**Contemporary:**		
Gene flow	Drift	Population	*F*	*M*
0.502	0.498	Southern	0.011 (0.005 - 0.021)	44.955
		Central	0.028 (0.012 - 0.038)	17.360
		Northern	0.214 (0.132 - 0.263)	1.836
		Historic:		
		Population	*F*	
0.558	0.442	Southern	0.037 (0.004 - 0.072)	13.014
		Central	0.045 (0.00002 - 0.108)	10.611
		Northern	0.035 (0.002 - 0.07)	13.786

Supplementary Table 3-7. Migration rate estimates (*m*), obtained from BayesAss using the contemporary and historical data sets. The 95% confidence interval is provided in parentheses.

		To:		
	Historic	South	Central	North
From:	South	0.896 (0.806 - 0.995)	0.12 (0.012 - 0.282)	0.097 (0.006 - 0.255)
	Central	0.065 (0.0004 - 0.153)	0.793 (0.669 - 0.955)	0.119 (0.002 - 0.258)
	North	0.039 (0.0008 - 0.152)	0.087 (0.004 - 0.267)	0.784 (0.687 - 0.95)

		To:		
	Contemporary	South	Central	North
From:	South	0.993 (0.973 - 0.999)	0.278 (0.182 - 0.327)	0.061 (0.001 - 0.286)
	Central	0.004 (0.00003 - 0.018)	0.706 (0.668 - 0.802)	0.199 (0.001 - 0.286)
	North	0.003 (0.00002 - 0.015)	0.016 (0.0004 - 0.059)	0.74 (0.668 - 0.982)

		To:			
	Contemporary	Alice & KWT	Lusikisiki	Creighton	Tzaneen
From:	Alice & KWT	0.991 (0.973 - 0.999)	0.222 (0.097 - 0.311)	0.267 (0.176 - 0.325)	0.042 (0.0002 - 0.17)
	Lusikisiki	0.002 (0.000005 - 0.013)	0.714 (0.097 - 0.311)	0.011 (0.00008 - 0.046)	0.029 (0.0002 - 0.109)
	Creighton	0.004 (0.000004 - 0.014)	0.033 (0.0004 - 0.122)	0.711 (0.667 - 0.8)	0.181 (0.001 - 0.307)
	Tzaneen	0.003 (0.000004 - 0.015)	0.031 (0.0003 - 0.116)	0.012 (0.00006 - 0.055)	0.748 (0.668 - 0.981)

CHAPTER 4: IDENTIFICATION OF MICROSATELLITE MULTIPLEXES FOR INDIVIDUAL IDENTIFICATION OF CAPE PARROTS (*POICEPHALUS ROBUSTUS*): PATERNITY TESTING AND MONITORING TRADE

4.1 Abstract

Illegal trade in rare wildlife species is a major threat to many parrot species around the world. Wildlife forensics plays an important role in the preservation of endangered or threatened wildlife species. Identification of illegally harvested or traded animals through DNA techniques is one of the many methods used during forensic investigations. In this study, 16 microsatellite markers specifically designed for the South African endemic Cape Parrot (*Poicephalus robustus*) are assessed for their utility in forensic casework. Using these 16 loci the genetic diversity of a subset of the captive Cape Parrot population was also assessed and compared to three wild Cape Parrot populations. It was determined that the full 16 locus panel has sufficient discriminatory power to be used in parentage analyses and can be used to determine if a bird has been bred in captivity and so can be legally traded or if has been illegally removed from the wild. In cases where birds have been removed from the wild, this study suggests that a reduced 12 locus microsatellite panel has sufficient power to assign confiscated birds to population of origin. The level of genetic diversity observed within the captive Cape Parrot population was similar to that observed in the wild populations, which suggests that the captive population is not suffering from decreased levels of genetic diversity. The captive Cape Parrots did however have double the amount of private alleles compared to that observed in the most genetically diverse wild population. This is accredited to the presence of rare alleles present in the founder population, which has not been lost due to genetic drift, as many of the individuals tested in this study are F1 to F3 wild descendants. The results from this study provide a suit of markers that can be used to aid conservation and law enforcement authorities to better control legal and illegal trade of this South African endemic.

Keywords: Cape Parrot, *Poicephalus robustus*, microsatellite, wildlife forensics, parentage, captive breeding

4.2 Introduction

The illegal wildlife trade includes the buying and selling of any wildlife product that has been captured alive, poached, and use as food, medicine, pets and trophies (TRAFFIC, 2008). The illegal trade in wildlife has a negative impact on wildlife and conservation programs worldwide (Alacs et al., 2010). The exact value of the illegal wildlife trade is unknown, but current estimates suggest that illegal transactions involving wildlife, and their products, is a multibillion US dollar enterprise (Broad et al., 2002, Interpol, 2014). This is particularly true for rare bird species, which are highly sought after (Cooney and Jepson, 2006, White et al., 2012). Major importers and exporters of exotic birds include regions of central and southern America, the Middle East, Southeast Asia and China (BirdLife International, 2012, Bush et al., 2014, Li and Jiang, 2014, Low, 2014, Regueira and Bernard, 2012). The third most internationally-traded bird in the world is the Grey Parrot (*Psittacus erithacus*) from equatorial Africa (BirdLife International, 2013), with these birds favoured as pets in Europe, the United States of America and China (BirdLife International, 2015a). Parrots (order Psittaciformes) are extremely popular as pets and have the highest reported trade figures among all traded avian orders (Bush et al., 2014). Of particular concern are the rare and enigmatic species, as half of the world's threatened or near-threatened parrot species are impacted by illegal trade (Pain et al., 2006). Although species from South America and Australasia make up a large proportion of the illegal trade (BirdLife International, 2008, Low, 2014, Weston and Memon, 2009), African species are increasingly becoming targets for exploitation. For example, in China a quarter of all imported parrots originated from South Africa (Li and Jiang, 2014). To date CITES has classified South Africa as a major importer and exporter of legally and illegally obtained birds (Warchol, 2004) and is regarded as the hub of wildlife trade in the region (Wynberg, 2002). It has been suggested that the South African endemic Cape Parrot is finding itself under tremendous pressure, not only due to habitat fragmentation, but also due to the illegal harvesting of wild birds and eggs for the pet trade (Martin et al., 2014, Wirminghaus et al., 1999).

The need to detect and punish perpetrators of illegal trade in wildlife is gaining momentum. The overexploitation of threatened bird species can lead to their extinction (BirdLife International, 2015b) as is the case for the great auk (*Pinguinus impennis*; Courchamp et al., 2006). Animal welfare is also a major issue with illegal harvesting (Bush et al., 2014, Cooney and Jepson, 2006), with large numbers of birds dying in transit before reaching their final destination (Cantú–Guzmán et al., 2007, Weston and Memon, 2009).

Captive breeding of exotic birds is a plausible alternative to sourcing wild animals, and it has been shown to be a viable practice (Pires, 2012). There are many difficulties linked to captive breeding programs, and it is not always easy to breed wild animals (Nogueira and Nogueira-Filho, 2011). Breeding of wildlife in captivity is also not always an alternative to wild harvesting, as there will always be a demand for new breeding stock from the wild (Bush et al., 2014, Nogueira and Nogueira-Filho, 2011). Captive breeding can lead to the accumulation of detrimental alleles, inbreeding and loss of genetic diversity (Williams and Hoffman, 2009), as a result of small founder populations (Witzenberger and Hochkirch, 2011). The addition of wild individuals to the captive populations will decrease the negative effects of genetic adaptation to captivity (Frankham, 2008). Many captive breeding programs, involving rare species, aim to use their captive bred animals for reintroduction purposes (Frankham et al., 2002, Robert, 2009). If reintroduction is the main purpose of a captive breeding program, then care should be taken not to include individuals into the captive population from only a few wild genetic lineages (Witzenberger and Hochkirch, 2011). The creation of self-sustaining captive populations, which resembles the wild genetic lineages as closely as possible, should be one of the main aims of captive breeding programs (Frankham, 2008, Robert, 2009). Regular assessment of the genetic health of captive populations is therefore important to ensure healthy populations exist for possible reintroductions. The legitimacy of some 'captive-bred' animals is also in question, as some breeding facilities produce more 'captive-bred' animals than is plausible (Lyons and Natusch, 2011, White et al., 2012). It is therefore imperative to monitor the legal trade of alleged captive bred birds to identify possible illegal activities.

Molecular forensic methods are widely used to identify suspected illegally obtained wildlife or wildlife products (Coghlan et al., 2012, Comstock et al., 2003, Dawnay et al., 2009, Gonçalves et al., 2015, Gupta et al., 2005, Lorenzini, 2005, Lorenzini et al., 2011, Mondol et al., 2014, Presti et al., 2015, White et al., 2012). A highly useful molecular forensic tool is genetic fingerprinting using microsatellite markers. Microsatellite markers are hypervariable nuclear markers suited for individual identification of specimens and assigning specimens to specific populations (Alacs et al., 2010). When a sufficient reference database is available, these markers have been successfully used to identify legally, and illegally, traded birds (for example Presti et al., 2015, White et al., 2012). It is necessary to consider the genetic sub-structuring within a species before releasing confiscated animals back into the wild, as the subpopulations could have acquired habitat specific fitness (for example, pathogen resistance Boyce et al., 2011). It is important, from a conservation viewpoint, to preserve genetically

distinct or evolutionary significant populations (Johnson, 2000). When a species is showing signs of genetic sub-structuring in the wild, it is important to reintroduce confiscated animals only to genetically similar populations to avoid the unnatural admixture of different genetic lineages. The use of microsatellite data to assign confiscated wildlife to their area of origin is a well-known technique used in wildlife forensic and conservation sciences (Manel et al., 2002, Mondol et al., 2014, Presti et al., 2015, White et al., 2012). For example, Presti et al. (2015) was able to assign 24 confiscated Hyacinth Macaw chicks to their populations of origin based on Bayesian clustering analysis using 10 microsatellite loci and White et al. (2012) were able to identify the kinship and area of origin of a White-tailed Black Cockatoo (*Calyptorhynchus baudinii / Calyptorhynchus latirostris*) using 20 microsatellite loci and kinship analyses.

A number of factors need to be considered when selecting a microsatellite panel for forensic studies. The quality of the data obtained from a set of markers should be assessed by considering the occurrence of genotyping errors such as null alleles and missing data which can lead to biased estimations of genetic diversity and false parentage assignments (Dakin and Avise, 2004). Additionally, the level of informativeness of each marker should also be assessed, focusing on the level of variation and the discriminatory power of each locus (Rosenberg et al., 2003).

The occurrence of genotyping errors, such as allelic dropout and null alleles, are major limitations of microsatellite markers which should be consider when performing forensic analyses (Alacs et al., 2010, Dakin and Avise, 2004). According to Dakin and Avise (2004), there are three general causes for null alleles; 1) poor primer annealing due to changes in nucleotide sequence, 2) shorter alleles being amplified more efficiently; and 3) polymerase chain reaction (PCR) failure due to low DNA quality or quantity. The occurrence of null alleles can be especially detrimental to studies focused on individual identification or parentage analyses, as erroneous genotypes will lead to misidentification of individuals biasing estimates of relatedness. Markers prone to such errors should therefore be identified and discarded from studies focusing on individual identification and parentage analyses. When null alleles occur in low frequencies, and it is unavoidable to use them, it is advisable to use parentage analysis methods that can compensate for these errors (Dakin and Avise, 2004).

A number of well-established methods are available to assess the informativeness of genetic markers, one of which is the polymorphic information content (PIC) estimate first used by Botstein et al. (1980). PIC estimates the frequency at which a marker is observed as being polymorphic (Buchanan and Thue, 1998), with markers with values higher than 0.6 considered

highly informative (Mateescu et al., 2005). The probability of identity (P_{ID}) calculated per locus is a method used to assess the discriminating ability of microsatellite markers used for individual identification (Taberlet and Luikart, 1999, Waits et al., 2001), and provides the probability that two random individuals will share the same genotype at a locus. Loci with low P_{ID} values should be considered for use in forensic studies, to increase the probability of accurately identifying individuals. Individual locus P_{ID} values in the range of 0.01 – 0.0001 are commonly used in wildlife forensics (Waits et al., 2001). The probability of exclusion (P_E) is another estimator used to assess the power of a marker, and gives the proportion of male or female individuals that could be excluded as the parent of an offspring, given the genotypic information observed (Fung et al., 2002). The P_{ID} and P_E estimates are well-established methods for assessing the ability of molecular markers to distinguish between individuals (Fung et al., 2002, Taberlet and Luikart, 1999,).

