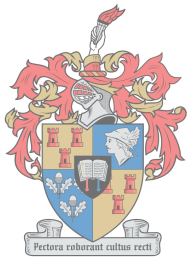
Species delineation in the southern African endemic catshark genus *Haploblepharus*

by

Michaela van Staden



Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in the Faculty of Natural Science at Stellenbosch University

Supervisor: Dr A.E. Bester-van der Merwe

Co-supervisor: Dr C. Rhode

Department of Genetics

December 2018

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2018

Summary

Accurate species identification is paramount for the effective implementation of conservation and management plans. Species identification in the genus *Haploblepharus* has historically been problematic due to the high degree of morphological conservatism between congeners, further complicated by the possibility of interspecific hybridisation. The research presented in this thesis addresses crucial knowledge gaps on species delineation in southern African endemic scyliorhinids by developing and applying molecular markers to assess species divergence in a morphologically conserved and threatened genus. Firstly, this study investigated the apparent lack of mitochondrial DNA sequence divergence previously reported among *Haploblepharus* species, using newly assembled mitochondrial genomes for *Haploblepharus edwardsii*, *Haploblepharus pictus*, *Halaelurus natalensis* and *Poroderma pantherinum*. The mitogenome assemblies for *H. edwardsii* and *H. pictus* contained single nucleotide polymorphism sequence variants in various mitochondrial genes. Following haplotype separation, interspecific sequence divergence was assessed for each protein-coding gene. Interestingly, divergence estimates between the mitogenome haplotypes recovered from a single *Haploblepharus* specimen met previously proposed species delineation thresholds. Accordingly, this study describes the presence of heteroplasmy in elasmobranchs, with evidence hinting at hybridisation and paternal leakage as possible factors responsible for the phenomenon. The phylogenetic reconstruction performed in this study illustrated that Scyliorhinidae, as presently recognised, is paraphyletic. Furthermore, the clustering of co-distributed southern African endemic scyliorhinids with alternate scyliorhinid species displaying distributions endemic to the northern Pacific provides valuable insight into the origin of these species. The observed genetic divergence between *P. pantherinum* and the rest of the study species suggests the occurrence of two separate colonisation events of the southern African coastline. In an attempt to provide evidence for the suspected hybridisation among *Haploblepharus* species, the second aspect of this project assessed species differentiation using novel microsatellite markers. Species-specific microsatellite markers were developed for a South African endemic catshark, *H. edwardsii*. Subsequently, the cross-species utility of these markers was assessed in *H. fuscus*, *H. pictus* and *H. natalensis*. A high cross-species amplification rate of success was observed, suggesting that these markers may also be useful in future population genetic studies for catsharks. In this study the null hypothesis of panmixia was rejected in only one of the study species, *H. pictus*, where genetic discontinuity was evident due to geographic distance. Accurate species assignment for *H. natalensis* illustrated the utility of these markers for species discrimination. In contrast, some of the *Haploblepharus* specimens presented a more complex assignment pattern. Interspecific genetic differentiation was statistically significant between all species; however, the level of differentiation between *H. fuscus* and *H. pictus* was low in comparison and seemed to be at a population level rather than at a species level. The index of admixture in Bayesian analysis has been

used to identify introgression, and by implementing *qi* thresholds this study was able to confidently identify pure and admixed individuals. While the presence of admixture among *Haploblepharus* taxa was evident, distinct genetic clusters were also present. Approximately 59% of 88 specimens genotyped were unambiguously assigned to a distinct genetic cluster that confirmed accurate taxonomic assignment. Accordingly, the research presented in this thesis provided valuable insights into the evolutionary relationships, genetic diversity and population connectivity of southern African endemic scyliorhinids.

Opsomming

Akkurate spesie identifikasie is noodsaaklik vir die effektiewe implementasie van bewarings- en bestuursplanne. Spesies identifikasie in die genus *Haploblepharus* was voorheen problematies te danke aan die hoë mate van morfologiese ooreenstemming tussen soortgelyke spesies. Hierdie tendens word verder gekompliseerd deur die moontlikheid van inter-spesie hibridisasie. Die navorsing in hierdie tesis het merkwaardige tekortkominge in kennis aangespreek in die spesie verwantskappe van endemiese kathaaie ("scyliorhinids") in suidelike Afrika. Dit was bereik deur die ontwikkeling en toepassing van molekulêre merkers om spesie diversifikasie in morfologies eenderse en bedreigde spesies te bestudeer. Die studie het eerstens ondersoek ingestel op die sogenaamde tekort van mitokondriese DNA volgorde divergensie, gevind in die *Haploblepharus* spesies, d.m.v. nuut bepaalde mitokondriese genome vir *Haploblepharus edwardsii*, *Haploblepharus pictus*, *Halaelurus natalensis* en *Poroderma pantherinum* te gebruik. Die mitogenomiese samestelling vir *H. edwardsii* en *H. pictus* het enkele nukleotied polimorfiese volgorde variasies in die mitokondriale gene bevat. Na haplotipe skeiding is interspesifieke volgorde divergensie vir elke proteïenkoderende geen geassesseer. Interessant genoeg stem die mate van divergensie tussen die mitogenoom haplotipes vanaf 'n enkele *Haploblepharus* individu ooreen met voorheen voorgestelde spesie-delinerings drempels. Verder beskryf hierdie studie die teenwoordigheid van heteroplasmie in kraakbeenvis met bewyse wat hibridisasie en "paternal leakage" as moontlike faktore identifiseer vir die verskynsel. Die filogenetiese rekonstruksie wat in hierdie studie uitgevoer is, het geïllustreer dat Scyliorhinidae, soos tans erken word, parafileties is. Daarbenewens bied die groepering van die endemiese kathaaie van suidelike Afrika met die vanaf die noordelike Stille Oseaan, waardevolle insig in die oorsprong van hierdie spesies. Die waargenome genetiese divergensie tussen *P. pantherinum* en die res van die studie spesies dui op die voorkoms van twee afsonderlike kolonisasies van die suidelike Afrikaanse kus. In 'n poging om bewyse voor te bring wat dui op die verdagte hibridisasie tussen *Haploblepharus* spesies het die tweede deel van die navorsing, spesie verskille geassesseer deur gebruik te maak van nuwe mikrosatelliet merkers. Spesie-spesifieke mikrosatelliet merkers was ontwikkel vir 'n skaam-haai spesie, *H. edwardsii*, endemies tot Suid Afrika. Gevolglik was die merkers getoets in *H. fuscus, H. pictus* en *H. natalensis*. 'n Hoë sukses van merker amplifisering in ander spesies is waargeneem, wat daarop dui dat hierdie merkers ook nuttig kan wees in toekomstige populasie genetiese studies vir ander kathaaie. In hierdie studie is die nulhipotese van panmiksia in slegs een van die studie spesies, *H. pictus*, verwerp, waar genetiese diskontinuïteit vanweë geografiese afstand duidelik was. Akkurate spesie toekenning vir *H. natalensis* het die nut van hierdie merkers vir spesie diskriminasie verder geïllustreer. In kontras, was daar sommige van die *Haploblepharus* individue wat 'n meer komplekse patroon van toedelings vertoon het. Inter-spesifieke genetiese differensiasie was statisties beduidend tussen alle spesies; nietemin, die vlak van genetiese differensiasie tussen *H. fuscus* en *H. pictus* in vergelyking was laag en was meer in lyn

met ‘n populasie eerder as op spesie-vlak. Die indeks van vermenging in Bayesiaanse analise is gebruik om introgressie te identifiseer, en deur die implementering van *qi*-drempels was hierdie studie in staat om individue te identifiseer wat suiwer en vermeng is. Terwyl die teenwoordigheid van vermenging tussen *Haploblepharus* taxa duidelik was, was daar ook verskillende genetiese groepe teenwoordig. Ongeveer 59% van die 88 individue is onvoorwaardelik toegeken aan 'n spesifieke genetiese groep wat ook akkurate taksonomiese toekenning bevestig het. Gevolglik het die navorsing wat in hierdie proefskrif aangebied word, waardevolle insig gegee in die evolusionêre verwantskappe, genetiese diversiteit en populasie konnektiwiteit van Suider-Afrikaanse endemiese kathaaie.

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions (in no particular order):

- My supervisor, Dr. Aletta Bester-van der Merwe, thank you for your patience, kindness and encouragement throughout the duration of this research; without your constant guidance the completion of this thesis would not have been possible.
- Dr. Clint Rhode, for your valuable inputs and assistance with my research.
- Collaborators and field scientists assisting with the collection of genetic samples vital to this research: Tinus Beukes (Two Oceans Aquarium), Rob Leslie (Department of Agriculture, Forestry and Fisheries), Matthew Parkinson (South African Institute for Aquatic Biodiversity), Ralph Watson (Rhodes University), Meaghan McCord (South African Shark Conservancy), and Mary Rowlinson (White Shark Diving Company).
- Colleagues (and friends) for providing me with immense support and advice: Katie Gledhill, Dr. Barbara van Asch, and the broader Molecular Breeding and Biodiversity (MBB) group.
- Family who have provided me with this opportunity to further my tertiary education, the love and support received has been invaluable.
- Funding which enabled this research as well as supported international travel was provided by the National Research Foundation, the Fisheries Society of the British Isles, Stellenbosch University and the Department of Genetics.

Preface

Scientific Contributions during Masters Candidature (2017-2018):

1. *Published or submitted papers, to date, directly emanating from the work presented in this thesis:*

van Staden M, Gledhill KS, Rhode C, Bester-van der Merwe AE. 2018. The complete mitochondrial genome and phylogenetic position of the leopard catshark, *Poroderma pantherinum*. *Mitochondrial DNA Part B: Resources* 3: 750-752.

van Staden M, Gledhill KS, Gennari E, McCord ME, Parkinson M, Watson RGA, Rhode C, Bester-van der Merwe AE. (in prep). Detecting signatures of admixture between three sympatric *Haploblepharus* species using newly developed microsatellite markers. *Aquatic conservation: Marine and Freshwater Ecosystems.*

2. *Published or submitted papers with indirect relevance to the work presented in this thesis*:

Gledhill KS, **van Staden M**, Booth DJ, McCord ME, Bester-van der Merwe AE. (submitted). Are molecular species identification markers able to differentiate among five South African endemic catsharks including the morphologically conserved genus *Haploblepharus*? *Marine Biodiversity*.

3. *Local conference presentations*:

van Staden M*, Gledhill KS, Rhode C, Bester-van der Merwe AE. Oral presentation: Comparative genomics of the southern African endemic catshark genus, *Haploblepharus*. *4th Southern African Shark and Ray Symposium*. September 2017. Hermanus, South Africa.

Gledhill KS*, **van Staden M**, Booth DJ, McCord ME, Bester-van der Merwe AE. Oral presentation: Molecular species identification of endemic catshark genera, *Haploblepharus* and *Poroderma*. *4th Southern African Shark and Ray Symposium*. September 2017. Hermanus, South Africa.

Gledhill KS, **van Staden M**, Booth DJ, McCord ME, Bester-van der Merwe AE*. Oral presentation: Molecular species identification of endemic catshark genera, *Haploblepharus* and *Poroderma*. *South African Marine Science Symposium*. July 2017. Port Elizabeth, South Africa.

4. *International conference presentations*:

van Staden M*, Gledhill KS, Rhode C, Bester-van der Merwe AE. Oral presentation: Comparative genomics of the southern African endemic catshark genus, *Haploblepharus. Sharks International*. June 2018. João Pessoa, Brazil.

Gledhill KS*, **van Staden M**, Booth DJ, McCord ME, Bester-van der Merwe AE. Oral presentation: Molecular species identification of endemic catshark genera, *Haploblepharus* and *Poroderma*. *Sharks International*. June 2018. João Pessoa, Brazil.