The South African endemic Cape Parrot (*Poicephalus robustus*) is a locally protected parrot species found in the South African mistbelt forests (Taylor, 2014, Wirminghaus, 1997). It has been estimated that there are fewer than 1600 individuals left in the wild (Downs et al., 2014). One of the factors linked to this low population number is illegal harvesting of nestlings and eggs for the pet bird trade (Wirminghaus et al., 1999). Recent genetic work has shown that the Cape Parrot should be elevated to species status (Coetzer et al., 2015), separate from the more widely distributed Grey-headed Parrot (*Poicephalus fuscicollis suahelicus*). This change in taxonomic status should provide the Cape Parrot with much needed conservation protection from international organisations like the IUCN and *Convention on International Trade in Endangered Species* of Wild Fauna and Flora (CITES). Cape Parrots have been successfully bred in captivity for a number of years, although it is a difficult enterprise with low breeding success among wild-caught birds (Wirminghaus et al., 1999). Captive breeding facilities provide the wildlife pet trade with legally obtained animals, and may also serve as source populations if future reintroductions to natural habitats are needed (Pires, 2012, Storfer, 1999, Williams and Hoffman, 2009). The occurrence and accumulation of deleterious mutations, as well as the effects of genetic adaptation to captivity (Williams and Hoffman, 2009) are major issues observed in captive populations. Genetic sub-structuring has been recently observed within the contemporary Cape Parrot population, with three genetic clusters which are geographically correlated along the current Cape Parrot distribution range (Chapter 3). It is therefore important to maintain captive populations which are genetically similar to these three genetic lineages if future reintroductions are needed. Proper studbook keeping and managing

of the captive populations are therefore essential for maintaining healthy captive bred wildlife populations (Ferrie et al., 2013). The regional Cape Parrot studbook currently holds records of 341 Cape Parrots, 216 of which are living (Wilkinson, 2015). The studbook is, however, currently lacking many records due to many breeders showing reluctance in sharing information with regards to their Cape Parrot stocks (S. Wilkinson pers. comm.).

In this study, three main aims are addressed. First, an optimum combination of 16 microsatellite markers previously designed specifically for Cape Parrots (Pillay et al., 2010) was identified for their utility in forensic analyses. These 16 loci were previously used in a higher-level taxonomic analysis of *Poicephalus* parrots (Coetzer et al., 2015) and in a phylogeographic assessment of the Cape Parrot (Chapter 3). Second, the utility of these 16 loci for use in assigning confiscated wild-caught birds to their area of origin was tested through a Bayesian assignment method. The approach outlined in this study will assist law enforcement and conservation authorities with the return of illegally harvested Cape Parrots to the wild. Third, the genetic differentiation between the wild Cape Parrot population and the captive population was assessed using 16 microsatellite loci. The proper management of captive populations of protected species is important, especially if these captive populations are to supplement the wild populations (Robert, 2009). It is known that the genetic composition of populations in captivity can change markedly from the wild populations (Hindar et al., 1991, Lynch and O'Hely, 2001), which can have serious implications when reintroductions are considered. It is therefore vital to assess the genetic composition of the captive Cape Parrot population. These results will aid in the management of the captive population and to ensure that the captive population can be self-sustaining with minimal or no supplementation from the wild.

4.3 Materials and methods

4.3.1 Ethics

Ethical clearance for this study was received from the University of KwaZulu-Natal Animal Ethics sub-committee (Ref numbers: 074/13/Animal, 017/14/Animal, 042/15/Animal). All sampling procedures followed the criteria laid out by this committee.

4.3.2 Sampling and DNA extraction

Samples were collected from 76 captive Cape Parrots (Supplementary Table 4-1). This includes samples taken from one international and five South African breeding facilities. The captive specimens included in this study comprises 22.3% of the Cape Parrot studbook (Wilkinson, 2015). The majority of these samples were sourced from one breeding facility, which holds one of the largest captive Cape Parrot breeding populations in the world. Five of the specimens included in this study were wild caught birds that were recently introduced into the captive breeding program. These five birds originated from the KwaZulu-Natal (KZN) province. To test the utility of the molecular markers, captive birds with known pedigree were included. Both parents of 31 offspring were sampled, with only the sire sampled for seven of the captive bred birds.

Whole blood was collected from 45 captive Cape Parrots using Whatman™ FTA™ Elute cards and was stored at room temperature in a dark cool storage area. Seventeen samples were whole blood stored in absolute ethanol at -20 °C. Samples were also collected from deceased birds provided by two breeding facilities (n = 14). Biopsies of 5 mm x 5 mm were collected from each carcass and stored in absolute ethanol at -20 °C.

DNA extraction from the Whatman™ FTA™ Elute cards were performed following the manufacturer's protocols. The DNA extraction from the tissue and whole blood stored in absolute ethanol was performed with the NucleoSpin® Tissue kit (Macherey-Nagel), following the manufacturer's protocols. All DNA extracts were stored at -20 °C.

To assess any genetic differences between captive-bred and wild Cape Parrots, 85 genotypes from wild Cape Parrot populations were taken from the three regional populations described in Chapter 3. This study assessed the phylogeographic relationships between wild Cape Parrot populations. Strong genetic sub-structuring was observed, with three distinct genetic clusters linked to three geographical regions within the Cape Parrot distribution range (Chapter 3). The wild genotypes consisted of 52 genotypes from the South genetic cluster (EC region), 19 from the Central cluster (KZN region) and five genotypes from the North cluster (Limpopo region). Details on these specimens are provided in Supplementary Table 4-2.

4.3.3 Microsatellite amplification

The 16 microsatellite loci (*Prob01*, *Prob06*, *Prob09*, *Prob15*, *Prob17*, *Prob18*, *Prob23*, *Prob25*, *Prob26*, *Prob28*, *Prob29*, *Prob30*, *Prob31*, *Prob34*, *Prob35* and *Prob36*) selected for

this study was specifically developed for use in Cape Parrots (Pillay et al., 2010). The markers were divided into six multiplex sets (Multiplex 1: *Prob06*, *Prob15* and *Prob26*; Multiplex 2: *Prob30* and *Prob36*; Multiplex 3: *Prob18, Prob25* and *Prob31*; Multiplex 4: *Prob01*, *Prob09* and *Prob17*; Multiplex 5: *Prob23* and *Prob28*; Multiplex 6: *Prob29*, *Prob34* and *Prob35*). The forward primer in each microsatellite pair was fluorescently labelled on the 5' end. The KAPA2G Fast Multiplex mix (KAPA Biosystems) was used for all amplifications, with each PCR reaction mixture consisting of: ~2-30 ng template DNA, 5 µl KAPA2G Fast Multiplex mix (KAPA Biosystems), 0.2 µM of each primer and dH_2O to give a final reaction volume of 10 μl. The PCR cycling conditions consisted of an initial denaturation step for 3 min at 94 °C followed by 30 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and a final extension step for 5 min at 72 °C. The whole PCR setup, excluding the DNA addition step, were performed in a DNA free area.

The amplified PCR products were sent to the Central Analytical Facility at Stellenbosch University, South Africa for fragment analysis. The software package GeneMarker® v2.4.0 (Soft Genetics) was used for all genotype scoring. I reamplified 20% of the dataset to check for genotype consistency.

4.3.4 Data analysis

4.3.4.1 Evaluating the best set of microsatellite loci

The Expectation Maximization algorithm (EM) for detection of null allele frequencies was used as implemented in the software program FreeNA (Chapuis and Estoup, 2007). To assess the informativeness of each locus, the polymorphic information content (PIC) and the allelic richness (Ar) of each locus was calculated using Cervus (Kalinowski et al., 2007) and FSTAT (Goudet, 2001) respectively. The rarefaction method was followed for the Ar estimation, to account for differences in sample size. The probability of identity (P_{ID}) and probability of exclusion (one parent known, P_{E2}) were estimated in GenAlEx (Peakall and Smouse, 2012) to evaluate the discriminatory power of each locus. The combined P_{ID} and P_{E2} values was also calculated. Deviations from Hardy-Weinberg equilibrium (HWD) was estimated using Genepop v4.2 (Rousset, 2008).

Each locus was ranked according to their null allele, PIC, Ar, P_{ID} and P_{E2} (one parent known) estimates (Table 4-1). A score of one (good) to 16 (bad) was given to each locus for each of these five estimates, with a minimum of 5 (highly informative) to a maximum of 80

(highly uninformative). Eight microsatellite panels were then assembled by selecting the highest ranking loci for each panel, containing 9 to 16 loci each (Supplementary Table 4-3). Each of these eight panels were tested in the subsequent parentage and assignment analyses.

4.3.4.2 Parentage testing of captive population

The eight selected microsatellite panels were evaluated by testing the accuracy of each panel during parentage assignments, using captive specimens with both known and unknown pedigrees. For this analysis, the full-pedigree maximum likelihood method implemented in Colony v2.0.4.6 (Jones and Wang, 2010) was used. This program compensates for genotyping errors and null alleles (Wang, 2004) and has been used previously to identify parentage in captive (Ferrie et al., 2013, Loughnan et al., 2015) and wild vertebrate populations (Bergner et al., 2014, Masello et al., 2002, Riehl, 2012). The offspring data set consisted of 38 individuals. For seven of these only the paternal parent was known. A monogamous mating system with no inbreeding was selected, using the full-likelihood method. A medium run length with no sibship prior was selected. The marker type and null allele frequencies for each locus was uploaded with an error rate of 0.02 as suggested by Wang (2004). I uploaded the genotypes of 38 offspring, 30 paternal candidates and 21 maternal candidates, with the probability of the sire or dame included in the data set at 0.75 and no paternal or maternal exclusion information.

4.3.4.3 Ability of microsatellite panel to detect origin of illegally traded birds

A partial Bayesian exclusion approach (Rannala and Mountain, 1997) as implemented in GeneClass2 (Piry et al., 2004) was used to further assess the eight microsatellite panels. This method estimates the likelihood that a test sample belongs to one of the reference populations provided for analysis and calculates a sample exclusion probability for each of the reference populations (Ogden and Linacre, 2015). All wild born individuals were excluded from the captive data set. To simulate an assignment study, six captive bred and six wild caught individuals were selected at random for the "samples to be assigned" data set. The captive population from the current study and the three wild populations from Chapter 3 were used as reference populations. The 12 individuals selected for the "samples to be assigned" data set were excluded from these data sets. The Bayesian method from Rannala and Mountain (1997) was followed, with probability computation done by using Monte-Carlo resampling and the simulation algorithm from Paetkau et al. (2004). The number of simulated individuals were set at 100000, with the Type I error set at 0.01 and the assignment threshold at 0.05. These parameters were used for each of the eight microsatellite panels tested.

4.3.4.4 Captive vs wild Cape Parrots

The genetic diversity of the captive bred sample group was compared to the three wild Cape Parrot populations identified in the recent phylogeographic study (Chapter 3). Values compared included the average number of alleles (N_A), number of private alleles (P_A), observed heterozygosity (H_O) and unbiased expected heterozygosity (uH_E) (estimated in GenAlEx), inbreeding coefficient (F_{IS}) using Genepop v4.2 (Rousset, 2008) and allelic richness (Ar) using rarefaction in FSTAT (Goudet, 2001). A Wilcoxon signed-ranked test was performed to assess the level of genetic differences between the captive population and the three wild populations using the per locus estimates for each of the N_A, H_O, uH_E, F_{IS} and Ar estimates. Pairwise F_{ST} values and analysis of molecular variance (AMOVA) was estimated to assess if the captive population constitutes a separate genetic unit using GenAlEx. A Bonferroni correction was implemented to all *p*-values to correct for problems with multiple testing (Rice, 1986). In this analysis, the five wild born individuals were removed from the captive data set.