[* Presenting author]

Table of Contents

List of Figures

List of Tables

List of Abbreviations

%	Percentage
<	Less than
=	Equal to
>	Greater than
~	Approximately
≥	Greater than or equal to
3′	Three primer
5′	Five prime
A	Adenine
AMOVA	Analysis of Molecular Variance
A_R	Allelic richness
ATP6	ATP synthase F_O subunit 6
ATP8	ATP synthase F_O subunit 8
BIC	Bayesian Information Criterion
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Data System
bp	Base pairs
C	Cytosine
℃	Degrees Celsius
CAF	Central Analytical Facility
CI	Confidence interval
COI	Cytochrome *c* oxidase subunit I
COII	Cytochrome *c* oxidase subunit II
COIII	Cytochrome *c* oxidase subunit III
CTAB	Cetyltrimethylammonium bromide
cytb	Cytochrome *b*
DAFF	Department of Agriculture, Forestry and Fisheries
DAPC	Discriminant Analysis of Principal Components
DFA	Discriminant Function Analysis
DNA	Deoxyribonucleotide Acid
dNTPs	Deoxynucleotide Triphosphates

E	East
EM	Expectation Maximisation
F	Forward primer
FAM	Blue (ABI-fluorescent label)
FAO	United Nations Food and Agriculture Organisation
F_{CT}	Derivative of Wright's Fixation Index adapted for hierarchical AMOVA (group of populations relative to the total population)
FDR	False discovery rate
F_{IS}	Wright's Fixation Index (individual relative to the population)
Fr_{NULL}	Frequency of null alleles
F_{SC}	Derivative of Wright's Fixation Index adapted for hierarchical AMOVA (subpopulation relative to the group of populations)
F_{ST}	Wright's Fixation Index (subpopulation relative to the total population)
G	Guanine
GB	Gigabyte
gDNA	Genomic DNA
GQS	genomic quality scores
HAB	Harmful Algal Bloom
H_E	Expected heterozygosity
HE	*Haploblepharus edwardsii*
HF	*Haploblepharus fuscus*
HN	*Halaelurus natalensis*
H_O	Observed heterozygosity
HP	*Haploblepharus pictus*
HWE	Hardy-Weinberg equilibrium
Inc.	Incorporation
ITS2	Internal transcribed spacer 2
IUCN	International Union for the Conservation of Nature
K2P	Kimura 2-parameter
km	Kilometre
Ltd.	Limited company
m	Metre
MCMC	Markov Chain Monte Carlo
$MgCl_2$	Magnesium Chloride

min	Minutes
mM	Millimole
MPCA	Multigroup Principal Component Analysis
MSATTOOLS	Microsatellite Excel Toolkit
mtDNA	Mitochondrial DNA
MUSCLE	Multiple Sequence Comparison by Log-Expectation
N_A	Number of alleles
NCBI	National Center for Biotechnology Information
ND1	Nicotinamide adenine dehydrogenase subunit 1
ND2	Nicotinamide adenine dehydrogenase subunit 2
ND3	Nicotinamide adenine dehydrogenase subunit 3
ND4	Nicotinamide adenine dehydrogenase subunit 4
ND4L	Nicotinamide adenine dehydrogenase subunit 4L
ND5	Nicotinamide adenine dehydrogenase subunit 5
ND6	Nicotinamide adenine dehydrogenase subunit 6
NED	Yellow (ABI-fluorescent label)
ng	Nanogram
ng/µL	Nanogram per microlitre
nm	Nanometre
NUMTs	Nuclear mitochondrial DNA segments
P-value	Probability value
PCL	Pre-caudal length
PCoA	Principal Co-ordinate Analysis
PCR	Polymerase chain reaction
PET	Red (ABI-fluorescent label)
P_{HW}	Probability of conforming to Hardy-Weinberg equilibrium
PIC	Polymorphic information content
pM	Picomole
PP	*Poroderma pantherinum*
Q	Phred quality score
Qi	Average coefficient of membership
qi	Individual membership coefficient
R	Reverse primer

rRNA	Ribosomal Ribonucleic Acid
s	Seconds
S	South
SASC	South African Shark Conservancy
SNP	Single Nucleotide Polymorphism
T	Thymine
T_A	Annealing temperature
TL	Total length
tRNA	Transcribed Ribonucleic Acid
U	Units
v	Version
V	Volts
VIC	Green (ABI-fluorescent label)
ΔK	Delta K
µL	Microlitre
µM	Micromole
®	Registered Trademark
™	Trademark

CHAPTER 1

Introduction: Literature survey, research aims and objectives

1.1 Chondrichthyans: Biodiversity and drivers of threat

Cartilaginous fish belong to the class Chondrichthyes, which is a highly diverse class comprising two subclasses: Elasmobranchii (sharks, skates and rays) and Holocephali (chimaeras) (Carroll 1988; Maisey 2012; Ebert and van Hees 2015). Evidence from fossil records suggest that sharks have persisted in Earth's oceans for approximately 420 million years, making them one of the oldest extant vertebrate lineages (Compagno 1990; Maisey 2012; Dulvy *et al*. 2017). Sharks radiated to become globally distributed, evolving morphologically and mechanically into many diverse types (Compagno 1990; White and Last 2012; Dulvy *et al*. 2017). Sharks remain one of the most speciose lineages of predators inhabiting coastal, demersal and pelagic habitats (Compagno 1990; Heupel *et al*. 2014). Additionally, sharks have an important functional role in the top-down control of coastal and oceanic ecosystems, in terms of both structure and function (Stevens *et al*. 2000; Ferretti *et al.* 2010; Heithaus *et al.* 2012). Knowledge of chondrichthyan biodiversity has expanded over the last decade, with approximately 230 new species being described; majority of these reported from the Indo-Australian region, the western North Pacific and southern African regions (Ebert and van Hees 2015). Despite the high number of new elasmobranch species being described in recent years, there is still a discrepancy over the exact number of valid chondrichthyan species (Weigmann 2016). Throughout the world's oceans and some freshwater ecosystems more than 1 188 species of chondrichthyans have been described (Weigmann 2016).

Coastal and ocean threat is largely driven by the rapid expansion of fisheries and global trade markets (Stevens *et al*. 2000; Baum *et al*. 2003; Poliodoro *et al*. 2008; McClenachan *et al*. 2012). The overfishing of some areas has drastically altered marine populations and ecosystems (Cortés 2000; Stevens *et al*. 2000; Dudley and Simpfendorfer 2006; Ferretti *et al.* 2010). Over the last 60 years, the increasing presence of several direct and indirect threats to sharks have placed these species at a high risk of becoming endangered or extinct (Myers and Worm 2003; Davidson *et al*. 2016). Extinction risk assessments of species are generated by Species Specialist Groups of the International Union for the Conservation of Nature (IUCN) using Red List categories and criteria (www.iucnredlist.org). The IUCN has been evaluating extinction risks of species since the 1950's, and this process has since grown into a robust and widely applicable classification scheme (Dulvy 2013). Dulvy *et al*. (2014) undertook a global assessment to review the conservation status of 1 041 chondrichthyan species, it was estimated that one quarter of chondrichthyans were threatened with an elevated risk of extinction. Furthermore, the IUCN determined that the primary drivers of threat to elasmobranchs is fishing (96.1%), including: directed commercial (31.7%), bycatch (57.9%), recreational (0.7%) and artisanal fishing (5.8%), followed by habitat destruction (2.9%) and pollution (0.4%; www.redlist.org).

Globally, chondrichthyan species are caught by marine fisheries either as direct or incidental catch (Chapman *et al*. 2005; da Silva *et al*. 2013; Worm *et al.* 2013), with numerous studies indicating a widespread and rapid decline of shark populations due to the direct and indirect effects of fishing (Baum *et al*. 2003; Dulvy *et al.* 2003; Ferretti *et al*. 2008; Costello *et al*. 2012; Davidson *et al*. 2016). Shark products have primarily been the result of bycatch in fisheries targeting other, more valuable and productive teleost species (Stevens *et al.* 2000; Dulvy *et al.* 2017). The historically low economic value of sharks led to poor baseline data on chondrichthyan landings (Stevens *et al*. 2000), as the majority of catches are often unrecorded, misidentified or aggregated (Clarke *et al*. 2006). For example, global elasmobranch landings reported to the United Nations Food and Agriculture Organisation (FAO) are often aggregated into larger taxonomic groups such as 'sharks and rays', with only 15% of landings reported at species level (Clarke *et al*. 2006; Dulvy *et al*. 2008). Consequently, population trends for many chondrichthyan species have not been well-documented (Stevens *et al*. 2000; Myers and Worm 2005; Dulvy *et al*. 2008). More recently, the harvesting of sharks has been driven by the relatively high, and increasing, value of shark fins in the Asian shark fin soup market (Clarke *et al.* 2006). This high value has driven the development of shark fisheries as well as the retention of incidental catch (Dulvy *et al*. 2017). Additionally, sharks are of further value as they yield a variety of other products, including: meat, fins, skin, liver oil, gill plates (from devil and manta rays), cartilage, jaws and teeth (Clarke *et al.* 2007). Chondrichthyans are particularly vulnerable to overexploitation as they demonstrate a low intrinsic rate of population increase in comparison to bony fishes (Myers and Worm 2005), due to *K*-selected life-history traits: slow growth, late sexual maturity, long gestation periods and low fecundity (Cortés 2000; Frisk *et al*. 2001; Ferretti *et al*. 2010). Chondrichthyan-directed fisheries have historically been characterised by overharvest with stock declines and limited population recovery, if any (Stevens *et al*. 2000). A further concern is that once a population is overfished, it may require several decades to recover (Cortés 2000; Stevens *et al*. 2000). Nevertheless, sustainable shark fisheries have been reported in cases where the targeted species is, to some extent, resilient to fishing pressure and timely management grounded on science-based limits has been enforced (Stevens *et al*. 2000; Simpfendorfer and Dulvy 2017).

Little is understood about the vulnerability of chondrichthyans to the emerging pressures of climate change; however, the number of published works focusing on climate change in the marine environment have increased significantly in recent years (Harley *et al*. 2006; Chin *et al*. 2010; Whitfield *et al*. 2016). Compelling evidence for the threat that climate change poses to coastal marine ecosystems already exists (Richardson and Poloczanska 2008; Last *et al*. 2011; Blamey *et al*. 2015). Until recently, the majority of climate change research in relation to marine ecosystems focused on temperature change, and its impact on species distribution and abundance patterns (Harley *et al*. 2006). It was predicted that, in response to an increasing temperature, species distribution and abundance will shift according to their thermal tolerance and adaptability (Fields *et al.* 1993). However, there is growing evidence suggesting that susceptibility to climate change is more complex and temperature is only a component

of potentially numerous variables interacting to drive ecological change (Harley *et al*. 2006; Chin *et al*. 2010). Many marine species, including both plant and animal taxa, are already displaying a shift in distribution ranges associated to climate change (Last *et al*. 2011; Blamey *et al*. 2015; Whitfield *et al*. 2016). To date, the New Caledonia catshark *Aulohalaelurus kanakorum* Séret 1990 is the only species of chondrichthyan to have a threatened status directly linked to climate change (Dulvy *et al*. 2014). However, using an integrated risk assessment, Chin *et al*. (2010) demonstrated that 30 elasmobranch species were either moderately or highly susceptible to climate change. Although the assessment suggested that vulnerability to climate change is dependent on a combination of components, the rarity of a species and its specialisation in terms of habitat dependency are thought to increase climate change vulnerability (Chin *et al*. 2010). While Chin *et al*. (2010) reported that climate change vulnerability was case specific, the study highlighted that rare and specialised species require special attention as these attributes have already been identified as increasing the risk of extinction for a species (Davies *et al*. 2004).

Changes in the marine environment, and the consequent loss of biodiversity, have been explored in numerous studies; in contrast, the formation of species has represented one of the most elusive subjects in evolutionary biology (Palumbi 1994; Rocha and Bowen 2008). While Darwin (1859) explained that natural selection was responsible for the origin of new species, Dobzhansky (1937) and Mayr (1942) focused their works on a now well-known model termed allopatric speciation. This mode of speciation occurs when a continuous population becomes separated into smaller populations by an extrinsic barrier, limiting gene flow and allowing populations to diverge. Independent evolution was then suggested to result in reproductive isolation, preventing genetic exchange between populations even in the absence of the original extrinsic barrier (Dobzhansky 1970). Mayr (1954) went on to describe allopatric speciation in marine species; however, few studies have since attempted to examine patterns and processes of speciation in marine habitats (Via 2001; Bowen *et al*. 2013). The marine environment represents a serious challenge to the allopatric model of speciation as many marine taxa inhabit large geographic ranges and maintain large population sizes with high rates of gene flow among geographically distant populations (Palumbi 1992, 1994; Mayr 2001; Rocha and Bowen 2008; Bowen *et al*. 2013). For species with large panmictic populations, allopatric speciation was thought to be slow and infrequent (Palumbi 1992); conversely, speciation in marine taxa exhibiting these life-history traits is common, with high numbers of closely related species displaying overlapping distribution ranges (Knowlton 1993; Bellwood and Wainwright 2002; Rocha and Bowen 2008). The topic of sympatric speciation, the formation of species in the absence of geographical barriers, has historically been controversial (Via 2001; Coyne and Orr 2004) with many studies proposing cases that are equally compatible with allopatric speciation (Barluenga *et al*. 2006). Recent studies have demonstrated that sympatric speciation can be exceptionally difficult to prove (Seehausen and van Alphen 1999; Barluenga *et al*. 2006); however, it most likely arises when species experience multiple forms of disruptive selection (Via 2001). Ecological factors were thus suggested as the driving force behind

sympatric speciation (Coyne and Orr 2004; Choat 2006; Bird *et al*. 2011), with several recent examples of ecological speciation (Munday *et al*. 2004; Nosil 2012) and speciation in the presence of gene flow (Feder *et al*. 2012; Abbott *et al*. 2013). In light heterogeneous environments, sensory drives have been implicated in the evolution of colour morphs (Seehausen *et al*. 2008). Distinctive colour and band patterning have played a significant role in generating and testing hypotheses central to evolutionary biology (Darwin 1859), and have been implicated as a driver for speciation (Forsman *et al*. 2008; Seehausen *et al*. 2008). Previous studies have indicated that different colour morphs have a selective advantage in different environments by being less visible to potential predators (Seehausen and van Alphen 1999; Ruxton *et al*. 2004). Furthermore, colouration has also been shown to act as a visual signal used in mate choice (Andersson 1994).