4.4 Results

4.4.1 Marker analysis

For this study, 76 captive Cape Parrots were successfully genotyped across 16 microsatellite loci. The data set contained less than 1% missing data, with a mean null allele frequency over all loci and samples of 3.9%. The per locus null allele frequencies ranged from 0 to 18.61% (Table 4-1). Only two loci showed null allele frequencies above 10% (*Prob15*, Na = 18.61%; *Prob36*, Na = 12.46%). The mean number of alleles per locus varied greatly among loci, ranging from 1.75 (*Prob36*) to 17 (*Prob17*) alleles. A large difference in allelic richness (Ar) values were observed across the loci, with values ranging from 2 (*Prob36*) to 22 (*Prob17*). Seven loci showed high levels of heterozygosity (Table 4-1), with negative F_{IS} values. Only two loci showed signs of heterozygote deficiency (*Prob09*, F_{IS} = 0.439; *Prob36*, F_{IS} = 0.471). Fourteen of the 16 loci were moderately to highly informative, with polymorphic information content (PIC) values ranging from 0.415 (*Prob29*) to 0.888 (*Prb17*). Only two loci (*Prob35* and *Prob36*) were identified as uninformative (PIC < 0.3; Table 4-1). The probability of identity (P_{ID}) values ranged from 0.019 (*Prob17*) to 0.591 (*Prob36*). A combined P_{ID} over all 16 loci was calculated as 1.831E-13 following the product rule. The probability of exclusion (with one parent known; P_{E2}) ranged from 0.658 (*Prob17*) to 0.032 *(Prob36),* with the combined P_{E2} at 0.995. It was observed that the PID and P_{E2} values improved as the number of

loci analysed increase (Figure 4-1). Five of the 16 loci significantly deviated from Hardy-Weinberg equilibrium (*Prob09, Prob15, Prob28, Prob30, Prob36*), following Bonferroni correction (p-value < 0.003; Rice, 1986).

Table 4-1. Primer details and genetic diversity estimates per locus as calculated from the captive data set used in Chapter 4. The standard error for the average number of alleles is provided in parentheses.

Locus	Primer sequence (5' – 3'):	Average number of alleles (N_A)	Allelic richness (Ar):	Probability of identity (P_{ID}):	Probability of exclusion (one parent known; P_{E2})	Polymorphic information content (PIC):	Null Allele Frequency as % (Na):	Inbreeding coefficient (F_{IS}):	Hardy-Weinberg deviation (*p*-value)	Locus rank:
Prob17	F: TGAACATGACTTATTTGTCTAGTCATACCTAATCC R: TTCCAAGGAGTAATATACAGATAATTGCTTCTACA	17 (3.559)	22[1]	0.018[1]	0.658[1]	0.888[1]	2.19[3]	0.017	0.045	1
Prob31	F: GCTGCAGTACAGGCAGTCTTTG R: CCCATGGCAGAAATTACAGTGA	5.25 (1.109)	6.997[5]	0.08[2]	0.404[2]	0.746[3]	0.00[1]	-0.058	0.096	2
Prob26	F: GATCCCCAAAACAGATGAGTCT R: GTTTCTTGATTCAGATTGGAGGCTGATG	7.25 (1.436)	9.877[3]	0.088[4]	0.370[4]	0.723[4]	0.00[1]	-0.109	0.188	3
Prob30	F: ACACTGAACCATGTCACACAAG R: GATCAGAAGGCTGCTTTGC	6 (0.707)	5.997[8]	0.081[3]	0.397[3]	0.751[2]	3.70[4]	0.044	0.0001	4
Prob23	F: CACCAGTCATGACAGATAAT R: AGTATAAATTCAGCCTAGTTATGT	5 (1.08)	5.997[7]	0.106[5]	0.341[5]	0.707[5]	1.01[2]	-0.091	0.034	5
Prob25	F: GATCCAGTGTGAAGCTAAAACAAGG R: GTTTCTTAAGGTAGATGTGGAGTGTAG	4.75 (0.629)	5.946[9]	0.113[6]	0.330[6]	0.691[7]	0.00[1]	-0.028	0.695	6
Prob18	F: GATCATTGAGAACTATTTGGAAG R: GTTTCTTATCAGTTGAACGCGAGAA	4.25 (0.479)	5[10]	0.112[7]	0.327[7]	0.694[6]	0.00[1]	0.035	0.198	7
Prob06	F: TCCAACCCACCTGAATTATCCAT R: GTTTCTTAGCTCCAATTCCGGGCTCT	6 (1.414)	7.957[4]	0.197[9]	0.213[9]	0.566[9]	0.00[1]	-0.022	0.606	8
Prob09	F: GAACGTTTGTAGGGATAGTCCAC R: GTTTCTTACCGTGTCCACCCCTTATTCG	7.25 (1.493)	10.833[2]	0.199[10]	0.198[10]	0.56[10]	6.04[6]	0.146	0.003	9
Prob15	F: GTGTCCCAGCCAGACCCAAT R: TCAGGTGTCCTGTCTCTGCTTCC	5.5 (1.323)	6[6]	0.135[8]	0.303[8]	0.656[8]	18.61[9]	0.439	0	10
Prob01	F: TGCTCCCCATTCTACAGGTC R: TGTTTCCATAATTTGGCTTGC	3 (0.408)	3.999[14]	0.207[11]	0.186[11]	0.559[11]	5.77[5]	0.129	0.016	11
Prob29	F: CAACACTGTGTATGCCCATGC R: GTTTCTTGTTTGGACCCAGCAATCACC	3.75 (0.629)	4[13]	0.338[13]	0.108[13]	0.415[13]	0[1]	-0.1	0.134	12
Prob34	F: GGTGCTGGAAGGTGGCTTCT R: GCTTTGGCTGGTGGTCCATT	4 (0.408)	4.999[11]	0.363[14]	0.095[14]	0.392[14]	0[1]	-0.055	0.004	13
Prob28	F: GATCAAGGTATCATTAATAAGC R: GAGCTCTCATTGTATGTCAA	3 (0.707)	4.957[12]	0.28[12]	0.167[12]	0.475[12]	8.11[7]	0.14	0.001	14
Prob35	F: ATTGCTGTATTGTGGGTAGG R: GATCAGCTCTTCACAGGAAT	2.5 (0.5)	3.995[15]	0.557[15]	0.034[15]	0.246[15]	0.00[1]	0.055	0.067	15
Prob36	F: GATCAAAAGCTATCTGACTGGACA R: GTTTCTTCCATATTCTCATTTGCTTTC	1.75 (0.25)	2[16]	0.59[16]	0.032[16]	0.221[16]	12.46[8]	0.471	0.001	16
Mean:		6.813 (1.089)	6.910 (1.149)	-	-	0.581 (0.047)	3.62 (1.37)	0.047	-	

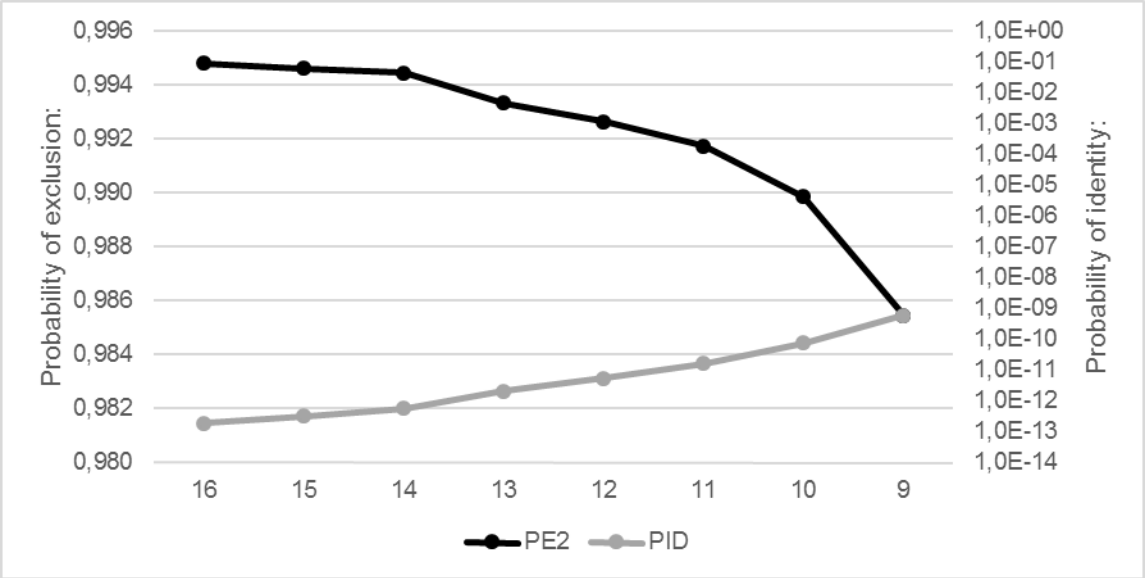


Figure 4-1. The probability of identity (P_{ID}) and probability of exclusion (one parent known, P_{E2}) estimates for the eight microsatellite panels tested in the current study. It can be observed that the full 16 locus panel has the most optimum P_{ID} and P_{E2} values compared to the other seven panels tested in this study.

4.4.2 Parentage analyses

All eight microsatellite panels showed very low combined probability of identity values, with moderate to high informativeness levels (PIC range: 0.581 to 0.703; Supplementary Table 4-4). The probability of identity values for the eight panels ranged from 1.8E-13 for the 16 locus panel to 5.7E-10 for the 9 locus panel (Supplementary Table 4-3). These values suggests 1 in 5.5E+12 (16 loci) to 1.8E+9 (9 loci) randomly chosen individuals will share the same genotype. The assessment from this parameter alone suggests that any of these panels could be suitable for forensic use, as the total number of wild Cape Parrots does not exceed 1600 individuals. The ability of these eight panels to successfully identify known parents, however, differed. The seven larger panels were generally equally successful in identifying parent pairs and individual parents, with only slight differences in the mean probability values and a slightly higher sire identification success rate for the 10 locus panel (Figure 4-2; Supplementary Table 4-4). The 9 locus panel was less successful in correctly identifying parent pairs, with only 71% of parent pairs correctly identified with high probability (probability > 0.75). The 9 locus panel also showed a lower success rate at identifying the correct sires and dames, with 73.7% of sires and 96.8% of dames correctly identified with high probability (probability > 0.75). All known dams were correctly identified using the seven larger panels. Although the seven larger panels had

similar assignment success rates (parent pair assignment success = 83.9%), the full 16 locus panel had overall higher mean probability rates making this panel most suited for use in future parentage analyses.

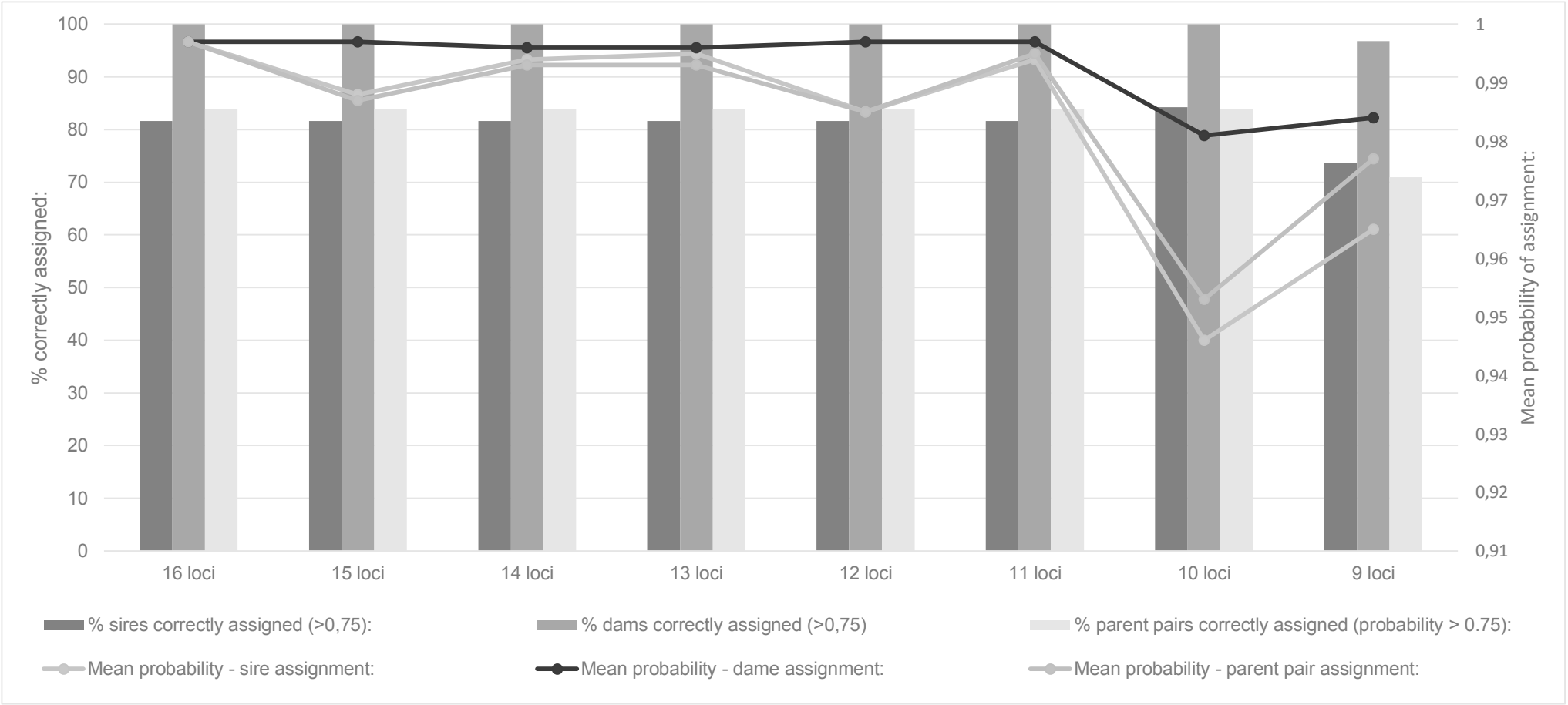


Figure 4-2. The parent pair and individual parentage assignment success of the eight microsatellite panels tested for use in *Poicephalus robustus*. The bars corresponds to the percentage known parents correctly assigned to each offspring, with high probability; the lines are representative of the probability values for each assignment type.