While much of the debate on the formation of species has revolved around allopatry versus sympatry, the incidence of hybridisation in nature has been widely understudied (Currat *et al*. 2008). Hybridisation refers to interspecific reproduction between sympatric species, indicating the presence of an incomplete reproductive barrier (Morgan *et al*. 2012); facilitated by the presence of semipermeable species boundaries which allow secondary contact among closely related species, providing extensive opportunity for introgression (Maddison 1997; Gardner 1997; Volmer and Palumbi 2002; Seehausen 2004; Hobbs *et al*. 2013). The study of introgression has received renewed attention due to the topic of human-induced climate change, resulting in rapid vegetation shifts and the creation of new hybrid zones with little-known consequences for the genetic integrity of species (Mank *et al*. 2004; Brumfield 2010). Although the evolutionary role of hybridisation remains a controversial topic, it is thought to play a significant role in diversification through the introduction of advantageous novelty into the gene pool (Arnold *et al*. 1999); additionally, hybrid offspring with increased fitness may adapt to new environments, resulting in novel evolutionary lineages (Seehausen 2004; Arnold and Martin 2010; Morgan *et al*. 2012; Pardo-Diaz *et al*. 2012). In contrast, high levels of interbreeding can lead to the loss of adaptive variants (Rhymer and Simberloff 1996) and reverse speciation (Seehausen 2006; Coleman *et al*. 2014). Consequently, hybridisation events in nature are thoroughly studied due to the potential of these events to illuminate processes such as speciation and adaptive evolution (Seehausen 2004; Rheindt and Edwards 2011; DiBattista *et al*. 2016; Corrigan *et al*. 2017; Walter *et al*. 2017). Morgan *et al*. (2012) was the first to assess hybridisation in the class Chondrichthyes, and suggested that the lack of documented cases was due to the elasmobranch reproductive strategy which relies on internal fertilisation, thus including mate choice as a pre-zygotic barrier to hybridisation. According to Allendorf *et al*. (2001) hybridisation can occur due to natural or anthropogenic effects, and managing the latter is an enormous challenge to biodiversity conservation.

1.2 The South African marine environment and chondrichthyan biodiversity

Southern Africa has been identified as a biodiversity hotspot for chondrichthyan species, with over 200 species occurring in the surrounding waters (Compagno 1999; Ebert and van Hees 2015). The rich

and diverse marine fauna present can partly be attributed to the variety of interconnected habitats along the coastline: estuaries, mangroves, sandy beaches, rocky shores, coral reefs, kelp beds and ocean depths of more than 5 km (Griffiths *et al*. 2010; Teske *et al*. 2011). South African waters have been divided into nine marine bioregions, with five inshore bioregions defined by means of faunistic and floristic analyses, and four offshore bioregions defined by physical criteria (*e.g.* temperature and depth) (Lombard 2004; **Figure 1.1**). Additionally, South Africa is dominated by two oceanic currents: the warm Agulhas Current on the east coast and the cold Benguela Current on the west coast (**Figure 1.1**). The Agulhas Current is a narrow, fast-flowing current considered to be one of the strongest flowing currents in the world; in contrast, the Benguela Current is a broad, slow-flowing current characterised by the upwelling of cold, nutrient-rich water (Lombard 2004; Griffiths *et al*. 2010; Briggs and Bowen 2012). The south coast and west coast oceanographic regimes overlap between Cape Point and Cape Agulhas, creating the Atlantic/Indian Ocean boundary or transition zone (Griffiths *et al*. 2010; Teske *et al*. 2011; **Figure 1.1**). This transition zone has been well-documented as a phylogeographic break separating distinct lineages of many species (Maduna *et al*. 2016; Soekoe 2016), with some lineages endemic to the transition zone (Teske *et al*. 2006; Teske *et al*. 2011). Interestingly, many species existing in more than one bioregion exhibit no genetic structuring (Teske *et al*. 2006; Benavides *et al*. 2011) or display phylogeographic breaks at different localities (Teske *et al*. 2011). Alternatively, some species with limited dispersal ability exhibit genetic structuring not coinciding with present-day phylogeographic breaks (Teske *et al*. 2006; Teske *et al*. 2007). These oceanographic regimes are also thought to influence species richness as the number of elasmobranch species differs significantly between the east and west coasts of South Africa, with Cape Point being the delimiting boundary between the two (Ebert and van Hees 2015). The west coast is inhabited by 96 species, in comparison, the east coast comprises 175 species; this greater diversity is attributed to coastal and continental shelf species, specifically species in the families Carcharhinidae and Dasyatidae (Ebert and van Hees 2015).

Globally, oceanic temperatures are thought to be rising (Belkin 2009); however, in some upwelling systems such as the southern Benguela ecosystem, opposite trends or mixed signals have been recorded (Belkin 2009; Leduc *et al*. 2010). South Africa's commercial fisheries, fuelled by the nutrient-rich waters of the southern Benguela, have a long and well-documented history (Griffiths *et al*. 2004); which allows the tracking of spatial and temporal changes of commercially valuable species in South Africa's marine ecosystem (Blamey *et al*. 2015). For example, small pelagic fish caught by the South African pelagic fishery, such as the sardine *Sardinops sagax* (Jenyns 1842) and anchovy *Engraulis encrasicolus* (Linnaeus 1758), have shown spatial distribution shifts (van der Lingen *et al*. 2002). However, these spatial distribution shifts in response to temperature fluctuations are not restricted to animal taxa. Following 50 years of unchanged biogeographical limits, the economically important, forest-forming kelp *Ecklonia maxima* exhibited an eastward range expansion around Cape Agulhas (Bolton *et al*.

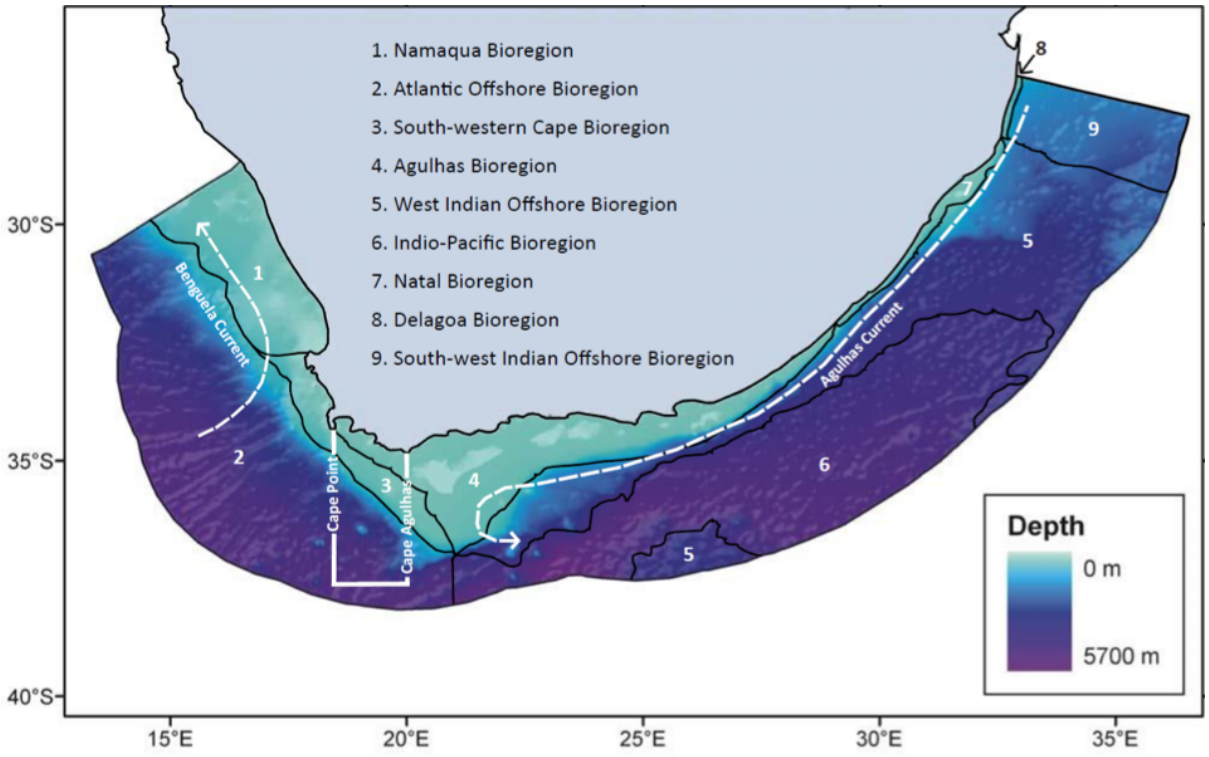


Figure 1.1 Map displaying South Africa's seafloor depths, the two major oceanic currents and nine marine bioregions, as described by Lombard (2004). The delimiting boundaries (Cape Agulhas and Cape Point) of the recognised phylogeographic break, known as the transition zone, are also illustrated. Modified from Griffiths *et al*. (2010).

2012). Even though the drivers of spatial changes are difficult to identify, particularly when multiple drivers are suspected to act in synergy (Harley *et al*. 2006), spatial shifts are assumed to create ripple effects on other parts of the marine ecosystem (Blamey *et al*. 2015). Research trawl surveys conducted by the Department of Agriculture, Forestry and Fisheries (DAFF) have reported a suspected decline in scyliorhinid populations distributed in the southern Benguela ecosystem, as scyliorhinid landings have decreased by up to 50% (da Silva C, perscomm). This decline could be due to a direct or indirect effect of climate change, such as the redistribution of a food source (*e.g.* small pelagic fishes) or habitat specificity (*e.g.* the spatial distribution shifts of forest-forming kelp). An additional phenomenon occurring within the southern Benguela are harmful algal blooms (HABs), which are characterised by toxicity due to the presence of certain phytoplankton species (Pitcher and Calder 2000; Pitcher and Probyn 2011). The associated toxicity is caused by intense localised depletion of oxygen, resulting in a profound negative impact on marine organisms within the environment (Pitcher and Probyn 2011), leading to large scale mortalities of many marine taxa (Cockcroft *et al*. 2008). Studies reporting the incidence of HABs have indicated a 6-fold increase in incidence as well as a concurrent increase of severity (Stephen and Hockey 2007). Previously, HABs were reported to occur most frequently on the west coast, affecting Lamberts Bay and Elands Bay, with a less frequent occurrence in False Bay (Pitcher and Calder 2000). Alternatively, a more recent study suggested that False Bay has become a hotspot for HABs (Blamey *et al*. 2015).

Worldwide, the majority of elasmobranch species inhabit continental shelves and slopes, making them highly vulnerable to trawl fishing (Shepherd and Myers 2005). While targeted fishing threatens one third of elasmobranchs, some of the most threatened species have experienced population declines due to incidental capture in fisheries (Dulvy *et al*. 2014). Fisheries management is dependent on the availability of quality information in the form of a resource assessment; however, resource assessments for chondrichthyans are often inadequate as there is a lack of basic data such as annual landings, catch rates and bycatch levels (Cortés 2007). In South Africa, chondrichthyans are targeted or harvested as bycatch by eight of 16 commercial fisheries (da Silva *et al*. 2015). Approximately 50% of southern Africa's known chondrichthyan fauna were reported in fisheries landings between 2010 and 2012; however, this catch data excludes chondrichthyans discarded at sea (da Silva *et al*. 2015). Actual catch numbers observed in shark-processing factories suggest that landings reported by fisheries can be as low as 25-50% (da Silva 2007).

Coastal species are vulnerable to the combined effects of fishing and habitat degradation (Dulvy *et al*. 2014). Consequently, the southern African coastline has been identified as a conservation priority due to the threats to chondrichthyans (Dulvy *et al*. 2014; Davidson and Dulvy 2017; Stein *et al*. 2018). The need for conservation action focused on imperilled endemics, a large proportion of which comprises scyliorhinids (Human 2003; Human 2007*a*; Ebert and van Hees 2015), has recently been highlighted (Davidson and Dulvy 2017; Stein *et al*. 2018). Stein *et al*. (2018) assessed spatial conservation risk in terms of species richness, endemic richness and evolutionary isolation of species; South Africa was identified as a 'triple hotspot' as well as a 'triple threatened hotspot' when additionally assessing species threat status (**Figure 1.2a** and **b**). A study conducted by Ebert and van Hees (2015) compared the threat status of 189 southern African chondrichthyans to that of 1 041 chondrichthyans assessed globally (Dulvy *et al*. 2014). Southern Africa was reported to have a higher number of species in the three threatened categories (Vulnerable, Endangered, Critically Endangered) when compared to the global assessment (29% threatened regionally versus 17.4% threatened globally; Ebert and van Hees 2015). Of the 24 southern African endemic shark species that have been assessed, five received threat status associated to an elevated risk of extinction (**Table 1.1**). Interestingly, four out of the five threatened species in this region belong to the catshark family Scyliorhinidae Gill 1862. The high percentage of southern African endemic species that are assessed in threatened categories, or as Data Deficient (33.3%), is concerning given several unresolved taxonomic issues; especially in groups including catsharks, dogfish sharks, stingrays, guitarfishes and chimaeras (Ebert and van Hees 2015). Additionally, the threat status of endemic species is exacerbated by a high habitat specificity and restricted geographic ranges (Davies *et al*. 2004). It was suggested that more research is required to ensure that the southern African chondrichthyan diversity is maintained; with special attention on endemic species as they inhabit small geographic ranges which are often restricted to areas subject to high trawling pressures (da Silva *et al*. 2015).