4.4.3 Tracing the origin of birds illegally removed from the wild

The eight microsatellite panels performed well with the Bayesian assignment analysis implemented in GeneClass2, with 83.33% to 66.67% of the test individuals assigned to the correct population of origin. The highest assignment success was achieved with the six larger microsatellite panels (16 loci to 11 loci), with 83.33% of the specimens correctly assigned (Figure 4-3; Supplementary Table 4-5). The 12 locus panel had the best average assignment probability value of the eight tested panels (Average assignment probability = 0.565, SE = 0.087), with five out of the 10 individuals correctly assigned had assignment probabilities above 0.6. The remaining five individuals assigned to the correct populations with assignment probabilities lower than 0.6 (assignment probability = 0.170 – 0.591; Supplementary Table 4-5). The two individuals (FH12 and FH32) that were wrongly assigned, were sampled from the Eastern Cape but assigned to the captive (FH12) and KZN (FH32) populations. The assignment probabilities of these individuals did, however, differ only slightly between the actual population of origin and the assigned population (Supplementary Table 4-5).

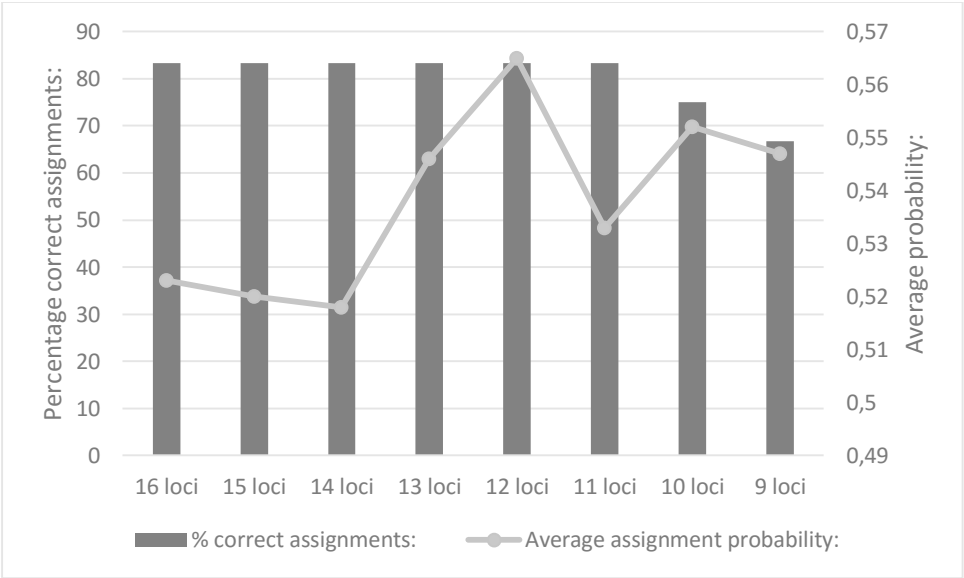


Figure 4-3. The assignment success rates for the eight microsatellite panels tested for their ability to correctly assign specimens to their area of origin in the current study. The black line represents the average assignment probability calculated from the correctly assigned specimens' probability values. The exact probability values for the assignments conducted with each of the eight panels are available in Supplementary Table 4-5.

4.4.4 Genetic diversity: captive versus wild populations

The genetic diversity estimates for the captive data set, using 16 microsatellite loci, was similar to that observed for the wild Cape Parrot populations (Table 4-2). Significant differences were observed between the Captive/Central and Captive/North per locus Na estimates (p-value < 0.0045). The average number of alleles observed for the captive data set was higher than that observed in the wild population (captive, N_A = 6.813, southern, N_A = 6.563; central, N_A = 5.313; northern, N_A = 2.875; Table 4-2). The significant differences observed for the Na estimates could, however, be influenced by differences in sample size (Captive born samples, n = 71; South, n = 60; Central, n = 20; North, n = 5). The allelic richness (Ar) estimates provide a more accurate estimation, with the only significant difference observed among the Captive/North per locus Ar estimates (*p*-value < 0.0045). The captive data set did, however, have the highest number of private alleles (P_A = 21), which was almost double that of the highest value observed among the wild populations (South, P_A = 13). No significant differences were observed between the Captive vs wild per locus observed heterozygosity (H_O), unbiased expected heterozygosity (uH_E) and inbreeding coefficient (F_{IS}) comparisons.

The H_O for the captive data set was only slightly lower than that observed for the three wild populations (captive, H_O = 0.591; southern, H_O = 0.605; central, H_O= 0.647; northern, H_O = 0.6), with an uH_E comparable to that observed for the South and Central wild populations (captive, uH_E = 0.625; southern, uH_E = 0.632; central, uH_E = 0.635). A low positive F_{IS} value was observed for the captive data set indicated only slight inbreeding (F_{IS} = 0.054), with low heterozygote deficiency, which could be expected from captive bred populations (Slade et al., 2014, Snyder et al., 1996). Low genetic differentiation was found only between the captive data set and the South population (F_{ST} = 0.017; *p*-value = 0.001). No significant genetic differentiation was observed between the captive and North populations (F_{ST} = 0.104; *p*-value = 0.004) or the captive and Central populations (F_{ST} = 0.01; *p*-value = 0.024), following a Bonferroni correction (*p*-value = 0.003). The global F_{ST} value calculated for the captive and three wild populations did not significantly differ from zero (F_{ST} = 0.008; *p*-value = 0.008). The analysis of molecular variance (AMOVA) indicated that 92% of the observed genetic variance occurred within individuals, with 5% of the genetic variance between individuals and only 3% among the populations. Similar AMOVA results was observed in a study focusing purely on the genetic variation among the wild Cape Parrot populations (Chapter 3).

Table 4-2. The genetic diversity estimates for each of the wild Cape Parrot populations and the captive data set, using 16 microsatellite loci. Standard error for average number of alleles, observed heterozygosity and unbiased expected heterozygosity is provided in parentheses.

Locality:	Average number of alleles (N_A):	Allelic richness (Ar):	Observed heterozygosity (H_O):	Unbiased expected heterozygosity (uH_E):	Inbreeding coefficient (F_{IS}):	Private alleles (P_A):
South	6.563 (1.252)	3.791 (0.400)	0.605 (0.055)	0.632 (0.053)	0.042	13
Central	5.313 (0.898)	3.708 (0.386)	0.647 (0.058)	0.635 (0.05)	-0.02	5
North	2.875 (0.34)	2.875 (0.340)	0.6 (0.063)	0.572 (0.052)	-0.055	2
Captive	6.813 (1.089)	3.673 (0.314)	0.591 (0.065)	0.625 (0.047)	0.054	21

4.5 Discussion

Microsatellite markers are widely used in parentage analyses, assignment tests and genetic diversity analyses and have been used successfully in parrots (Presti and Wasko, 2014). To investigate the usefulness of a range of species specific microsatellite markers for forensic applications in the Cape Parrot, 16 microsatellite loci were analysed and tested across 76 captive Cape Parrots. The optimum combination and number of loci were identified for use in parentage analysis and population assignment analysis, by assessing the informativeness of each locus. Only slight genetic differences were detected between the captive Cape Parrot data set and the wild populations.

4.5.1 Microsatellite loci for parentage analysis

The level of variation and informativeness observed in the current study is comparable to that observed in other studies. The PIC values observed in the current study falls within the same range as the values observed by Klauke et al. (2013) during a study investigating the breeding system of the endangered El Oro parakeet from southwest Ecuador. The combined P_{ID} observed by Russello et al. (2007a) for 14 loci used in the South American Monk parakeet was lower than that observed for Cape Parrots in the current study.

The locus informativeness analyses performed on the 16 microsatellite loci, allowed for the ranking of the 16 loci according to their level of informativeness. The full locus set of 16 markers showed to be the best combination of markers for parentage analysis, of the eight panels tested. This panel had the highest average assignment probabilities for parent pair, sire

and dame assignment tests (Figure 4-2). The 16 locus panel had a combined P_{ID} of 1.8E-13 and a combined P_{E2} of 0.995. This microsatellite panel has a probability of identity value suggesting that 1 in 5.5E+12 individuals will share the same genotype, indicating that this panel is highly suited for individual-level identification. All the known dames were positively identified with this panel, but only 31 of the 38 offspring's known sires were identified with high probability. It was observed that the 10 locus panel did have a better assignment rate for the known sires, with 32 of the 38 sires identified, but the average assignment probability value was much lower than that observed for the 16 locus panel (Figure 4-2). The failure to assign a parent, or assignment of a parent with low probability, can be linked to the occurrence of amplification errors during PCR causing allelic dropout, null alleles or stuttering (Dewoody et al., 2006, Ferrie et al., 2013). It is possible to compensate for such errors in the parentage analysis program Colony by importing the expected error rates of each locus, including null allele frequencies, prior to analysis. When this was done in the current study, failed or incorrect assignments were still observed and it is advisable to not only rely on genetic data, but also make use of a complete studbook of legally registered captive bred birds, as suggested by Ferrie et al. (2013). It is therefore important to compile a complete studbook of all captive bred Cape Parrots, complemented by a complete DNA data base using the full 16 locus microsatellite panel described in this study.

4.5.2 Population of origin analysis

The use of assignment methods to identify the population or area of origin of confiscated wildlife is a well-known tool in wildlife forensics (Alacs et al., 2010, Ogden and Linacre, 2015). The partial Bayesian assignment analysis implemented in GeneClass2 is a well-known method for assignment of specimens to their population of origin. This method has been used on a wide range of wildlife species, including fish (Grobler et al., 2005, Primmer et al., 2000, Renshaw et al., 2006), birds (Chan et al., 2002, Leader et al., 2008, Pruett et al., 2010), reptiles (Davy et al., 2014, Graciá et al., 2011, Russello et al., 2007b), marsupials (Eldridge et al., 2001, Piggott et al., 2006), placental mammals (Bilgmann et al., 2011, Fischer et al., 2015, Joshi et al., 2013, Kotze et al., 2008) and monotremes (Furlan et al., 2012). An example of the forensic application of this method can be taken from a study by Primmer et al. (2000). These authors used the methods implemented in GeneClass to investigate a case of fishing competition fraud and successfully excluded the fishing competition location as the origin of the fish in question and supporting the claim of competition fraud (Primmer et al., 2000).

The assignment analysis performed with six of the eight microsatellite panels (16 loci to 11 loci) all had a success rate of 83.33%. The average assignment probabilities of the correct assignments did however differ, with the 12 locus panel outperforming the rest (Figure 4-3). The two Eastern Cape individuals were not assigned to their population of origin, with sample *FH12* assigned to the Captive population and *FH32* assigned to KZN (Supplementary Table 4-5). The probabilities that these two samples should be assigned to the Captive and KZN populations were only marginally higher than the probabilities observed for assignment to the Eastern Cape population. This could be due to the occurrence of ancestral admixture, as it is shown that the southern (Eastern Cape) populations form a source to the central (KZN) populations (Chapter 3), and the captive bred populations in turn is largely sourced from the KZN populations (C.T. Downs pers. comm.). These individuals could therefore have ancestral links to individuals in the central (KZN) and the captive populations (via the KZN populations).