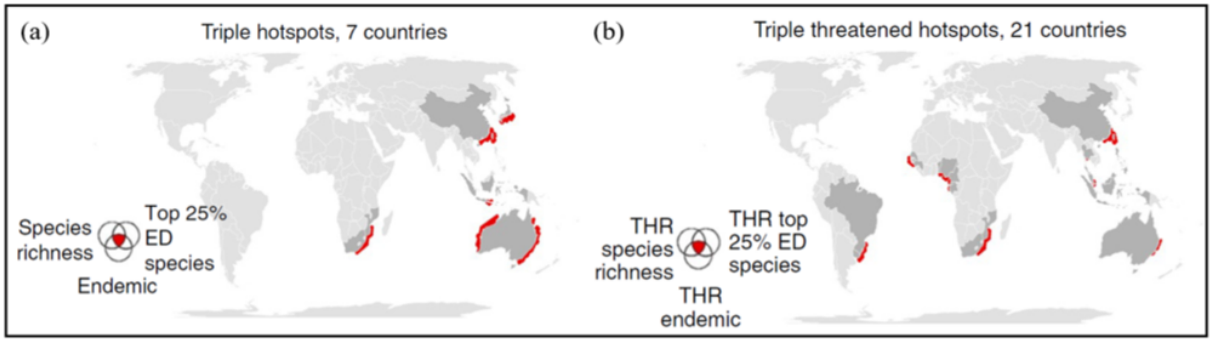


Figure 1.2 Location of chondrichthyan hotspots shown in red as determined by three conservation metrics: species richness, endemicity and upper quartile of evolutionary distinct (ED) species. (a) Global locations of hotspot congruence. (b) Global locations of hotspot congruence for threatened (THR) chondrichthyans. Countries shaded in dark grey have jurisdiction over the hotspots. Modified from Stein *et al*. (2018).

Table 1.1 Threat status, as assessed by the International Union for the Conservation of Nature (IUCN), of five southern African endemic elasmobranch species.

Species	Common name	IUCN threat status	Year assessed
Haploblepharus fuscus	brown shyshark	Vulnerable	2008
Scylliogaleus quecketti	flapnose houndshark	Vulnerable	2005
Holohalaelurus favus	honeycomb Izak catshark	Endangered	2008
Holohalaelurus punctatus	African spotted catshark	Endangered	2008
Haploblepharus kistnasamyi	Natal shyshark	Critically endangered	2008

1.3 An introduction to Scyliorhinidae Gill 1862

The non-charismatic family Scyliorhinidae Gill 1862 is one of the most speciose families of chondrichthyans, comprising at least 17 genera and 152 species which are colloquially referred to as catsharks (Compagno *et al*. 2005; Ebert and van Hees 2015; Weigmann 2016). Scyliorhinids are globally distributed, small, demersal sharks, and range from rare to abundant in tropical to cool temperate waters (Compagno 1999; Human 2003). Scyliorhinids are typically found on the continental shelf or slope, in waters greater than 100m in depth and down to at least 2 000m (Springer 1979). With the exception of morphological information provided in species descriptions, the biology of scyliorhinid species remains poorly known (Ebert *et al*. 2006; Flammang *et al*. 2008). However, more recent studies have illustrated the diversity of this family with descriptions of phenomena such as biofluorescence (Gruber *et al*. 2016) and facultative parthenogenesis in captive sharks (Feldheim *et al.* 2016).

Globally, a high number of scyliorhinid species are taken as bycatch by several fisheries; however, they are not regarded as valuable and are often discarded at sea (Human 2003; Compagno 1988). Consequently, as for many chondrichthyans, fisheries statistics most likely do not represent the true extent to which scyliorhinids are caught (Human 2003; da Silva *et al*. 2015). While Scyliorhinidae was identified as one of the least threatened families globally (Dulvy *et al*. 2014), threat status ranges from Least Concern to Critically Endangered in southern African waters. The southern African coastline is

inhabited by at least 19 scyliorhinid species, contributing approximately 8% to the southern African chondrichthyan biodiversity (Ebert and van Hees 2015), with at least 16 endemic species and two endemic genera (Compagno 1999; Ebert *et al*. 2006; Human 2007*a*). The inshore habit that southern African scyliorhinids demonstrate is a unique behaviour; in particular, *Haploblepharus* Garman 1913 and *Poroderma* Smith 1837 species are commonly seen close to shore in kelp forests and/or rocky reefs, rendering these species vulnerable to recreational fishing (Human 2007*a*; Beukes T, perscomm). Sharks belonging to both of the aforementioned genera are frequently caught and discarded by recreational fisherman, who regard them as pests that compete with target fish for bait (Human 2003; Gledhill KS, perscomm). Only limited catch data is available for *Haploblepharus* as they are commonly grouped into a 'shyshark' or 'unspecified shark' category (Guastella 1993; Pradervand 1999; Human 2007*a*; da Silva *et al*. 2015). The lack of accurate reporting underestimates the catch rates of these species. For example, species within *Haploblepharus* are commonly referred to as shysharks or doughnut sharks due to their behaviour when threatened, as they cover their eyes with their tails. Common names such as these have invalidated catch data, from both commercial and recreational fishermen, as both the genera *Haploblepharus* and *Poroderma* display this behaviour (Human 2003). Accordingly, the low catch rates of *Haploblepharus* species are suspected to be due to the lack of accurate reporting rather than actual low catch rates (Human 2003; Human 2007*a*). The frequent occurrence of scyliorhinids as incidental catch in commercial fisheries warrants future research into the biology and distribution ranges of these species (Rogers and Ellis 2000; Ebert 2003).

Although the systematics of the family Scyliorhinidae have been reviewed numerous times (Springer 1979; Bass *et al*. 1975; Human *et al*. 2006); Maisey (2012) suggested that the phylogeny be re-evaluated as morphological and molecular studies have indicated that Scyliorhinidae, as presently recognised, may be paraphyletic (Chen *et al*. 2016; van Staden *et al.* 2018). Naylor *et al.* (2012) described two major groupings of Scyliorhinidae based on a nicotinamide dehydrogenase subunit 2 (*ND2*) phylogeny, which corresponds to the morphological based clustering of the two subfamilies Scyliorhininae Gill 1862 and Pentanchinae Smith and Radcliffe 1912 (Human *et al.* 2006). Human *et al*. (2006) reported further resolution of Pentanchinae, illustrating the presence of two main tribes based on a cytochrome *b* (*cytb*) dataset, namely: Pentanchini Smith and Radcliffe 1912 and Halaelurini Compagno 1988 (**Figure 1.3**). The presence of the subfamily Scyliorhininae was well supported and although morphological differences between Scyliorhininae and Pentanchinae do exist, the presence of the tribe Pentanchini was not statistically well supported (Human *et al*. 2006). Moreover, the presence of the tribe Halaelurini was well supported; however, interrelationships between species within this tribe were not resolved. Human *et al*. (2006) stated that the paraphyly illustrated by molecular studies contrasts the morphological conservation displayed by the members of this family, and the relative recency of the family, which was first recorded from the end of the Jurassic period (Cappetta 1987).

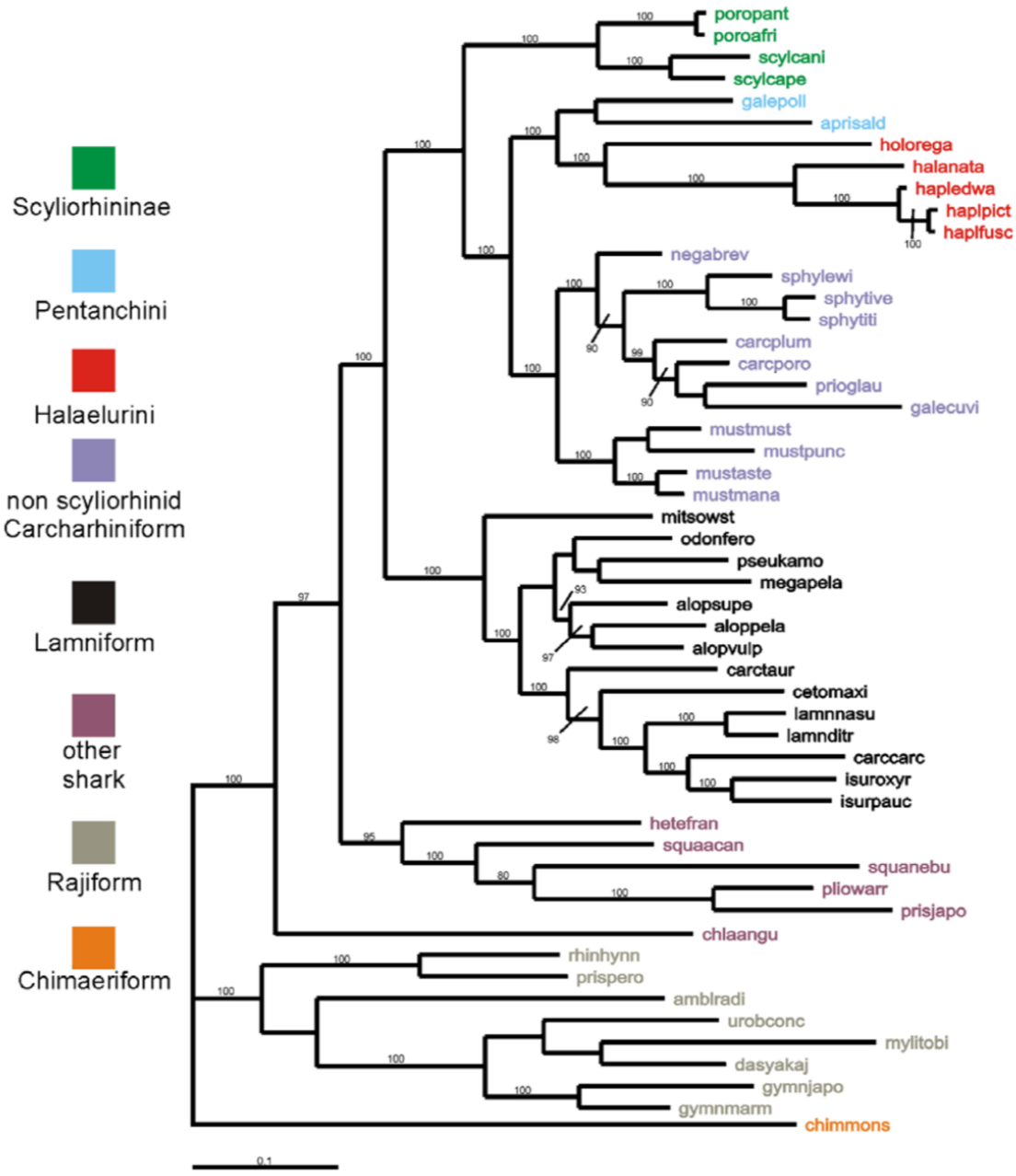


Figure 1.3 Phylogenetic interrelationships among chondrichthyan taxa based on Bayesian inference using a cytochrome *b* dataset. Percentage support values greater than 80% are only displayed. Scale bar indicates relative branch lengths. Modified from Human *et al*. (2006).

1.4 The morphologically conserved genus *Haploblepharus* Garman 1913

The genus *Haploblepharus* Garman 1913 is an understudied group comprising small to medium sized catsharks endemic to the region of southern Africa, occurring along the coasts of Namibia and South Africa (Human and Compagno 2006). *Haploblepharus* species are easily recognised as slender, broad-headed catsharks, distinguished from other southern African scyliorhinids by the expansion and fusion of the anterior nasal lobes into a flap (Human 2007*a*). The genus currently contains four recognised species: *H. edwardsii* (Schinz 1822), *H. fuscus* Smith 1950, *H. pictus* (Müller and Henle

1838) and *H. kistnasamyi* Human and Compagno 2006. Members belonging to this genus are morphologically conserved and display highly variable colour patterns (Human 2003; Human 2007*a*). Species identification within this group has historically been problematic, stemming from the use of colour patterns and unreliable morphological characters in species identification keys (Human and Compagno 2006); leading to recurrent species misidentification which has filtered through to the voucher specimens of some of these taxa (Human 2003; Human 2007*a*). The possibility of interspecific hybridisation was raised by Human (2007*a*) due to the difficulty in classifying some specimens which shared morphological and/or colour patterns of different *Haploblepharus* taxa. Additionally, the identification of juvenile or sub-adult specimens has been highlighted as further complicating accurate species identification (Human 2007*a*). In an attempt to increase species identification accuracy in this genus, a revised taxonomic dichotomous key was described for *Haploblepharus* (Human 2007*a*); however, misidentification between *Haploblepharus* species remains widespread (Gledhill *et al*. submitted).

Human (2007*b*) used morphometric and meristic data from a total of 73 *Haploblepharus* specimens to examine species group clustering and discrimination. Both multigroup principal component analyses (MPCA) and discriminant function analyses (DFA) were performed to numerically discriminate and classify specimens. Human (2007*b*) reported that the shape variation analyses reflected the high degree of morphological conservation among congeners, with only 69.9% of individuals correctly classified into their assigned species group. The analyses were unable to accurately classify any of the species to their assigned group with a 100% success rate, with the number of correctly assigned specimens per species ranging from as low as 38.5% to ~85% (Human 2007*b*). A more recent study was unable to accurately differentiate between three *Haploblepharus* species (*H. edwardsii*, *H. fuscus* and *H. pictus*) based on three mitochondrial gene regions (cytochrome *c* oxidase subunit I (*COI*), *cytb* and *ND2*) as well as the internal transcribed spacer 2 (*ITS2*) nuclear gene (Gledhill *et al*. submitted). Both of the aforementioned studies were also performed for *Poroderma*, another South African endemic catshark genus (Human 2003; Ebert and van Hees 2015). The shape variation analyses were able to classify *Poroderma* specimens into their assigned species groups with a 100% success rate (Human 2006). Although genetic distances between *P. africanum* (Gmelin 1789) and *P. pantherinum* (Smith *in* Müller and Henle 1838) were low, the taxa were accurately identified based on fixed nucleotide differences at all four molecular markers (Gledhill *et al*. submitted). The ability of these analyses to accurately distinguish between the two closely related *Poroderma* species illustrates and supports the utility of these methods for species discrimination.