4.5.3 Captive vs Wild Cape Parrots

The majority of the genetic differentiation estimates showed little to no genetic difference between the captive data set and the three wild Cape Parrot populations. A clear difference was, however, observed for the private allele estimate. The captive data set contained almost double the amount of private alleles observed in the southern wild population, which the recent phylogeographic study suggests is the most genetically diverse wild population (Chapter 3). In theory, the higher number of private alleles in the captive population could be due to rare alleles, which are generally not often seen in the wild, being present in the founders of the captive populations. Gautschi et al. (2003) observed a similar trend in a captive bearded vulture (*Gypaetus barbatus*) population, with a higher level of genetic diversity in the captive population when compared to that observed in the wild population. This was linked to founder individuals, who are still present in the breeding population, carrying rare alleles and thereby passing these alleles down to their offspring (Gautschi et al., 2003). It is therefore possible that the captive Cape Parrots have not been in captivity for an appropriate amount of time to lose the observed rare alleles, and that these alleles are still being passed down to the new generations. New wild birds are also regularly introduced to the captive stock, through the addition of injured or confiscated wild birds (Wilkinson, 2015). These introductions could then also supplement the genetic diversity of the captive population, especially if the birds originate from different regions of the Cape Parrot's natural distribution range. Many of the birds in the

captive data set used in the current study are F1 to F3 descendants from wild birds, and could therefore still carry these rare alleles.

The captive and wild birds are also subjected to different environmental forces, which can lead to genetic adaptation to captivity (Frankham, 2008). The difference in selective pressures like lack of predators, food availability and pathogenic exposure could promote the selection of certain traits in captive animals, which would normally be detrimental in the wild (Lynch and O'Hely, 2001). It is possible for selection of certain rare, fitness linked, loci to influence the genetic diversity of neutral loci like microsatellites, although it was observed to mostly decrease the genetic diversity of neutral loci (Montgomery et al., 2010). It could, therefore, also be argued that the large number of private alleles observed in the captive sample set could in some way be linked to the selection of rare alleles, due to human mediated mate selection of breeding pairs. Further fitness analyses using fitness linked loci, like the major histocompatibility complex (MHC) genes or toll like receptor (TLR) genes, should be performed to better understand the effects captive breeding has on the genetic health of the Cape Parrot population.

4.6 Conclusions

The assessment of the 16 microsatellite loci tested in the current study identified the full 16 locus panel as the best set of markers for use in parentage analysis. Such analyses should be performed on traded birds suspected of being illegally harvested from the wild. It is therefore important to have a data base of all legally owned Cape Parrots and a complete studbook for future use. Using this set of loci, birds suspected of being illegally harvested from the wild can be traced to region of origin through implementation of the partial Bayesian approach in GeneClass2 for individual assignment analysis. The 12 locus microsatellite panel is most appropriate for this analysis. It is, however, recommended to increase the reference data sets, for both the wild and captive populations, thereby increasing the accuracy of the individual assignment analysis using the assignment methods implemented in GeneClass2. This recommendation is based on the low level of differentiation observed between the wild and captive populations. The use of additional highly polymorphic loci could improve these results (Cornuet et al., 1999). The high number of private alleles observed in the captive population highlights its distinctiveness. Reintroductions to the wild from the current captive population is not recommended until further analyses of fitness related loci are performed, as accumulation

of certain rare alleles could have detrimental effects on the wild populations. It is further recommended that, for reintroduction purposes, captive populations from the three Cape Parrot populations should be kept separate to prevent unnatural admixture of the different genetic lineages.

The results from this study will help conservation and law enforcement authorities to better police and identify cases of illegal trafficking in South Africa's only endemic parrot. The information obtained here also highlights the genetic distinctiveness of the captive population, and the effect these birds will have on wild populations should be considered before any future re-introductions plans are made.

4.7 Acknowledgements

I would like to thank all the private Cape Parrot breeders for their support in providing samples for this study; Mr. William Horsfield (Amazona Birds Cc), Mr T. Davies and Mrs L. Davies (private breeders) and Mr Shaun Wilkinson (Montecasino Bird Gardens, Johannesburg). I would also like to thank all the funders of this project; University of KwaZulu-Natal, National Research Foundation and the South African National Biodiversity Institute.

4.8 References

ALACS, E. A., GEORGES, A., FITZSIMMONS, N. N. & ROBERTSON, J. 2010. DNA detective: a review of molecular approaches to wildlife forensics. *Forensic Science, Medicine, and Pathology,* 6**,** 180-194.

BERGNER, L., JAMIESON, I. & ROBERTSON, B. 2014. Combining genetic data to identify relatedness among founders in a genetically depauperate parrot, the Kakapo (Strigops habroptilus). *Conservation Genetics***,** 1-8.

BILGMANN, K., MÖLLER, L., HARCOURT, R., KEMPER, C. & BEHEREGARAY, L. 2011. The Use of Carcasses for the Analysis of Cetacean Population Genetic Structure: A Comparative Study in Two Dolphin Species. *PLoS ONE,* 6**,** e20103.

BIRDLIFE INTERNATIONAL. 2008. *The illegal parrot trade remains a problem in Latin America* [Online]. BirdLife International. Available: http://www.birdlife.org/datazone/sowb/casestudy/165 [Accessed 20 August 2015].

BIRDLIFE INTERNATIONAL. 2012. *Unsustainable exploitation of birds is most prevalent in Asia* [Online]. BirdLife International. Available: http://www.birdlife.org/datazone/sowb/casestudy/163 [Accessed 18 August 2015].

BIRDLIFE INTERNATIONAL. 2013. *Trade in Africa's Grey Parrots and Timneh Parrots is currently not sustainable* [Online]. BirdLife International. Available: http://www.birdlife.org/datazone/sowb/casestudy/568 [Accessed 23 November 2015].

BIRDLIFE INTERNATIONAL. 2015a. *Species factsheet: Psittacus erithacus* [Online]. BirdLife International. Available: http://www.birdlife.org/datazone/speciesfactsheet.php?id=112657 [Accessed 23 November 2015].

BIRDLIFE INTERNATIONAL. 2015b. *Wild bird trade and CITES* [Online]. Birdlife International. Available: http://www.birdlife.org/worldwide/policy/wild-bird-trade-and-cites [Accessed 23 November 2015].

BOTSTEIN, D., WHITE, R. L., SKOLNICK, M. & DAVIS, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics,* 32**,** 314.

BOYCE, W. M., WEISENBERGER, M. E., PENEDO, M. C. T. & JOHNSON, C. K. 2011. Wildlife translocation: the conservation implications of pathogen exposure and genetic heterozygosity. *BMC Ecology,* 11**,** 5.

BROAD, S., MULLIKEN, T. & ROE, D. 2002. The nature and extent of legal and illegal trade in wildlife. *In:* OLDFIELD, S. (ed.) *The trade in wildlife: regulation for conservation.* London: Earthscan Publications Ltd.

BUCHANAN, F. C. & THUE, T. D. 1998. Intrabreed polymorphic information content of microsatellites in cattle and sheep. *Canadian Journal of Animal Science,* 78**,** 425-428.

BUSH, E. R., BAKER, S. E. & MACDONALD, D. W. 2014. Global Trade in Exotic Pets 2006–2012. *Conservation Biology,* 28**,** 663-676.

CANTÚ–GUZMÁN, J., SALDANA, M. E. S., GROSSELET, M. & GAMEZ, J. S. 2007. *The illegal parrot trade in Mexico: A comprehensive assessment,* Mexico and Washington, DC: Defenders of Wildlife.

CHAN, Y., ARCESE, P. & HACKETT, S. 2002. Subspecific differentiation and conservation of song sparrows (*Melospiza melodia*) in the San Francisco Bay region inferred by microsatellite loci analysis. *The Auk,* 119**,** 641-657.

CHAPUIS, M.-P. & ESTOUP, A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution,* 24**,** 621-631.

COETZER, W. G., DOWNS, C. T., PERRIN, M. R. & WILLOWS-MUNRO, S. 2015. Molecular Systematics of the Cape Parrot (*Poicephalus robustus*): Implications for Taxonomy and Conservation. *PLoS ONE,* 10**,** e0133376.

COGHLAN, M. L., WHITE, N. E., PARKINSON, L., HAILE, J., SPENCER, P. B. & BUNCE, M. 2012. Egg forensics: an appraisal of DNA sequencing to assist in species identification of illegally smuggled eggs. *Forensic Science International: Genetics,* 6**,** 268-273.

COMSTOCK, K. E., OSTRANDER, E. A. & WASSER, S. K. 2003. Amplifying nuclear and mitochondrial DNA from African elephant ivory: a tool for monitoring the ivory trade. *Conservation Biology,* 17**,** 1840-1843.

COONEY, R. & JEPSON, P. 2006. The international wild bird trade: what's wrong with blanket bans? *Oryx,* 40**,** 18-23.

CORNUET, J. M., PIRY, S., LUIKART, G., ESTOUP, A. & SOLIGNAC, M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics,* 153**,** 1989-2000.

COURCHAMP, F., ANGULO, E., RIVALAN, P., HALL, R. J., SIGNORET, L., BULL, L. & MEINARD, Y. 2006. Rarity value and species extinction: the anthropogenic Allee effect. *PLoS Biology,* 4**,** e415.

DAKIN, E. E. & AVISE, J. C. 2004. Microsatellite null alleles in parentage analysis. *Heredity,* 93**,** 504-509.

DAVY, C. M., BERNARDO, P. H. & MURPHY, R. W. 2014. A Bayesian approach to conservation genetics of Blanding's turtle (*Emys blandingii*) in Ontario, Canada. *Conservation Genetics,* 15**,** 319-330.

DAWNAY, N., OGDEN, R., WETTON, J. H., THORPE, R. S. & MCEWING, R. 2009. Genetic data from 28 STR loci for forensic individual identification and parentage analyses in 6 bird of prey species. *Forensic Science International: Genetics,* 3**,** e63-e69.

DEWOODY, J., NASON, J. D. & HIPKINS, V. D. 2006. Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes,* 6**,** 951-957.

DOWNS, C. T., PFEIFFER, M. & HART, L. A. 2014. Fifteen years of annual Cape Parrot *Poicephalus robustus* censuses: current population trends and conservation contributions. *Ostrich,* 85**,** 273-280.

ELDRIDGE, M. D. B., KINNEAR, J. E. & ONUS, M. L. 2001. Source population of dispersing rock-wallabies (*Petrogale lateralis*) identified by assignment tests on multilocus genotypic data. *Molecular Ecology Notes,* 10**,** 2867-2878.

FERRIE, G. M., COHEN, O. R., SCHUTZ, P., LEIGHTY, K. A., PLASSE, C., BETTINGER, T. L. & HOFFMAN, E. A. 2013. Identifying parentage using molecular markers: Improving accuracy of studbook records for a captive flock of marabou storks (Leptoptilus crumeniferus). *Zoo Biology,* 32**,** 556-564.

FISCHER, M. L., HOCHKIRCH, A., HEDDERGOTT, M., SCHULZE, C., ANHEYER-BEHMENBURG, H. E., LANG, J., MICHLER, F.-U., HOHMANN, U., ANSORGE, H., HOFFMANN, L., KLEIN, R. & FRANTZ, A. C. 2015. Historical Invasion Records Can Be Misleading: Genetic Evidence for Multiple Introductions of Invasive Raccoons (*Procyon lotor*) in Germany. *PLoS ONE,* 10**,** e0125441.

FRANKHAM, R. 2008. Genetic adaptation to captivity in species conservation programs. *Molecular Ecology,* 17**,** 325-333.

FRANKHAM, R., BALLOU, J. D. & BRISCOE, D. A. 2002. *Introduction to conservation genetics,* UK, Cambridge University Press.

FUNG, W. K., CHUNG, Y. & WONG, D. 2002. Power of exclusion revisited: probability of excluding relatives of the true father from paternity. *International Journal of Legal Medicine,* 116**,** 64-67.

FURLAN, E., STOKLOSA, J., GRIFFITHS, J., GUST, N., ELLIS, R., HUGGINS, R. M. & WEEKS, A. R. 2012. Small population size and extremely low levels of genetic diversity in island populations of the platypus, *Ornithorhynchus anatinus*. *Ecology and Evolution,* 2**,** 844-857.

GAUTSCHI, B., MÜLLER, J., SCHMID, B. & SHYKOFF, J. 2003. Effective number of breeders and maintenance of genetic diversity in the captive bearded vulture population. *Heredity,* 91**,** 9-16.

GONÇALVES, P. F. M., OLIVEIRA-MARQUES, A. R., MATSUMOTO, T. E. & MIYAKI, C. Y. 2015. DNA barcoding identifies illelgal parrot trade. *Jounal of Heredity,* 106**,** 560-564.

GOUDET, J. 2001. *FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3)* [Online]. Available: http://www.unil.ch/zea/softwares/fstat.html.

GRACIÁ, E., GIMÉNEZ, A., ANADÓN, J. D., BOTELLA, F., GARCÍA-MARTÍNEZ, S. & MARÍN, M. 2011. Genetic patterns of a range expansion: The spur-thighed tortoise *Testudo graeca graeca* in southeastern Spain. *Amphibia-Reptilia,* 32**,** 49-61.

GROBLER, J. P., KOTZE, A., SWART, H. & HALLERMAN, E. M. 2005. The application of microstellite DNA markers for forensic analysis of koi carp (*Cyprinus carpio*): research in action. *South African Journal of Science,* 101**,** 19-21.

GUPTA, S. K., VERMA, S. K. & SINGH, L. 2005. Molecular insight into a wildlife crime: the case of a peafowl slaughter. *Forensic Science International,* 154**,** 214-217.

HINDAR, K., RYMAN, N. & UTTER, F. 1991. Genetic effects of cultured fish on natural fish populations. *Canadian Journal Of Fish and Aquatic Science,* 48**,** 945-957.

INTERPOL. 2014. *Illegal trade in wildlife and timber products finances criminal and militia groups, threatening security and sustainable development* [Online]. Available: http://www.interpol.int/News-and-media/News/2014/N2014-119 [Accessed 20 August 2015].

JOHNSON, M. S. 2000. Measuring and interpreting genetic structure to minimize the genetic risks of translocations. *Aquaculture Research,* 31**,** 133-143.

JONES, O. R. & WANG, J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources,* 10**,** 551-555.

JOSHI, A., VAIDYANATHAN, S., MONDOL, S., EDGAONKAR, A. & RAMAKRISHNAN, U. 2013. Connectivity of Tiger (*Panthera tigris*) Populations in the Human-Influenced Forest Mosaic of Central India. *PLoS ONE,* 8**,** e77980.

KALINOWSKI, S. T., TAPER, M. L. & MARSHALL, T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology,* 16**,** 1099-1106.

KLAUKE, N., SEGELBACHER, G. & SCHAEFER, H. M. 2013. Reproductive success depends on the quality of helpers in the endangered, cooperative El Oro parakeet (*Pyrrhura orces*). *Molecular Ecology,* 22**,** 2011-2027.

KOTZE, A., EHLERS, K., CILLIERS, D. C. & GROBLER, J. P. 2008. The power of resolution of microsatellite markers and assignment tests to determine the geographic origin of cheetah (*Acinonyx jubatus*) in Southern Africa. *Mammalian Biology,* 73**,** 457-462.

LABUSCHAGNE, C., NUPEN, L., KOTZÉ, A., GROBLER, P. J. & DALTON, D. L. 2015. Assessment of microsatellite and SNP markers for parentage assignment in ex situ African Penguin (*Spheniscus demersus*) populations. *Ecology and Evolution,* 5**,** 4389-4399.

LEADER, N., GEFFEN, E., MOKADY, O. & YOM-TOV, Y. 2008. Song dialects do not restrict gene flow in an urban population of the orange-tufted sunbird, *Nectarinia osea*. *Behavioral Ecology and Sociobiology,* 62**,** 1299-1305.

LI, L. & JIANG, Z. 2014. International Trade of CITES Listed Bird Species in China. *PLoS ONE,* 9**,** e85012.

LORENZINI, R. 2005. DNA forensics and the poaching of wildlife in Italy: A case study. *Forensic Science International,* 153**,** 218-221.

LORENZINI, R., CABRAS, P., FANELLI, R. & CARBONI, G. L. 2011. Wildlife molecular forensics: identification of the Sardinian mouflon using STR profiling and the Bayesian assignment test. *Forensic Science International: Genetics,* 5**,** 345-349.

LOUGHNAN, S. R., SMITH-KEUNE, C., JERRY, D. R., BEHEREGARAY, L. B. & ROBINSON, N. A. 2015. Genetic diversity and relatedness estimates for captive barramundi (*Lates calcarifer*, Bloch) broodstock informs efforts to form a base population for selective breeding. *Aquaculture Research*.

LOW, B. W. 2014. The global trade in native Australian parrots through Singapore between 2005 and 2011: a summary of trends and dynamics. *Emu,* 114**,** 277-282.

LYNCH, M. & O'HELY, M. 2001. Captive breeding and the genetic fitness of natural populations. *Conservation Genetics,* 2**,** 363-378.

LYONS, J. A. & NATUSCH, D. J. D. 2011. Wildlife laundering through breeding farms: Illegal harvest, population declines and a means of regulating the trade of green pythons (Morelia viridis) from Indonesia. *Biological Conservation,* 144**,** 3073-3081.

MANEL, S., BERTHIER, P. & LUIKART, G. 2002. Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. *Conservation Biology,* 16**,** 650-659.

MASELLO, J. F., SRAMKOVA, A., QUILLFELDT, P., EPPLEN, J. T. & LUBJUHN, T. 2002. Genetic monogamy in burrowing parrots Cyanoliseus patagonus? *Journal of Avian Biology,* 33**,** 99-103.

MATEESCU, R., ZHANG, Z., TSAI, K., PHAVAPHUTANON, J., BURTON-WURSTER, N., LUST, G., QUAAS, R., MURPHY, K., ACLAND, G. M. & TODHUNTER, R. J. 2005. Analysis of allele fidelity, polymorphic information content, and density of microsatellites in a genome-wide screening for hip dysplasia in a crossbreed pedigree. *Journal of Heredity,* 96**,** 847-853.

MONDOL, S., SRIDHAR, V., YADAV, P., GUBBI, S. & RAMAKRISHNAN, U. 2014. Tracing the geographic origin of traded leopard body parts in the indian subcontinent with DNA-based assignment tests. *Conservation Biology,* 29**,** 556-564.

MONTGOMERY, M. E., WOODWORTH, L. M., ENGLAND, P. R., BRISCOE, D. A. & FRANKHAM, R. 2010. Widespread selective sweeps affecting microsatellites in *Drosophila* populations adapting to captivity: Implication for captive breeding programs. *Biological Conservation,* 143**,** 1842-1849.

NOGUEIRA, S. S. & NOGUEIRA-FILHO, S. L. 2011. Wildlife farming: an alternative to unsustainable hunting and deforestation in Neotropical forests? *Biodiversity and Conservation,* 20**,** 1385-1397.

OGDEN, R. & LINACRE, A. 2015. Wildlife forencis science: A review of genetic geogrphic origin assignment. *Forensic Science International,* 18**,** 152-159.

PAETKAU, D., SLADE, R., BURDEN, M. & ESTOUP, A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology,* 13**,** 55-65.

PAIN, D., MARTINS, T., BOUSSEKEY, M., DIAZ, S., DOWNS, C., EKSTROM, J., GARNETT, S., GILARDI, J., MCNIVEN, D. & PRIMOT, P. 2006. Impact of protection on nest take and nesting success of parrots in Africa, Asia and Australasia. *Animal Conservation,* 9**,** 322-330.

PEAKALL, R. & SMOUSE, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* 28**,** 2537-2539.

PIGGOTT, M. P., BANKS, S. C. & TAYLOR, A. C. 2006. Population structure of brush-tailed rock-wallaby (*Petrogale penicillata*) colonies inferred from analysis of faecal DNA. *Molecular Ecology Notes,* 15**,** 93-105.

PILLAY, K., DAWSON, D. A., HORSBURGH, G. J., PERRIN, M. R., BURKE, T. & TAYLOR, T. D. 2010. Twenty-two polymorphic microsatellite loci aimed at detecting illegal trade in the Cape parrot, *Poicephalus robustus* (Psittacidae, AVES). *Molecular Ecology Resources,* 10**,** 142-149.

PIRES, S. F. 2012. The illegal parrot trade: a literature review. *Global Crime,* 13**,** 176-190.

PIRY, S., ALAPETITE, A., CORNUET, J. M., PAETKAU, D., BAUDOUIN, L. & ESTOUP, A. 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity,* 95**,** 536-539.

PRESTI, F. T., GUEDES, N. M., ANTAS, P. T. & MIYAKI, C. Y. 2015. Population genetic structure in Hyacinth Macaws (*Anodorhynchus hyacinthinus*) and identification of the probable origin of confiscated individuals. *Journal of Heredity,* 106**,** 491-502.

PRESTI, F. T. & WASKO, A. P. 2014. A review of microsatellite markers and their application on genetic diversity studies in parrots. *Open Journal of Genetics,* 4**,** 69-77.

PRIMMER, C. R., KOSKINEN, M. T. & PIIRONEN, J. 2000. The one that did not get away: individual assignment using microsatellite data detects a case of fishing competition fraud. *Proceedings of the Royal Society of London B: Biological Sciences,* 267**,** 1699-1704.

PRUETT, C. L., TURNER, T. N., TOPP, C. M., ZAGREBELNY, S. V. & WINKER, K. 2010. Divergence in an archipelago and its conservation consequences in Aleutian Island rock ptarmigan. *Conservation Genetics,* 11**,** 241-248.

RANNALA, B. & MOUNTAIN, J. L. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences,* 94**,** 9197-9201.

REGUEIRA, R. F. S. & BERNARD, E. 2012. Wildlife sinks: Quantifying the impact of illegal bird trade in street markets in Brazil. *Biological Conservation,* 149**,** 16-22.

RENSHAW, M. A., SAILLANT, E., BROUGHTON, R. E. & GOLD, J. R. 2006. Application of hypervariable genetic markers to forensic identification of 'wild' from hatchery-raised red drum, *Sciaenops ocellatus*. *Forensic Science International,* 156**,** 9-15.

RICE, W. 1986. Analyzing tables of statistical tests. *Evolution,* 1**,** 223-225.

RIEHL, C. 2012. Mating system and reproductive skew in a communally breeding cuckoo: hard-working males do not sire more young. *Animal Behaviour,* 84**,** 707-714.

ROBERT, A. 2009. Captive breeding genetics and reintroduction success. *Biological Conservation,* 142**,** 2915-2922.

ROSENBERG, N. A., LI, L. M., WARD, R. & PRITCHARD, J. K. 2003. Informativeness of Genetic Markers for Inference of Ancestry. *The American Journal of Human Genetics,* 73**,** 1402-1422.

ROUSSET, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources,* 8**,** 103-106.

RUSSELLO, M., SARANATHAN, V., BUHRMAN-DEEVER, S., EBERHARD, J. & CACCONE, A. 2007a. Characterization of polymorphic microsatellite loci for the invasive monk parakeet (Myiopsitta monachus). *Molecular Ecology Notes,* 7**,** 990-992.

RUSSELLO, M. A., BRAZAITIS, P., GRATTEN, J., WATKINS-COLWELL, G. J. & CACCONE, A. 2007b. Molecular assessment of the genetic integrity, distinctiveness and phlogeographic context of the Saltwater crocodile (*Crocodylus porosus*) on Palau. *Conservation Genetics,* 8**,** 777-787.

SLADE, B., PARROTT, M. L., PAPROTH, A., MAGRATH, M. J., GILLESPIE, G. R. & JESSOP, T. S. 2014. Assortative mating among animals of captive and wild origin following experimental conservation releases. *Biology Letters,* 10**,** 20140656.

SNYDER, N. F., DERRICKSON, S. R., BEISSINGER, S. R., WILEY, J. W., SMITH, T. B., TOONE, W. D. & MILLER, B. 1996. Limitations of captive breeding in endangered species recovery. *Conservation Biology***,** 338-348.

STORFER, A. 1999. Gene flow and endangered species translocation: a topic revisited. *Biological Conservation,* 87**,** 173-180.

TABERLET, P. & LUIKART, G. 1999. Non-invasive genetic sampling and individual identification. *Biological Journal of the Linnean Society,* 68**,** 41-55.

TAYLOR, M. 2014. Eskom Red data book of birds of South Africa, Lesotho and Swaziland. Birdlife South Africa, Johannesburg.

TRAFFIC. 2008. *Our Work: wildlife trade* [Online]. Available: http://www.traffic.org/trade/ [Accessed 23 November 2015].

WAITS, L. P., LUIKART, G. & TABERLET, P. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology,* 10**,** 249-256.

WANG, J. 2004. Sibship Reconstruction From Genetic Data With Typing Errors. *Genetics,* 166**,** 1963-1979.

WARCHOL, G. L. 2004. The transnational illegal wildlife trade. *Criminal Justice Studies,* 17**,** 57-73.

WESTON, M. & MEMON, M. 2009. The illegal parrot trade in Latin America and its consequences to parrot nutrition, health and conservation. *Bird populations,* 9**,** 76-83.