1.4.1 Comparative overview of Haploblepharus species

A taxonomic revision of *Haploblepharus* revealed that the described distributions of some species had included those of other *Haploblepharus* taxa, leading to the over- or underestimation of both distribution ranges and abundances of the individual species (Human 2003; Human 2007*a*). The

puffadder shyshark *Haploblepharus edwardsii* was previously thought to be distributed from the Western Cape to as far north as Durban, KwaZulu-Natal (Bass *et al*. 1975; Compagno 1988; Compagno *et al*. 1989); however, this distribution included misidentified specimens from a more recently described species, *H. kistnasamyi* (Human and Compagno 2006; Human 2007*a*). Bass *et al.* (1975) illustrated a *Haploblepharus* specimen from Kwa-Zulu Natal, which was identified as the 'Natal' form of the more abundant 'Cape' form of *H. edwardsii*. The 'Natal' form was discussed as being a regional colour variant of *H. edwardsii* (Springer 1979), however, it was later described as *Haploblepharus kistnasamyi* due to significant morphological differences between the two forms (Human 2003; Human and Compagno 2006). The Natal shyshark *Haploblepharus kistnasamyi* is thus the most northerly ranging *Haploblepharus* shark on the east coast, occurring in inshore waters from northern Kwa-Zulu Natal to the Western Cape (Human and Compagno 2006; Human 2007*a*). *Haploblepharus kistnasamyi* is assumed to be rare, described from only three adult specimens, all of which were collected from a small area in Durban (Human and Compagno 2006). Due to the apparent rarity of *H. kistnasamyi*, it was assessed as Critically Endangered using the IUCN Red List criteria as the small population size and restricted distribution range renders this species highly susceptible to habitat degradation (Human 2009*a*). Although juvenile specimens have been tentatively assigned to this species, it was suggested that genetic studies were required to confirm this (Human 2007*a*).

The distribution range of *H. edwardsii* has since been re-described by Human (2007a), with verified specimens occurring from the Langebaan Lagoon, Western Cape, to as far east as the western shore of Algoa Bay, Eastern Cape. Following the described range reduction, and given that the suspected distribution range is restricted to waters subjected to high fishing pressures, *H. edwardsii* was assessed as Near Threatened according to the IUCN Red List criteria (Human 2009*b*). *Haploblepharus edwardsii* is thought to demonstrate habitat preferences, as the species exploits different habitat niches either side of Cape Agulhas by occurring at different depths, preferring deeper waters east of Cape Agulhas (Human 2007*a*). Compagno (1988) and Human (2007*a*) suspected that *H. edwardsii* occurred at different depths to avoid competition with other *Haploblepharus* sharks occurring in the Eastern Cape. The brown shyshark *Haploblepharus fuscus* is frequently caught by shore anglers in the Eastern Cape, from Storms River mouth to East London (Human 2007*a*). Additionally, Human (2007*a*) described the possible rare occurrence of *H. fuscus* in the Western Cape as a verified specimen was identified in the Langebaan Lagoon; however, it was further cautioned that this relied on the assumption that specimen locality was accurately reported. Due to the apparent high site-fidelity, and suspected population fragmentation, *H. fuscus* was assessed as Vulnerable by the IUCN using Red List criteria (Human 2009*c*).

The dark shyshark *Haploblepharus pictus* displays a highly variable colour pattern which can superficially resemble *H. edwardsii* or *H. fuscus* in some specimens (Human 2007*a*), often leading to species misidentification, particularly involving *H. edwardsii* (Human 2007*b*). *Haploblepharus pictus*

was previously thought to occur along the southern African coastline from north of Lüderitz, Namibia, extending into South Africa as far east as Cape Agulhas (Bass *et al*. 1975; Compagno 1988; Compagno *et al*. 1989); however, a more recent study verified the distribution of *H. pictus* to as far east as Storms River mouth, South Africa (Human 2007*a*). Given its apparent abundance and lack of significant fishing related threats, *H. pictus* was assessed as Least Concern according to the IUCN Red List criteria (Human 2009*d*). While no information is available on population structure, the presence of substructure is suspected due to the sedentary nature of this species (Human 2003). It was therefore suggested that further monitoring of species abundance is required due to its endemicity and habitat preference (Human 2009*d*).

Given the endemicity of *Haploblepharus* species to a narrow geographic range and their occurrence in coastal areas experiencing significant fishing pressure (Human 2007*a*), it should be considered a priority to gather biological data for these species. Furthermore, given the frequent occurrence of species misidentification, leading to confusion over species abundance (Human 2003), an accurate method of species identification is required to investigate population trends within these species.

1.5 Molecular approaches and applications in elasmobranchs

Elasmobranchs are a poorly studied group in relation to other vertebrate classes (Naylor *et al*. 2012), possibly owing to their historically low economic value (Walker 1998). More recently, emerging conservation concern for many commercially harvested species as well as recognition of the key role of sharks in marine ecosystem functioning has led to a surge in elasmobranch research (Last 2007). The majority of research has been systematic and descriptive in approach, with approximately one third of chondrichthyan fauna being described over the last 30 years (Last 2007); further reflecting the relative immaturity of chondrichthyan taxonomy (Naylor *et al*. 2012). However, advances in molecular biology over the last decade have provided a variety of tools for aiding conservation (Clarke *et al*. 2006). The increasing affordability of molecular genetic approaches, and pressing need to address critical conservation issues, has led to a surge in genetic-based studies on elasmobranchs (Dudgeon *et al*. 2012).

The first genetic-based study on elasmobranchs was published 32 years ago (Smith 1986). The study investigated genetic diversity in smoothhound *Mustelus lenticulatus* Phillips 1932 and blue sharks *Prionace glauca* (Linnaeus 1758) using allozymes. While allozyme research dominated literature for over a decade, the advent of the polymerase chain reaction (PCR) provided a powerful way to examine specific DNA regions (Saiki *et al*. 1985). Highly conserved gene regions were used to investigate the origins of vertebrates (Bernardi and Powers 1992), while studies using more variable gene regions aimed to resolve phylogenetic placements and relationships within elasmobranchs (Naylor *et al*. 1997; Corrigan *et al*. 2008; Corrigan and Beheregaray 2009; Velez-Zuazo and Argnarsson 2011). More recent studies have described regional and global phylogeographic patterns using both mitochondrial DNA (mtDNA) and nuclear markers; broadening knowledge on the historical and contemporary processes driving distribution of genetic variation in elasmobranch populations (Dudgeon *et al*. 2012; Vignaud *et*

al. 2013; Dudgeon and Ovenden 2015). The use of hypervariable regions in the genome have enabled the study of reproduction, relatedness, movement patterns and philopatry; additionally, offering increased resolving power in population structure assessment (Ferguson and Danzmann 1998; Karl *et al*. 2011; Abdul-Muneer *et al*. 2014; Veríssimo *et al*. 2017). Molecular markers also have forensic applications, allowing species identification of shark fins and a more accurate monitoring of the shark fin trade (Shivji *et al*. 2002; Clarke *et al*. 2006; Holmes *et al*. 2009). Recent advances in high-throughput sequencing technologies have enabled the efficient development of molecular markers as well as a shift from studies using gene fragments to those investigating whole genomes (Allendorf *et al*. 2010; Ouborg *et al*. 2010; Stapley *et al*. 2010; McMahon *et al*. 2014); with a concurrent shift from neutral to adaptive variation (Dudgeon *et al*. 2012; Narum *et al*. 2013; Benestan *et al*. 2016).

1.5.1 Phylogenetics and evolutionary placement

Phylogenetics refers to the study of evolutionary history and relationships among individuals or groups of individuals, which is central to the understanding of biodiversity, evolution, ecology and genomes. Observable traits such as DNA sequences or morphological characters are used to infer these relationships under an evolutionary model. The basal phylogenetic placement of chondrichthyans to bony fishes (Inoue *et al*. 2010) makes chondrichthyans a critical reference to understanding features characterising the ancestral vertebrate as well as vertebrate genome evolution (Larsson *et al*. 2009; Ravi *et al*. 2009). While the monophyly of modern elasmobranchs is well-established, many competing hypotheses over the interrelationships among them still exist (Pavan-Kumar *et al*. 2014). The majority of molecular phylogenies have been at the order or family level, with few studies investigating species level interrelationships (Eitner 1995; Human *et al*. 2006; Dosay-Akbulut 2008; Stelbrink *et al*. 2009); however, phylogenetic analyses can provide valuable insights into traits that are unique to particular lineages. For example, phylogenetic studies investigating the genus *Mustelus* Linck 1790 have shown a paraphyletic relationship between species groups, with separation evident between placental and aplacental clades (López *et al*. 2006; Velez-Zuazo and Agnarsson 2011; Boomer *et al*. 2012; Naylor *et al*. 2012).

Recent studies distinguishing new species based on single-locus sequences have prompted debates about the validity of such species designations (Rubinoff 2006) as discordance between gene trees and species trees is a well-recognised problem (Maddison 1997). Empirical and simulation studies have indicated that certain DNA sequence properties influence the phylogenies yielded under parsimony. These include the degree of among-site-rate-variation (Yang 1994, 1996; Sullivan *et al*. 1995, 1996), the percentage of sites free to vary (Shoemaker and Fitch 1989; Palumbi 1989), base composition (Sidow and Wilson 1990; Collins *et al*. 1994), stationarity (Saccone *et al*. 1990; Steel 1994), and the length of the gene (Comeron *et al*. 1999). While molecular markers have been widely used to assess intra- and interspecific differentiation in teleost and elasmobranch species, few studies have compared the differences in divergence estimates provided by different gene regions (Feutry *et al*. 2014). Studies

have since employed multilocus approaches in an attempt to investigate differing divergence estimates provided by gene regions experiencing varying levels of functional constraints (*e.g.*, Richards *et al*. 2009; Corrigan *et al*. 2017; Walter *et al*. 2017). High-throughput sequencing technologies now allow for the cost effective and efficient recovery of whole mitochondrial genomes (mitogenomes) (Feutry *et al*. 2014). In comparison to single gene approaches, the use of whole mitogenomes can potentially provide a higher resolution of phylogenies as well as an increased precision in divergence estimates (Arnason *et al*. 2008).

1.5.2 DNA barcoding, species delineation and hybrid identification

Accurate species identification is the first step towards the implementation of effective biodiversity monitoring and management plans (Dayrat 2005). Species identification has previously relied on morphology as it is often the fastest and cheapest method (Dudgeon *et al*. 2012); however, this method is problematic when examining specimens at different life stages or when species are phenotypically similar (Ward *et al*. 2009; Steinke *et al*. 2016). Species identification in elasmobranchs is a widespread concern as misidentification between morphologically conserved species results in unreliable abundance estimates, distribution ranges and fisheries catch data; impeding species-specific conservation and management plans (Abercrombie *et al*. 2005; Bester-van der Merwe and Gledhill 2015; Smart *et al*. 2016). Molecular methods have since gained recognition as a tool for species identification, and several studies have demonstrated the utility of these methods in identifying species (*e.g.*, Ward *et al*. 2005; Naylor *et al*. 2012; Steinke *et al*. 2016).

DNA barcoding is a widely accepted tool that seeks to assist species identification through the use of a standardised gene region (Teletchea 2010), namely, cytochrome *c* oxidase subunit I (*COI*) (Hebert *et al*. 2003). For the successful application of DNA barcoding, sequences are required to exhibit a higher interspecific variation than intraspecific variation; however, the success of molecular species identification is limited to the existing knowledge available in taxon-specific reference libraries. To obtain species identity, sequences produced for specimens under study can be compared to reference sequences available on open access databases such as the Barcode of Life Data System (BOLD; Ratnasingham and Hebert 2007) or GenBank (Benson *et al*. 2013); often resulting in species being identified by a single sequence or a tight cluster of sequences (Ward *et al*. 2008). The utility of the *COI* gene for species identification has been validated in numerous vertebrate and invertebrate taxa (Hebert *et al*. 2004; Ward *et al*. 2005, 2008; Hajibabaei *et al*. 2006; Steinke *et al*. 2016). Alternatively, molecular identification methods for sharks have also previously included the use of the mitochondrial cytochrome *b* (*cytb*) (Human *et al*. 2006; Blanco *et al*. 2008) and nicotinamide adenine dehydrogenase subunit 2 (*ND2*) (Naylor *et al*. 2012; Giresi *et al*. 2013), as well as the internal transcribed spacer 2 (*ITS2*) nuclear region (Pank *et al*. 2001; Shivji *et al*. 2002; Abercrombie *et al*. 2005). In chondrichthyans, the *COI* gene fragment was shown to be more slowly evolving than the *ND2* fragment (Moore *et al*. 2011); thus the use of the fast-evolving *ND2* gene region in sharks was suggested as it allows for the discrimination of

closely related species, cryptic species, species complexes as well as geographic variants (Naylor *et al*. 2012; Straube *et al*. 2013; Giles *et al*. 2016; Henderson *et al*. 2016).

Previously, species identification based on mtDNA has been favoured due to its haploid mode of inheritance (Saccone *et al*. 1999) as well as the rapid evolution of the mitochondrial genome (mitogenome) in comparison to nuclear DNA (Moore 1995; Galtier *et al*. 2009). In particular, the use of mtDNA protein-coding genes limits the presence of indels due to functional constraints (Hebert *et al*. 2003), simultaneously allowing the occurrence of synonymous nucleotide changes which provide a unique discrimination ability (Ward and Holmes 2007). While the use of molecular markers for species discrimination has become a standard tool in conservation biology (Moritz 1994; Rubinoff 2006), limitations to the utility of mtDNA sequences have been raised (Naylor *et al*. 2012). Furthermore, Naylor *et al*. (2012) emphasised that mtDNA data comprises only a part of a suite of tools required to assess biodiversity and that the methods shortcomings need to be acknowledged to prevent misleading interpretations of data.

Potential limitations of using mtDNA to infer species boundaries include, but are not limited to, introgression following hybridisation, retention of ancestral polymorphism, and rate variation among lineages (Moritz and Cicero 2004). Hybridisation can lead to discordance between molecular and taxonomic species identification, depending on the direction of gene flow between species, due to the maternal inheritance of mtDNA (Avise *et al*. 1987; Ward *et al*. 2005; Morgan *et al*. 2012). Therefore, for species identification in closely related and co-distributed elasmobranchs, it was recommended that DNA barcoding be accompanied by the use of a bi-parentally inherited nuclear marker (Li *et al*. 2010; Morgan *et al*. 2012). If ancestral polymorphism persists over more than two nodes in an evolutionary tree, subsequently becoming differentially fixed in descendant lineages, the presence of paraphyletically distributed alleles can be detected (Patton and Smith 1994; Funk and Omland 2003). This results in the apparent paraphyly of species which require further investigation using multiple independent nuclear markers (Degnan and Rosenberg 2009; Liu *et al*. 2009). Finally, numerous studies have reported that substantial rate variation among lineages is evident, preventing the description of a set 'sequence divergence threshold' for species distinctness (Naylor *et al*. 2012). Nevertheless, species delineation thresholds of 3.5% (Zemlak *et al*. 2009) or 10x (Hebert *et al*. 2004) have been suggested. Some well-established species display genetic distances lower than proposed thresholds, however, can be readily distinguished by phylogenetic analyses or by the presence of fixed nucleotide differences (Henderson *et al*. 2016; Gledhill *et al*. submitted). While some species do not meet previously described thresholds, Naylor *et al*. (2012) described that the higher the overall sequence divergence found between forms, the more likely that the two forms represent different species.

The inability of mtDNA markers to accurately identify species due to haplotype sharing has previously been reported in elasmobranchs (Morgan *et al*. 2012; Cruz *et al*. 2015; Marino *et al*. 2015, 2017; Corrigan *et al*. 2017). Evidence for hybridisation among closely related elasmobranch species

(Morgan *et al*. 2012; Corrigan *et al*. 2017), as well as the increasing documentation of cryptic species and species complexes (Ward *et al*. 2008; Naylor *et al*. 2012; Henderson *et al*. 2016; Jabado 2018), highlights the need for an integrative approach to species identification. Mitochondrial DNA markers have been used to highlight the presence of species complexes or cryptic speciation events (*e.g*., Iglésias *et al*. 2009; Richards *et al*. 2009; Naylor *et al*. 2012; Henderson *et al*. 2016); however, such cases still require subsequent taxonomic investigation. While revealing population structure and identifying isolated populations is an important application of microsatellites, these markers have also successfully been applied in the identification of hybrid individuals. In closely related species, it is difficult to discern whether the detected admixture is attributed to contemporary hybridisation or a consequence of incomplete lineage sorting due to recent speciation. This topic has received much interest in recent years (Sang and Zhong 2000; Buckley *et al*. 2006; Holland *et al*. 2008), particularly due to the increasing detection of introgression events; however, few effective approaches exist for distinguishing between these two processes (DiBattista *et al*. 2016). An increased resolution can be achieved through range-wide sampling with mtDNA and microsatellite markers in conjunction with morphological and meristic data (DiBattista *et al*. 2016).

The identification of hybridisation events and species complexes resulting in the descriptions of new species is crucial to the overall management and conservation of these elasmobranchs in the given region. In light of the new taxonomic understanding of the respective group, management plans require reassessment as distribution ranges and population sizes are often smaller than previously thought (White and Last 2012).

1.5.3 Genetic diversity and population connectivity

There is an increasing conservation concern over the potential genetic effects of overharvesting marine fauna and flora (Law 2007; Allendorf *et al*. 2008; Palkovacs 2011); with genetic changes potentially arising from natural selection or genetic drift resulting from a reduction in population size (Allendorf *et al*. 2014). Allelic diversity is directly influenced by a reduction in population size, with the greatest effect experienced by loci with many alleles (Allendorf *et al*. 2014). Such genetic changes are predicted to increase species extinction risks as well as reduce recovery rates following the overharvesting of populations (Walsh *et al*. 2006). Molecular approaches have proven useful for characterising genetic variability and defining reproductively isolated populations in marine organisms, which aids in elucidating historical and contemporary processes responsible for the observed patterns of spatial genetic differentiation (Veríssimo *et al*. 2010; Ovenden *et al*. 2011; Kousteni *et al*. 2015; Maduna *et al*. 2017). However, there is a lack of adequate scientific information on chondrichthyan population structure (Dudgeon *et al*. 2012) and few molecular markers are available for the group, especially for southern African endemics (Bester-van der Merwe and Gledhill 2015).

Microsatellites comprise short, tandem repeat motifs with repeat sizes ranging from one to six base pairs in length (Tautz 1989; Edwards *et al*. 1991). The high level of variability associated with

microsatellites allows the ability to detect differences among closely related populations (Abdul-Muneer 2014). Recent advances in high-throughput sequencing technologies have allowed the accurate and efficient recovery of microsatellite loci from non-model organisms (Boomer and Stow 2010; Chabot and Nigenda 2011; Pirog *et al*. 2015; Maduna *et al*. 2017, 2018). Furthermore, owing to highly conserved microsatellite flanking sequences (Martin *et al*. 2002; Ellegren 2004), cross-species amplification is an effective alternate approach to *de novo* development and has been tested in elasmobranchs with relatively high success rates (Griffiths *et al*. 2011; Maduna *et al*. 2014; Blower *et al*. 2015; Pirog *et al*. 2015). The use of cross-amplified markers in a standardised panel allows for molecular species identification and comparative population genetics (Maduna *et al*. 2014, 2017; Marino *et al*. 2014; Giresi *et al*. 2015).

Microsatellites have successfully been applied in quantifying within and between population genetic variations (Abdul-Muneer *et al*. 2009; Hoffman and Nichols 2011), and in differentiating geographically isolated populations and sibling species (Zardoya *et al*. 1996; Supungul *et al*. 2000; Fernandes *et al*. 2012; Lu *et al*. 2014). The use of microsatellites is however not without limitations, including: the presence of stutter bands or null alleles, homoplasy, as well as the possible presence of length variations in the flanking regions producing inaccurate length variations (Zardoya *et al*. 1996; Hoffman and Nichols 2011). Nonetheless, the use of microsatellite markers in generating knowledge on population structure and genetic variation provides a significant step towards the conservation of species in their natural populations. A combination of evolutionary forces, life-history traits and environmental features are suspected to govern the degree of population genetic structuring among natural populations (Dudgeon *et al*. 2012; Ovenden 2013). Numerous hypotheses have been proposed to describe varying levels of genetic connectivity. Isolation by distance predicts that gene flow between populations in close proximity is more likely to occur than between geographically distant populations; further describing that populations are connected *via* a series of 'stepping stones' (Wright 1943). Alternatively, limited gene flow across a biogeographic barrier resulting in a sudden change in genetic variation between adjacent populations can occur (Ovenden 2013). Finally, gradual differences in allele frequencies along a geographical gradient can be explained by clinal variation due to differential adaptation to environmental conditions such as temperature, salinity or depth (Storz 2002; Teske *et al*. 2011); possibly leading to divergent evolution and the development of ecotypes (Lowry 2012; Louis *et al*. 2014).

1.6 Research aims and objectives

The overall aim of the research presented in this thesis is to address crucial knowledge gaps on species delineation in southern African endemic scyliorhinids by developing and applying molecular markers to assess species divergence in a morphologically conserved and threatened genus, *Haploblepharus*.

The first experimental chapter (Chapter 2) investigates the apparent lack of mitochondrial DNA sequence divergence evident among *Haploblepharus* species, further attempting to identify a more variable region in the mitochondrial genome for future use in species identification. The study describes the mitochondrial genome assembly for four southern African endemic scyliorhinids, the identification of heteroplasmy and the subsequent separation of mitochondrial haplotypes. Furthermore, sequence divergence in terms of *p*-distances are assessed for each protein-coding gene, and the phylogenetic placement of each study species within the order Carcharhiniformes is illustrated.

In Chapter 3, species differentiation is assessed using bi-parentally inherited markers to provide evidence for the suspected hybridisation among *Haploblepharus* species. This study describes the *de novo* development of polymorphic microsatellite markers from reduced genome sequencing data for the puffadder shyshark *Haploblepharus edwardsii*. Furthermore, a cross-species amplification approach is employed to assess the utility of these markers for future population genetics studies in other scyliorhinid species. This chapter applied traditional differentiation statistics (F_{ST} and AMOVA), multivariate analyses (PCoA and DAPC) and a Bayesian clustering model-based method (STRUCTURE) to define genetic structure between populations and species.

CHAPTER 2

Mitochondrial genome assembly and comparative mitogenomics for four southern African endemic scyliorhinids

Abstract

The catshark family Scyliorhinidae is one of the most speciose shark families, contributing approximately 8% to southern Africa's rich elasmobranch biodiversity. Accurate species identification is required prior to addressing any ecological or evolutionary questions; moreover, it is paramount for the effective implementation of conservation and management plans. Species misidentification is frequent among scyliorhinid species belonging to the morphologically conserved genus *Haploblepharus*; attributed to suspected interspecific hybridisation among sympatric species. Little to no sequence divergence was previously reported for three *Haploblepharus* species based on four molecular species identification markers (cytochrome *c* oxidase subunit I, nicotinamide adenine dehydrogenase subunit 2, cytochrome *b* and the internal transcribed spacer 2). However, discordance between gene trees and species trees is a well-recognised problem due to variable mutation rates and the use of a small number of molecular markers when inferring species relationships. Accordingly, this study reports on the first complete mitochondrial genome for a South African endemic catshark, *Poroderma pantherinum*, as well as partial mitogenomes for *Halaelurus natalensis*, *Haploblepharus edwardsii* and *Haploblepharus pictus*. Mitogenome assemblies for *H. edwardsii* and *H. pictus* contained single nucleotide polymorphism sequence variants in various mitochondrial genes, including both synonymous and nonsynonymous variants. Following haplotype separation, interspecific sequence divergence was assessed for each protein-coding gene, with interspecific evolutionary distances being congruent among all genes assessed. Interestingly, divergence estimates between the haplotypes recovered from a single *Haploblepharus* specimen met previously proposed species delineation thresholds. Accordingly, this is the first study to describe the presence of heteroplasmy due to suspected hybridisation in elasmobranchs. Finally, the phylogenetic reconstruction performed in this study confirmed that Scyliorhinidae, as presently recognised, is paraphyletic.

2.1 Introduction

Chondrichthyans are one of the oldest extant vertebrate lineages and remain one of the most speciose lineages of predators (Compagno 1990; Maisey 2012), with over 1186 species described throughout the world's oceans (Weigmann 2016). Marine populations and ecosystems have changed drastically over recent years, largely driven by the rapid expansion of fisheries and other anthropogenic effects (Cortés 2000; Stevens *et al*. 2000; Dudley and Simpfendorfer 2006; Ferretti *et al.* 2010; Dulvy *et al*. 2017). Even though targeted fishing threatens one third of elasmobranchs, Dulvy *et al*. (2014) reported that some of the most threatened species have experienced population declines due to incidental capture in fisheries. Accordingly, an estimated one quarter of chondrichthyan species are threatened with an elevated risk of extinction (Dulvy *et al*. 2014).