WHITE, N. E., DAWSON, R., COGHLAN, M. L., TRIDICO, S. R., MAWSON, P. R., HAILE, J. & BUNCE, M. 2012. Application of STR markers in wildlife forensic casework involving Australian black-cockatoos (*Calyptorhynchus* spp.). *Forensic Science International: Genetics,* 6**,** 664-670.

WILKINSON, S. 2015. Regional Studbook: Cape Parrot Studbook (*Poicephalus robustus*). Montecasino Bird Gardens (under the auspices of PAAZA).

WILLIAMS, S. E. & HOFFMAN, E. A. 2009. Minimizing genetic adaptationin captive breeding programs: A review. *Biological Conservation,* 142**,** 2388-2400.

WIRMINGHAUS, J. O. 1997. Cape Parrot *Poicephalus robustus*. *In:* HARRISON, J. A., ALAN, D. G., UNDERHILL, L. L., HERREMANS, M., TREE, A. J., PARKER, V. & BROWN, C. J. (eds.) *The Atlas of Southern African Birds.* Johannesburg: BirdLife South Africa.

WIRMINGHAUS, J. O., DOWNS, C. T., SYMES, C. T. & PERRIN, M. R. 1999. Conservation of the Cape parrot in southern Africa. *South African Journal of Wildlife Research,* 29**,** 118-129.

WITZENBERGER, K. A. & HOCHKIRCH, A. 2011. Ex situ conservation genetics: a review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. *Biodiversity and Conservation,* 20**,** 1843-1861.

WYNBERG, R. 2002. A decade of biodiversity conservation and use in South Africa: tracking progress form the Rio Earth Summit to the Johannesburg World Summit on Sustainable Development: review article. *South African Journal of Science,* 98**,** 233-243.

4.9 Supplementary Information

Supplementary Table 4-1. Sample information for all samples used in Chapter 4; a) identification and origins of all captive specimens used in the current study and b) number of specimens from each of the breeding facilities (BF).

a)

Received specimen ID:	ID from current study:	Captive bred (CB) or Wild born (WB)	Breeding facility (BF):
L+T 138	Cap01	CB	BF1
L+T 156	Cap02	CB	BF1
L+T 157	Cap03	CB	BF1
L+T 402	Cap04	CB	BF1
08 WAH 17 ZA-0	Cap05	CB	BF2
WAH 35	Cap06	CB	BF2
WAH 36	Cap07	CB	BF2
WAH 20	Cap08	CB	BF2
20 WAH 10	Cap09	CB	BF2
43671B7C54	Cap10	CB	BF6
None	Cap11	CB	BF3
None	Cap12	CB	BF3
None	Cap13	CB	BF3
3155	Cap14	CB	BF4
3907	Cap15	CB	BF4
4029	Cap16	CB	BF4
4020	Cap17	CB	BF4
4835	Cap18	CB	BF4
3157	Cap19	CB	BF4
10 WAH 25	Cap20	CB	BF2
22343	Cap21	CB	BF5
22345	Cap22	CB	BF5
22346	Cap23	CB	BF5
24382	Cap24	CB	BF5
435A0F1075	Cap25	CB	BF6
433F1C6B2D	Cap26	CB	BF6
43592F1837	Cap27	CB	BF6
442F10027B	Cap28	CB	BF6
43681A3B71	Cap29	CB	BF6
06 WAH 47	F1	CB	BF2
GT 984	F2	CB	BF2
04 WAH 48	F3	CB	BF2
L+T 112 ZA-0	F5	CB	BF2
08 WAH 19 ZA-0	F6	CB	BF2
46047A4221	F7	CB	BF2
48681A2352	F8	CB	BF2
000102FFF7	F9	CB	BF2
500C596124	F10	WB	BF2
0001D23107	F11	CB	BF2
4359237E38	F15	WB	BF2
03 WAH 7	M1	CB	BF2
CPWG	M2	WB	BF2
97 LZ 3	M3	CB	BF2

Supplementary Table 4-1.a (Cont.)			
WAH 45	M5	CB	BF2
00-0124-A357	M6	CB	BF2
00-01D2-F647	M7	CB	BF2
02 WAH 01	M8	CB	BF2
4 356 475 761	M10	WB	BF1
0001245B22	M11	CB	BF2
00-01D1-F720	M12	CB	BF2
43670C6372	M15	WB	BF2
13 WAH 10	O1i	CB	BF2
13 WAH 11	O1ii	CB	BF2
13 WAH 39	O1iii	CB	BF2
13 WAH 41	O1iv	CB	BF2
L+T 172	O2i	CB	BF2
L+T 174	O2ii	CB	BF2
13 WAH 4	O3i	CB	BF2
13 WAH 5	O3ii	CB	BF2
Chick of 03 WAH 07	O4i	CB	BF2
14 WAH 5	O5i	CB	BF2
14 WAH 6	O5ii	CB	BF2
14 WAH 7	O5iii	CB	BF2
14 WAH 10	O6i	CB	BF2
14 WAH 11	O6ii	CB	BF2
14 WAH 18	O6iii	CB	BF2
11 WAH 38	O7i	CB	BF2
13 WAH 44	O7ii	CB	BF2
13 WAH 45	O7iii	CB	BF2
15 WAH 1	O8i	CB	BF2
02 WAH 11	O9i	CB	BF2
02 WAH 20	O9ii	CB	BF2
WAH 15	O9iii	CB	BF2
WAH 32	O10i	CB	BF2
WAH 39	O11i	CB	BF2
WAH 40	O11ii	CB	BF2

b)

Breeding facility (BF):	Number of specimens:	% contribution to data set:
BF1	5	6.579
BF2	52	68.421
BF3	3	3.947
BF4	6	7.895
BF5	4	5.263
BF6	6	7.895

Supplementary Table 4-2. Wild caught Cape Parrot genotypes sourced from Chapter 3 for the captive vs wild Cape Parrot analyses in Chapter 4. Each sample's membership to one of the three genetic clusters identified in Chapter 3 is provided.

Sample code:	Museum ID:	Location:	GPS coordinates:	Date sampled:	Sourced:	Genetic cluster:
FH01	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH02	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH03	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH04	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH05	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH06	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH07	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH08	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH09	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH10	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH11	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH12	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH13	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH14	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH15	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH16	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH17	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH18	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH19	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH20	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH21	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH22	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH23	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH24	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH25	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH26	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH27	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH28	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH29	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH30	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH31	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH32	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH33	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH34	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH35	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH36	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH37	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH38	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH39	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH40	NA	Alice, Eastern Cape	-32.796097, 26.850024	2010	University of Cape Town	South
FH41	NA	Alice, Eastern Cape	-32.796097, 26.850024	2013	This study (University of KwaZulu-Natal)	South
KWT01	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South

Supplementary Table 4-2 (Cont.)

KWT02	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South
KWT03	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South
KWT04	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South
KWT05	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South
KWT06	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South
KWT07	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South
KWT08	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South
KWT09	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South
KWT10	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2011	University of Cape Town	South
KWT11	NA	King William's Town, Eastern Cape	-32.880202, 27.398856	2013	This study (University of KwaZulu-Natal)	South
ECH12	6162	Needs Camp, Amathole, East London area, Eastern Cape	-32.995257, 27.647519	1958	East London Museum	South
ECH13	6163	Needs Camp, Amathole, East London area, Eastern Cape	-32.995257, 27.647519	1958	East London Museum	South
ECH14	7201	Cambridge district, East London, Eastern Cape	-33.008834, 27.802254	1959	East London Museum	South
ECH15	13276	Lusikisiki, Eastern Cape	-31.366218, 29.570018	1968	East London Museum	South
ECH16	13277	Lusikisiki, Eastern Cape	-31.366218, 29.570018	1968	East London Museum	South
ECH17	P.r.r 8266	Eastern Cape	Unknown	1960	Durban Natural Sciences Museum	South
ECH18	P.r.r 8267	Eastern Cape	Unknown	1954	Durban Natural Sciences Museum	South
ECH21	TM 40942	Eastern Cape	-31.366218, 29.570018	1951	Ditsong National Museum of Natural History	South
KZN01	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal	Central
KZN02	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal	Central
KZN03	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal	Central
KZN04	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal	Central
KZN05	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal	Central
KZN06	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2005	University of KwaZulu-Natal	Central
KZN07	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2013	This study (University of KwaZulu-Natal)	Central
KZN08	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN09	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN10	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN11	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN12	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN13	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN14	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN15	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN16	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN17	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN18	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KZN19	NA	Creighton, KwaZulu-Natal	-30.027832, 29.838148	2014	This study (University of KwaZulu-Natal)	Central
KH04	TM 40930	KwaZulu-Natal	Unknown	1957	Ditsong National Museum of Natural History	Central
Lim01	NA	Tzaneen, Limpopo	-23.859859, 30.006596	1999	Craig Symes (Wits University, RSA)	North
Lim02	NA	Tzaneen, Limpopo	-23.859859, 30.006596	1999	Craig Symes (Wits University, RSA)	North
Lim03	NA	Tzaneen, Limpopo	-23.859859, 30.006596	1999	Craig Symes (Wits University, RSA)	North
Lim04	NA	Tzaneen, Limpopo	-23.859859, 30.006596	1999	Craig Symes (Wits University, RSA)	North
Lim05	TM80817	Tzaneen, Limpopo	-23.822019, 30.131136	2014	Ditsong National Museum of Natural History	North

Supplementary Table 4-3. The eight microsatellite panels tested in Chapter 4, with the polymorphic information content (PIC) and combined probability of identity (P_{IDcom}) for each panel.

Locus rank:	16 locus panel:	15 locus panel:	14 locus panel:	13 locus panel:	12 locus panel:	11 locus panel:	10 locus panel:	9 locus panel:
1	Prob17	Prob17	Prob17	Prob17	Prob17	Prob17	Prob17	Prob17
2	Prob31	Prob31	Prob31	Prob31	Prob31	Prob31	Prob31	Prob31
3	Prob26	Prob26	Prob26	Prob26	Prob26	Prob26	Prob26	Prob26
4	Prob30	Prob30	Prob30	Prob30	Prob30	Prob30	Prob30	Prob30
5	Prob23	Prob23	Prob23	Prob23	Prob23	Prob23	Prob23	Prob23
6	Prob25	Prob25	Prob25	Prob25	Prob25	Prob25	Prob25	Prob25
7	Prob18	Prob18	Prob18	Prob18	Prob18	Prob18	Prob18	Prob18
8	Prob06	Prob06	Prob06	Prob06	Prob06	Prob06	Prob06	Prob06
9	Prob09	Prob09	Prob09	Prob09	Prob09	Prob09	Prob09	Prob09
10	Prob15	Prob15	Prob15	Prob15	Prob15	Prob15	Prob15	
11	Prob01	Prob01	Prob01	Prob01	Prob01	Prob01		
12	Prob29	Prob29	Prob29	Prob29	Prob29			
13	Prob34	Prob34	Prob34	Prob34				
14	Prob28	Prob28	Prob28					
15	Prob35	Prob35						
16	Prob36							
Polymorphic information content (PIC):	0.581	0.605	0.63	0.642	0.663	0.686	0.698	0.703
Combined probability of identity (P_{IDcom}):	1.8E-13	3.1E-13	5.4E-13	2.0E-12	5.4E-12	1.6E-11	7.3E-11	5.7E-10

Supplementary Table 4-4. The parentage assignment success rates for all parentage assignments performed in Chapter 4. Standard error values are provided in parentheses.

	16 loci	**15 loci**	**14 loci**	**13 loci**	**12 loci**	**11 loci**	**10 loci**	**9 loci**
% parent pairs correctly assigned (probability > 0.75):	83.87	83.87	83.87	83.87	83.87	83.87	83.87	70.97
Mean probability:	0.997 (0.001)	0.987 (0.005)	0.993 (0.002)	0.993 (0.002)	0.985 (0.006)	0.995 (0.002)	0.953 (0.021)	0.977 (0.011)
% sires correctly assigned (>0,75):	81.58	81.58	81.58	81.58	81.58	81.58	84.21	73.68
Mean probability:	0.997 (0.001)	0.988 (0.004)	0.994 (0.002)	0.995 (0.002)	0.985 (0.005)	0.994 (0.001)	0.946 (0.018)	0.965 (0.010)
% dams correctly assigned (>0,75)	100	100	100	100	100	100	100	96.77
Mean probability:	0.997 (0.001)	0.997 (0.001)	0.996 (0.001)	0.996 (0.001)	0.997 (0.001)	0.997 (0.001)	0.981 (0.008)	0.984 (0.007)

Supplementary Table 4-5. The population of origin results for each of the eight microsatellite panels tested in Chapter 4; a) The percentage (%) of correct assignments, with the average assignment probabilities of the correct assignments. Standard error (SE) is given in parentheses; b) The GeneClass2 assignment results for each of the eight panels tested, with correct assignments marked by green and incorrect assignments marked by red.

a)

	16 loci	**15 loci**	**14 loci**	**13 loci**	**12 loci**	**11 loci**	**10 loci**	**9 loci**
% correct assignments:	83.33	83.33	83.33	83.33	83.33	83.33	75	66.67
Average assignment probability:	0.523 (0.085)	0.520 (0.089)	0.518 (0.088)	0.546 (0.074)	0.565 (0.087)	0.533 (0.090)	0.552 (0.086)	0.547 (0.092)

b)

Correct assignment:
Incorrect assignment:

16 loci

Sample ID:	Assigned sample	Captive	Eastern Cape	KwaZulu-Natal	Limpopo
Cap02	Cap01	0.587	0.278	0.079	0.000
O3i	Cap02	0.377	0.140	0.150	0.000
O6i	Cap03	0.793	0.274	0.261	0.001
O8i	Cap04	0.879	0.609	0.116	0.002
O11ii	Cap05	0.668	0.231	0.399	0.002
Cap14	Cap06	0.057	0.021	0.022	0.000
FH12	EC01	0.507	0.439	0.127	0.000
FH32	EC02	0.148	0.397	0.446	0.000
KWT08	EC03	0.059	0.306	0.030	0.000
KZN02	KZN01	0.539	0.449	0.674	0.000
KZN08	KZN02	0.098	0.204	0.213	0.014
KZN13	KZN03	0.328	0.089	0.677	0.002

15 loci:

Sample ID:	Assigned sample	Captive	Eastern Cape	KwaZulu-Natal	Limpopo
Cap02	Cap01	0.556	0.249	0.070	0.000
O3i	Cap02	0.349	0.117	0.134	0.000
O6i	Cap03	0.840	0.347	0.333	0.001
O8i	Cap04	0.855	0.578	0.103	0.002
O11ii	Cap05	0.636	0.203	0.378	0.002
Cap14	Cap06	0.063	0.011	0.029	0.000
FH12	EC01	0.563	0.545	0.166	0.000
FH32	EC02	0.139	0.364	0.426	0.000
KWT08	EC03	0.057	0.276	0.025	0.000
KZN02	KZN01	0.507	0.416	0.656	0.000
KZN08	KZN02	0.093	0.178	0.195	0.014
KZN13	KZN03	0.368	0.096	0.776	0.005

Supplementary Table 4-5.b (Cont.)

14 loci:

Sample ID:	Assigned sample	Captive	Eastern Cape	KwaZulu-Natal	Limpopo
Cap02	Cap01	0.522	0.231	0.063	0.000
O3i	Cap02	0.325	0.110	0.123	0.000
O6i	Cap03	0.895	0.370	0.333	0.001
O8i	Cap04	0.833	0.547	0.094	0.002
O11ii	Cap05	0.601	0.189	0.355	0.002
Cap14	Cap06	0.101	0.045	0.085	0.000
FH12	EC01	0.633	0.572	0.167	0.000
FH32	EC02	0.164	0.390	0.427	0.000
KWT08	EC03	0.057	0.296	0.026	0.000
KZN02	KZN01	0.475	0.390	0.630	0.000
KZN08	KZN02	0.106	0.193	0.196	0.012
KZN13	KZN03	0.427	0.105	0.777	0.004

13 loci:

Sample ID:	Assigned sample	Captive	Eastern Cape	KwaZulu-Natal	Limpopo
Cap02	Cap01	0.519	0.270	0.070	0.000
O3i	Cap02	0.271	0.102	0.111	0.000
O6i	Cap03	0.904	0.426	0.358	0.001
O8i	Cap04	0.790	0.521	0.084	0.001
O11ii	Cap05	0.541	0.177	0.329	0.001
Cap14	Cap06	0.583	0.360	0.448	0.000
FH12	EC01	0.573	0.546	0.153	0.000
FH32	EC02	0.152	0.387	0.438	0.000
KWT08	EC03	0.055	0.294	0.027	0.000
KZN02	KZN01	0.413	0.367	0.601	0.000
KZN08	KZN02	0.099	0.191	0.203	0.008
KZN13	KZN03	0.367	0.098	0.752	0.002

12 loci:

Sample ID:	Assigned sample	Captive	Eastern Cape	KwaZulu-Natal	Limpopo
Cap02	Cap01	0.761	0.344	0.150	0.000
O3i	Cap02	0.217	0.072	0.091	0.000
O6i	Cap03	0.917	0.407	0.348	0.000
O8i	Cap04	0.804	0.505	0.080	0.001
O11ii	Cap05	0.462	0.137	0.284	0.001
Cap14	Cap06	0.789	0.493	0.581	0.000
FH12	EC01	0.494	0.480	0.126	0.000
FH32	EC02	0.117	0.324	0.384	0.000
KWT08	EC03	0.034	0.240	0.021	0.000
KZN02	KZN01	0.407	0.345	0.591	0.000
KZN08	KZN02	0.070	0.150	0.170	0.005
KZN13	KZN03	0.301	0.068	0.700	0.001

Supplementary Table 4-5.b (Cont.)

11 loci: Sample ID:	Assigned sample	Captive	Eastern Cape	KwaZulu-Natal	Limpopo
Cap02	Cap01	0.707	0.286	0.110	0.000
O3i	Cap02	0.181	0.058	0.063	0.000
O6i	Cap03	0.938	0.435	0.359	0.000
O8i	Cap04	0.756	0.441	0.054	0.000
O11ii	Cap05	0.405	0.112	0.223	0.000
Cap14	Cap06	0.818	0.530	0.608	0.000
FH12	EC01	0.436	0.415	0.090	0.000
FH32	EC02	0.113	0.342	0.397	0.000
KWT08	EC03	0.027	0.249	0.018	0.000
KZN02	KZN01	0.351	0.288	0.520	0.000
KZN08	KZN02	0.051	0.122	0.126	0.004
KZN13	KZN03	0.253	0.055	0.634	0.001

10 Loci: Sample ID:	Assigned sample	Captive	Eastern Cape	KwaZulu-Natal	Limpopo
Cap02	Cap01	0.659	0.250	0.092	0.000
O3i	Cap02	0.158	0.051	0.051	0.000
O6i	Cap03	0.926	0.608	0.509	0.001
O8i	Cap04	0.709	0.396	0.044	0.000
O11ii	Cap05	0.365	0.097	0.197	0.000
Cap14	Cap06	0.794	0.710	0.779	0.000
FH12	EC01	0.398	0.397	0.078	0.000
FH32	EC02	0.096	0.303	0.365	0.000
KWT08	EC03	0.019	0.233	0.015	0.000
KZN02	KZN01	0.321	0.270	0.503	0.000
KZN08	KZN02	0.041	0.113	0.111	0.003
KZN13	KZN03	0.229	0.052	0.618	0.000

9 loci: Sample ID:	Assigned sample	Captive	Eastern Cape	KwaZulu-Natal	Limpopo
Cap02	Cap01	0.677	0.660	0.254	0.000
O3i	Cap02	0.115	0.049	0.038	0.000
O6i	Cap03	0.902	0.609	0.462	0.001
O8i	Cap04	0.736	0.359	0.046	0.000
O11ii	Cap05	0.674	0.120	0.411	0.000
Cap14	Cap06	0.794	0.644	0.805	0.000
FH12	EC01	0.381	0.333	0.073	0.000
FH32	EC02	0.071	0.251	0.371	0.000
KWT08	EC03	0.211	0.263	0.146	0.000
KZN02	KZN01	0.238	0.208	0.430	0.000
KZN08	KZN02	0.022	0.110	0.088	0.001
KZN13	KZN03	0.180	0.050	0.576	0.000

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

This study is, to date, the most comprehensive genetic assessment of the South African endemic Cape Parrot (*Poicephalus robustus*). This species has been the topic of much debate over the last two decades, specifically with regards to its taxonomic and conservation status. The taxonomic assessment of the Cape Parrot (Chapter 2) is the most inclusive study done on the taxonomic relationships within the *Poicephalus robustus* clade using molecular data. The previously published differences in morphology, habitat and dietary needs between *P. r. robustus*, *P. r. fuscicollis* and *P. r. suahelicus*, coupled with the clear genetic differentiation observed in the current study provides strong evidence for the elevation of *P. r. robustus* to full species status i.e. *P. robustus* sensu stricto. It was further suggested in this study that *P. r. fuscicollis* and *P. r. suahelicus* should remain as subspecies under *P. fuscicollis*, namely *P. f. fuscicollis* stat. nova and *P. f. suahelicus* stat. nova.

It was established, in Chapter 3, that the genetic history of the Cape Parrot has been affected by two significant bottleneck events. One of these major population declines occurred during the mid-Holocene. During this same time period, a significant decline in yellowwood forests was observed. Signs of a more recent bottleneck event were also detected. This was linked to a second decline in yellowwood forests during the early 1900's due to colonial logging practices. It was, however, observed that the effective population size of the current Cape Parrot population is three times higher than estimates of a 100 years BP, indicating an increase in Cape Parrot population size. The more recent bottleneck event led to the isolation of the northern population from the remaining populations. It was shown that this northern population is clearly genetically distinct from the rest of the Cape Parrot population, and it is therefore important to monitor the genetic health of this population to ensure its continued survival. Breeding success surveys should be conducted on a regular basis to establish the breeding success of the birds in this population, simultaneously collecting DNA samples from nest sites for genetic analysis. The two most western Cape Parrot populations (Alice and King William's Town, Eastern Cape) were shown to play an important role as source populations for migrants moving into the more central populations. The southern populations also showed the highest levels of genetic diversity, highlighting the importance of these populations for the preservation of genetic integrity of the global Cape Parrot population. These southern populations can possibly be used as source populations for translocations, if the need arises.

The assessment of 16 microsatellite loci for use in forensic case work for Cape Parrots identified two marker panels for use in parentage (16 loci) and individual assignment (12 loci) of suspected illegally traded birds. The effectiveness of these tests will be enhanced by the availability of a complete genotype data base of all legally owned captive Cape Parrots, as well as a complete studbook of all captive Cape Parrot individuals. Adequate reference data bases of the three wild Cape Parrot lineages is also needed for the accurate assignment of confiscated birds to their region of origin. Low levels of genetic differences were observed between the captive and wild populations. The captive population did, however, have a high number of private alleles, which might be as a result of a founder individuals possessing rare alleles, not often seen in the wild. It is recommended that the current captive population should not be used for reintroduction purposes, until further analyses of fitness related loci are performed. Due to the level of genetic sub-structuring observed in the wild Cape Parrot population, it is further recommended that captive populations for use in reintroductions should resemble the wild populations as closely as possible to prevent the unnatural mixing of distinct lineages.

The findings from this thesis will provide local, and international, conservation and law enforcement agencies with additional information to help in better protecting this South African endemic species. The results obtained from Chapter 2 will provide conservation authorities with enough evidence to view the South African endemic Cape Parrot as a Vulnerable species of conservation priority. Recognition of the Cape Parrot as a separate species, and the phylogeographic assessment of this species, will assist the biodiversity conservation sector to prioritize, plan and implement appropriate conservation strategies focused on the Cape Parrot. The microsatellite panels identified in Chapter 4 will further assist the relevant conservation and law enforcement authorities to better assess the legality of trade in this species. The genetic assessment of the captive population will aid parrot breeders, and conservation authorities, to better manage captive populations kept for possible future reintroductions into the wild.

The way forward

The phylogeographic analyses performed on the wild Cape Parrot population and the genetic comparison between the captive and three wild Cape Parrot lineages were all done using neutral microsatellite loci. Neutral markers do, however, not provide any information with regards to evolutionary adaptive variability within or among populations. The use of fitness linked loci such as the major histocompatibility complex (MHC) and Toll-like receptor (TLR) gene

families is recommended to better understand the level of adaptive differences within and among captive and wild Cape Parrot populations. These two gene families both play important roles in vertebrate immune responses. Certain genes in these gene families are known for their involvement in viral and others in bacterial immunity. Variation in these genes will provide more insights into how different Cape Parrot populations have adapted to pathogens found in the different distribution ranges. Assessment of fitness linked genes will also provide information on how the captive birds have adapted to captivity, for instance their ability to respond to various types of pathogens.