The first step towards biodiversity monitoring and conservation is accurate species identification (Dayrat 2005). However, species misidentification in elasmobranchs is a widespread concern, stemming from difficulties in unambiguously identifying congeneric species due to a high degree of interspecific morphological conservatism (Pank *et al*. 2001; Human 2003; Abercrombie *et al*. 2005). Species misidentification results in unreliable abundance estimates, distribution ranges and fisheries catch data; impeding species-specific conservation and management plans (Abercrombie *et al*. 2005; Bester-van der Merwe and Gledhill 2015; Smart *et al*. 2016). Species identification has previously relied on morphological and meristic characteristics; however, these methods are problematic when examining specimens at different life stages or when species are phenotypically similar (Ward *et al*. 2009; Steinke *et al*. 2016). Molecular methods have since gained recognition as a tool for species identification, and several studies have demonstrated the utility of these approaches in identifying species (Ward *et al*. 2005; Naylor *et al*. 2012; Steinke *et al*. 2016). DNA barcoding is a widely accepted tool that seeks to assist in species identification through the use of a standardised gene region (Teletchea 2010), namely, cytochrome *c* oxidase subunit I (*COI*) (Hebert *et al*. 2003). The utility of the *COI* gene for species identification has been validated in numerous vertebrate and invertebrate taxa (Hebert *et al*. 2004; Ward *et al*. 2005; Hajibabaei *et al*. 2006; Ward *et al*. 2008; Steinke *et al*. 2016). Alternatively, molecular identification methods for sharks have also previously included the use of the mitochondrial cytochrome *b* (*cytb*) (Human *et al*. 2006) and nicotinamide adenine dehydrogenase subunit 2 (*ND2*) (Naylor *et al*. 2012; Giresi *et al*. 2013), as well as the nuclear ribosomal internal transcribed spacer 2 (*ITS2*) region (Pank *et al*. 2001; Shivji *et al*. 2002; Abercrombie *et al*. 2005). The use of the fast-evolving *ND2* region in sharks is favourable as it allows for the discrimination of closely related species, cryptic species, species complexes as well as geographic variants (Naylor *et al*. 2012; Straube *et al*. 2013; Giles *et al*. 2016; Henderson *et al*. 2016).

While the use of molecular markers for species discrimination has become a standard tool in conservation biology (Moritz 1994; Rubinoff 2006), the inability of these markers to accurately identify species due to haplotype sharing has previously been reported in elasmobranchs (Morgan *et al*. 2012;

Cruz *et al*. 2015; Marino *et al*. 2015, 2017; Corrigan *et al*. 2017). Corrigan *et al*. (2017) stated that numerous factors could be responsible for the *ND2* haplotype sharing found between *Carcharhinus galapagensis* (Snodgrass and Heller 1905) and *C. obscurus* (Lesuer 1818), including: mitochondrial introgression, incomplete lineage sorting, selection at the mitochondrial level, or that the two forms do not represent valid species, but are instead ecologically driven morphological variants of the same species. However, following the use of >2000 nuclear single nucleotide polymorphic (SNP) loci, species were readily assigned into species clusters consistent with existing taxonomy, suggesting that historical mitochondrial introgression had occurred (Corrigan *et al*. 2017). Similarly, Marino *et al*. (2017) reported *COI* haplotype sharing between two sympatric smooth-hound sharks, *Mustelus mustelus* (Linneaus 1758) and *M. punctulatus* (Risso 1827); however, microsatellite markers were able to accurately assign individuals to distinct species clusters consistent with morphological identification. It was suggested that the discordance between mitochondrial and nuclear results could be attributed to possible heteroplasmy and introgression due to past hybridisation events (Marino *et al*. 2017). Accordingly, Morgan *et al*. (2012) cautioned that the potential for interspecific hybridisation should be taken into account when mitochondrial DNA (mtDNA) is used for species identification in closely related and co-distributed elasmobranchs.

Hybridisation refers to interspecific reproduction that occurs between sympatric species, indicating the presence of an incomplete reproductive barrier (Morgan *et al*. 2012), often facilitated by secondary contact between two recently diverged species (Gardner 1997; Volmer and Palumbi 2002; Hobbs *et al*. 2013). Hybridisation has previously been recorded in viral, prokaryotic and eukaryotic groups; however, it appears to be more common in plants than in animals (Arnold 2006). As of 2012, hybridisation had not been reported in the class Chondrichthyes. Morgan *et al*. (2012) suggested that this was due to the elasmobranch reproductive strategy, which relies on internal fertilisation; thus including mate choice as a pre-zygotic barrier to hybridisation. Hybridisation can lead to discordance between molecular and taxonomic species identification, depending on the direction of gene flow between species, as mtDNA is maternally inherited (Avise *et al*. 1987; Ward *et al*. 2005; Morgan *et al*. 2012). Therefore, for species identification in closely related and co-distributed elasmobranchs, it was recommended that DNA barcoding be accompanied by the use of a bi-parentally inherited nuclear marker (Morgan *et al*. 2012). Hybridisation is thought to play a significant role in evolutionary diversification; hybrid offspring with high fitness may adapt to new environments, resulting in novel evolutionary lineages (Seehausen 2004; Arnold and Martin 2010; Morgan *et al*. 2012; Pardo-Diaz *et al*. 2012). In contrast, high levels of interbreeding can lead to the loss of adaptive variants (Rhymer and Simberloff 1996) and reverse speciation (Seehausen 2006; Coleman *et al*. 2014). Consequently, hybridisation events in nature are thoroughly studied due to the potential of these events to illuminate processes such as speciation and adaptive evolution (Morgan *et al*. 2012; Marino *et al*. 2015, 2017; Corrigan *et al*. 2017; Walter *et al*. 2017). According to Allendorf *et al*. (2001) hybridisation can occur

due to natural or anthropogenic effects, and managing the latter is an enormous challenge to biodiversity conservation.

The non-charismatic family Scyliorhinidae Gill 1862 is one of the most diverse families of elasmobranchs, represented by approximately 152 species (Ebert and van Hees 2015; Weigmann 2016). Despite the diversity within this family, little is known about their biology or population trends (Ebert *et al*. 2006). Although the systematics of Scyliorhinidae have been reviewed numerous times (Springer 1979; Bass *et al*. 1975; Human 2003; Human *et al*. 2006), Maisey (2012) suggested that the phylogeny be re-evaluated as morphological and molecular studies have indicated that Scyliorhinidae, as presently recognised, may be paraphyletic (Human *et al.* 2006; Naylor *et al.* 2012; Chen *et al*. 2016; van Staden *et al*. 2018). Therefore, an in-depth phylogenetic analysis as well as a revision of morphological characters is required to clarify the relationships between species within this family (Human *et al*. 2006; Bester-van der Merwe and Gledhill 2015). Even though Scyliorhinidae was identified as one of the least threatened families globally (Dulvy *et al*. 2014), threat status in southern African waters ranges from Least Concern to Critically Endangered according to the International Union for the Conservation of Nature (IUCN) Red List assessments, particularly within the genus *Haploblepharus* Garman 1913. Before reliable population trend data can be recorded, the high incidence of species misidentification in this genus needs to be addressed. A phylogeny based on morphological data examined by Compagno (1988) indicated that *Haploblepharus*, along with *Halaelurus* Gill 1862 and *Holohalaelurus* Fowler 1934, belong to the tribe Halaelurini; however, interrelationships within Halaelurini could not be resolved (Human *et al*. 2006). Following a taxonomic revision of *Haploblepharus*, Human (2007*a*) raised the possibility of hybridisation among *Haploblepharus* species due to the difficulty in classifying some specimens that shared morphological and/or colour patterns of different *Haploblepharus* taxa. More recently, Gledhill *et al*. (submitted) was unable to differentiate between three *Haploblepharus* species (*H. edwardsii* (Schinz 1822), *H. fuscus* Smith 1950, and *H. pictus* (Müller and Henle 1838)) based on three mitochondrial gene regions (*COI*, *cytb* and *ND2*) as well as the nuclear *ITS2* gene region.

Molecular data can provide a powerful tool for distinguishing between closely related species; however, different gene regions exhibit variable mutation rates which can result in discordance between gene trees and species trees (Maddison 1997). While molecular markers have been widely used to assess intra- and interspecific differentiation in teleost and elasmobranch species, few studies have compared the differences in divergence estimates provided by different gene regions (Feutry *et al*. 2014). Gledhill *et al*. (submitted) suggested that the use of an integrated approach provided by the use of a combination of molecular markers may be required to assess species differentiation in closely related or recently diverged species. Recent advances in high-throughput sequencing technologies allow the cost effective and efficient recovery of whole mitochondrial genomes (mitogenomes) (Feutry *et al*. 2014). In comparison to single gene approaches, the use of whole mitogenomes can potentially provide a higher

resolution of phylogenies as well as an increased precision in divergence estimates (Arnason *et al*. 2008).

Considering the lack of sequence divergence between *Haploblepharus* species using the traditional molecular species identification markers, additional molecular markers are required to re-evaluate species divergence in *Haploblepharus*. Accordingly, this chapter reports on the assembly of partial mitogenomes for two morphologically similar scyliorhinids, the puffadder shyshark *H. edwardsii* and the dark shyshark *H. pictus*; as well as for a more distantly related scyliorhinid, the tiger catshark *Halaelurus natalensis* Regan 1904. Additionally, the assembly of the first complete mitogenome for a South African endemic scyliorhinid, the leopard catshark *Poroderma pantherinum* (Smith *in* Müller and Henle 1838), is discussed. A comparison of the scyliorhinid mitogenomes, with special focus on *Haploblepharus*, allowed for the exploration of close interspecific relationships from a molecular perspective. Finally, to investigate the molecular phylogeny and reported paraphyly in Scyliorhinidae, the position of the study species within the order Carcharhiniformes was assessed using the newly assembled mitogenomes.

2.2 Materials and Methods

2.2.1 Taxon sampling

Tissue samples were collected from four southern African endemic scyliorhinid species: *Halaelurus natalensis*, *Haploblepharus edwardsii*, *Haploblepharus pictus* and *Poroderma pantherinum* (**Figure 2.1**). The *H. edwardsii* and *H. natalensis* specimens were sampled by scientific officers on-board the research vessel *Africana* during a demersal trawl survey of the Agulhas Bank, South Africa, by the South African Department of Agriculture, Forestry and Fisheries (DAFF). The *H. pictus* and *P. pantherinum* specimens were sampled opportunistically by trained shark biologists at the South African Shark Conservancy (SASC) during a snorkelling survey in Hermanus, South Africa. Species identification was performed based on external morphology and colour patterns according to taxonomic keys described by Human (2007*a*) for both *Haploblepharus* species, Ebert *et al*. (2013) for *H. natalensis* and Human (2006) for *P. pantherinum*. For *Haploblepharus* species, only specimens morphologically identified as *H. edwardsii* are recorded at offshore locations as deep as 130m (Bass *et al*. 1975), while *H. pictus* is presumed to be confined to shallow inshore waters (Human 2007*a*). Due to frequent species misidentification, the offshore *Haploblepharus* whole specimen was retained for voucher purposes and selected as a representative for *H. edwardsii* for high-throughput sequencing. Although the *H. pictus* specimen was sampled in an area of sympatric distribution, it was unambiguously identified as *H. pictus* according to taxonomic keys (dark dorsal surface colouration, uniform cream ventral surface colouration, robust body and rounded snout; **Figure 2.1b**, **c**) (Human 2007*a*).

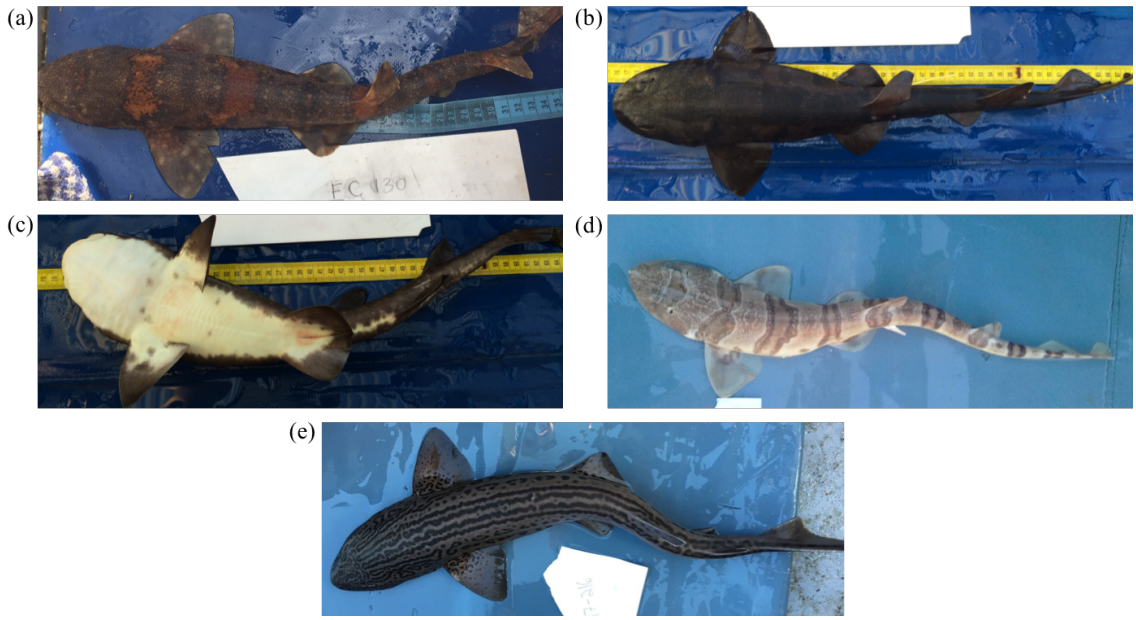


Figure 2.1 Photographs of four southern African endemic scyliorhinid species for voucher purposes, illustrating morphological characteristics used to assign specimens to correct taxonomic groups. (a) *Haploblepharus edwardsii*. (b) Dorsal surface of *Haploblepharus pictus*. (c) Ventral surface of *H. pictus*. (d) *Halaelurus natalensis*. (e) *Poroderma pantherinum*.

2.2.2 DNA extraction and molecular species identification

Total genomic DNA (gDNA) was isolated using a standard cetyltrimethylammonium bromide (CTAB) extraction protocol (Sambrook and Russell 2001) from fin tissue which had been stored in 90% ethanol at room temperature. The concentration of gDNA was quantified on the Qubit 4.0 Fluorometer using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA; www.thermofisher.com), according to the manufacturer's protocol (MAN0002326 Rev. B.0.). The purity of extracted DNA was determined by measuring its optical density at 230 nm (A_{230}), 260 nm (A_{260}) and 280 nm (A_{280}) using a NanoDrop™ ND 2000 spectrophotometer (Thermo Fisher Scientific). For *H. natalensis* and *P. pantherinum*, genomic quality scores (GQS) were determined on the LabChip GXII Touch using the DNA Extended Range LabChip (PerkinElmer, Waltham, MA, USA) according to the manufacturer's protocol (CLS140166 Rev. C.). DNA integrity was determined through agarose gel electrophoresis: 2 µL of gDNA was loaded on to a 0.8% agarose gel and subjected to electrophoresis at 70V for two hours. The gels were visualised and photographed under a Gel Documentation System (Gel Doc™ XR+, Bio-Rad, South Africa).

To confirm accurate species identification ~655 bp were amplified from the 5′ region of the *COI* gene using the universal *FishF1* and *FishR1* primers, according to the protocol outlined in Ward *et al*. (2005). Cycle sequencing was conducted on a GeneAmp® PCR System 2700 using the standard Sanger sequencing chemistry (BigDye® Terminator v3.1 Cycle Sequencing Kit, Life Technologies, South Africa) and following the manufacturer's instructions. Capillary electrophoresis was conducted at the Central Analytical Facility (CAF), Stellenbosch University. Using the Basic Local Alignment Search Tool (BLAST), a BLASTn (query nucleotide against nucleotide database) search was performed against the National Center for Biotechnology Information (NCBI) database to determine the sequence identity of each sample.

2.2.3 Library construction and Ion Torrent sequencing

For each sample, library preparation was performed from ~300 ng of gDNA using the Ion Plus Fragment Library Kit according to the manufacturer's protocol (MAN0006846 Rev. B.0.). Libraries with a mean insert size of 400 bp were prepared for *H. edwardsii* and *H. pictus*, while insert sizes of 600 bp were used for *H. natalensis* and *P. pantherinum*. In brief, gDNA was sheared by sonication using a Covaris S2 Focused-Ultrasonicator (Covaris® Inc., Woburn, MA, USA). The fragmented gDNA was end-repaired in preparation for blunt-end ligation using the Ion Xpress™ Barcode Adaptors Kit and the Ion Code™ Barcode Adaptors Kit for the 400 and 600 bp libraries, respectively. The adaptor-ligated, barcoded libraries were purified using the Agencourt™ AMPure™ XP reagent (Beckman Coulter, Life Sciences, South Africa). To select library fragments of the target size, a 2% gel cassette was used with marker L on a PippenPrep (Sage Science Inc., Beverly, MA, USA) with a tight selection profile for 475 or 675 bp fragments respectively. Purified, size-selected library fragments were

amplified and quantified using the Ion Library TaqMan™ Quantification Kit on a StepOnePlus Real-Time PCR System. For the 400 bp libraries, library fragment size distribution was verified on the PerkinElmer LabChip GXII Touch using the DNA NGS 3K LabChip and Reagent Kit according to the manufacturer's protocol (CLS145099 Rev. D.). While for the 600 bp libraries, library fragment size distribution was assessed on the BioAnalyzer 2100 (Agilent Technologies, South Africa) using the High Sensitivity DNA Kit according to the manufacturer's protocol (G2938-90322 Rev. C.).

For template preparation using the Ion 520™ & Ion 530™ Chef Kit, libraries were diluted to a target concentration (80 pM for 400 bp and 20 pM for 600 bp). Briefly, the diluted, pooled libraries were loaded on to the Ion Chef Liquid Handler with 4 μL of Ion S5T ExT Calibration Standard using reagents, solutions and supplies following the manufacturer's protocol (MAN0010846 Rev. D.0. and MAN0015805 Rev. C.0. for the 400 and 600 bp libraries respectively). Enriched, template positive ion sphere particles were loaded onto an Ion 530™ Chip for massively parallel sequencing using the Ion S5™ Sequencing Solutions and Sequencing Reagents Kit according to the manufacturer's protocol (MAN0010846 Rev. D.0. and MAN0015805 Rev. C.0. for the 400 and 600 bp libraries, respectively).

Low coverage whole genome sequencing was performed on the Ion Torrent S5™ (Thermo Fisher Scientific) high-throughput sequencing platform at CAF. Flow space calibration and basecaller analyses were performed using the default analysis parameter settings in Torrent Suite™ v5.6.0 Software (Thermo Fisher Scientific).

2.2.4 Mitochondrial genome assembly and annotation

The total reads for each sample were quality filtered using Torrent Suite™ Software and mapped to the reference mitogenomes from *Halaelurus buergeri* (Müller and Henle 1838) (accession KU892589.1) for the *Haploblepharus* and *Halaelurus* datasets, and *Scyliorhinus canicula* (Linnaeus 1758) (accession NC_001950.1) for the *P. pantherinum* dataset, using the Medium Sensitivity option in Geneious Mapper (Kearse *et al*. 2012). Final assemblies were annotated using MitoAnnotator (Iwasaki *et al*. 2013). A MUSCLE alignment with eight iterations was performed in Geneious including newly assembled and publicly available mitogenomes (**Table A2.1**). From this alignment, positions of the tRNA, rRNA, control region and protein-coding genes were confirmed. Protein-coding sequences were further analysed to minimise the presence of nuclear mitochondrial DNA segments (NUMTs) in the dataset: sequences were translated into amino acid sequences to check for the presence of premature stop codons and frameshift insertions or deletions. Graphs of each consensus sequence were generated in Geneious to visualise gene organisation and GC content.

2.2.5 Haplotype identification, separation and sequence divergence

The presence of multiple DNA templates was detected in the *H. edwardsii* and *H. pictus* mitogenome assemblies (**Figure 2.2**). To ensure the presence of alternate nucleotides was not due to sequencing errors, the presence of the nucleotides was assessed using the Find Variations/SNPs feature in Geneious.

The parameters for this analysis included: Minimum Coverage = 30, Minimum Variant Frequency = 0.05, Maximum Variant *P*-value = 10^{-6}, and Minimum Strand-bias *P*-value = 10^{-5}. The analysis was performed for regions both within and flanking the protein-coding sequences, and the 'Ignore Reference Sequence' option was used (only find variations within the sample). Additionally, the 'Homopolymer Quality Reduction' was enabled: this setting reduces the quality of the end bases by a specified percentage, attempting to counteract the overestimation of quality scores assigned by high-throughput sequencing machines.

A consensus sequence generated based on a majority threshold (the most common nucleotides) was extracted from the draft assembly. Using the consensus as a new reference, the sequence reads were re-mapped using the Custom Sensitivity option in Geneious Mapper. This was performed using the default settings with the exception that 'Maximum Mismatches Per Read' was set to 0% (no mismatches between the sequence reads and reference are allowed). The new assembly represented a single, most frequent state of the mitogenome referred to as Haplotype 1 (**Figure 2.3a**). Reads with alternate nucleotides identified as single nucleotide polymorphisms (SNPs) in the initial assembly were extracted and re-mapped to the consensus reference using the default Medium-High Sensitivity option in Geneious Mapper; resulting in a draft assembly of the second, less frequent mitogenome state, referred to as Haplotype 2 (**Figure 2.3b**). Basic sequence statistics for Haplotype 1 and 2 are summarised in **Table A2.2** for the three mitochondrial gene regions (*ND2*, *COI* and *cytb*) commonly used for species discrimination. To exclude cross-contamination due to human error as a possible explanation for the presence of multiple haplotypes, the newly separated haplotypes were compared to previously generated *COI* Sanger sequences from Gledhill *et al*. (submitted).

To assess sequence divergence for each protein-coding gene, uncorrected *p*-distances (Jukes and Cantor 1969) were estimated in MEGA v7.0.14 (Kumar *et al*. 2016). This model simply represents the proportion of nucleotide differences between two sequences without estimating any unobserved changes (multiple hits), thus emphasizing differences between closely related species (Naylor *et al*. 2012). Furthermore, the ~655 bp barcoding region (*COI*) was aligned and assessed using the standard Kimura 2-parameter (K2P; Kimura 1980) to allow for comparison with other barcoding studies. Finally, *cytb* sequences generated by Human (2003) were included in the *p*-distance estimates as a previous study reported that the *cytb* gene region provided the best phylogenetic resolution among scyliorhinid species for the gene regions included in Human *et al*. (2006). For each dataset, standard errors were calculated from 1 000 bootstrapped values and the complete deletion of missing data option was selected.

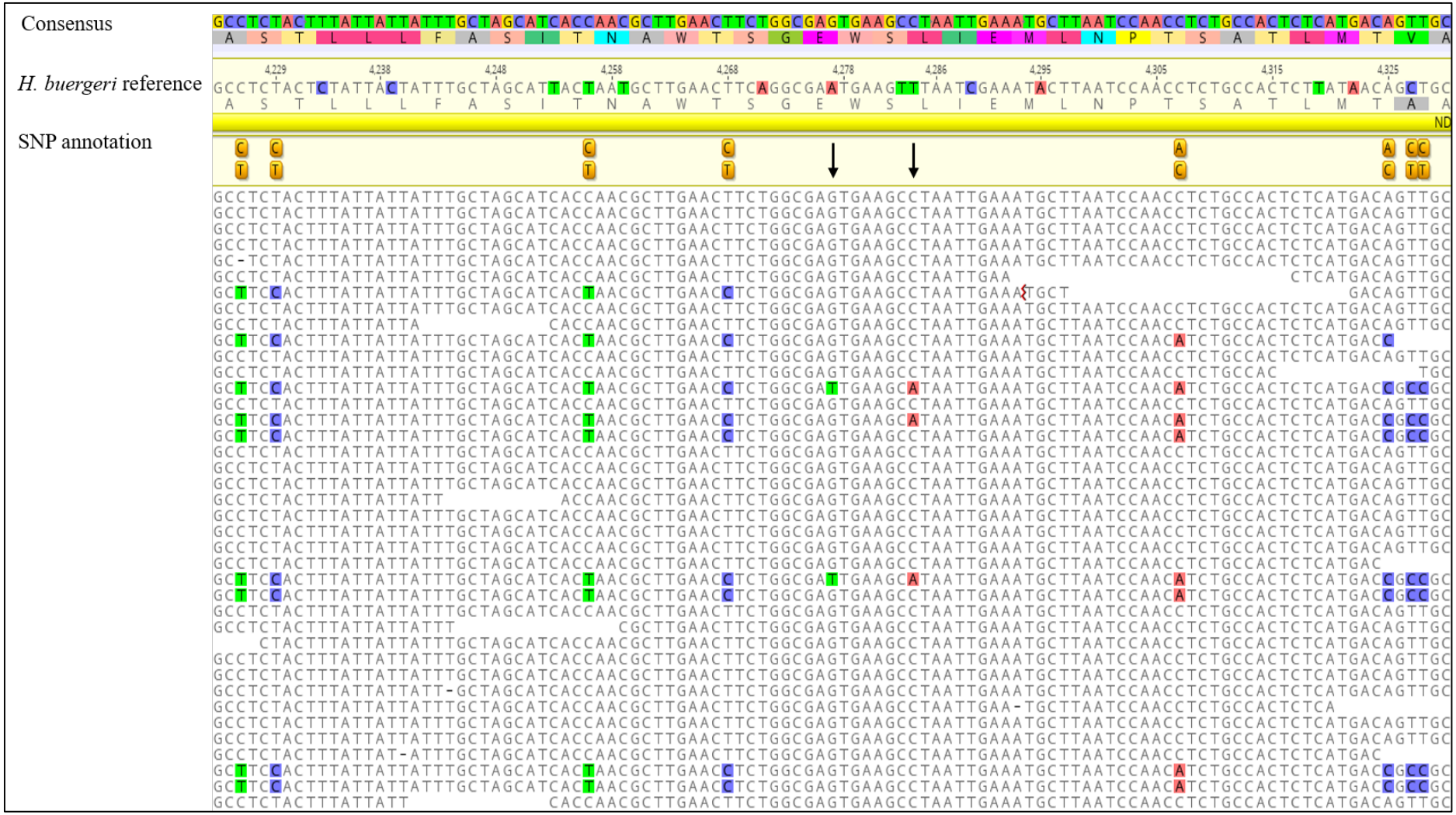


Figure 2.2 Draft assembly for the mitochondrial genome of the puffadder shyshark *Haploblepharus edwardsii* indicating the presence of multiple mitochondrial genomes as detected by the Find Variations/SNPs feature in Geneious® v10.2.3 (Kearse *et al*. 2012). The black arrows indicate potential variants that did not meet the 5% variant frequency threshold, and were thus dismissed during the final Haplotype calling.

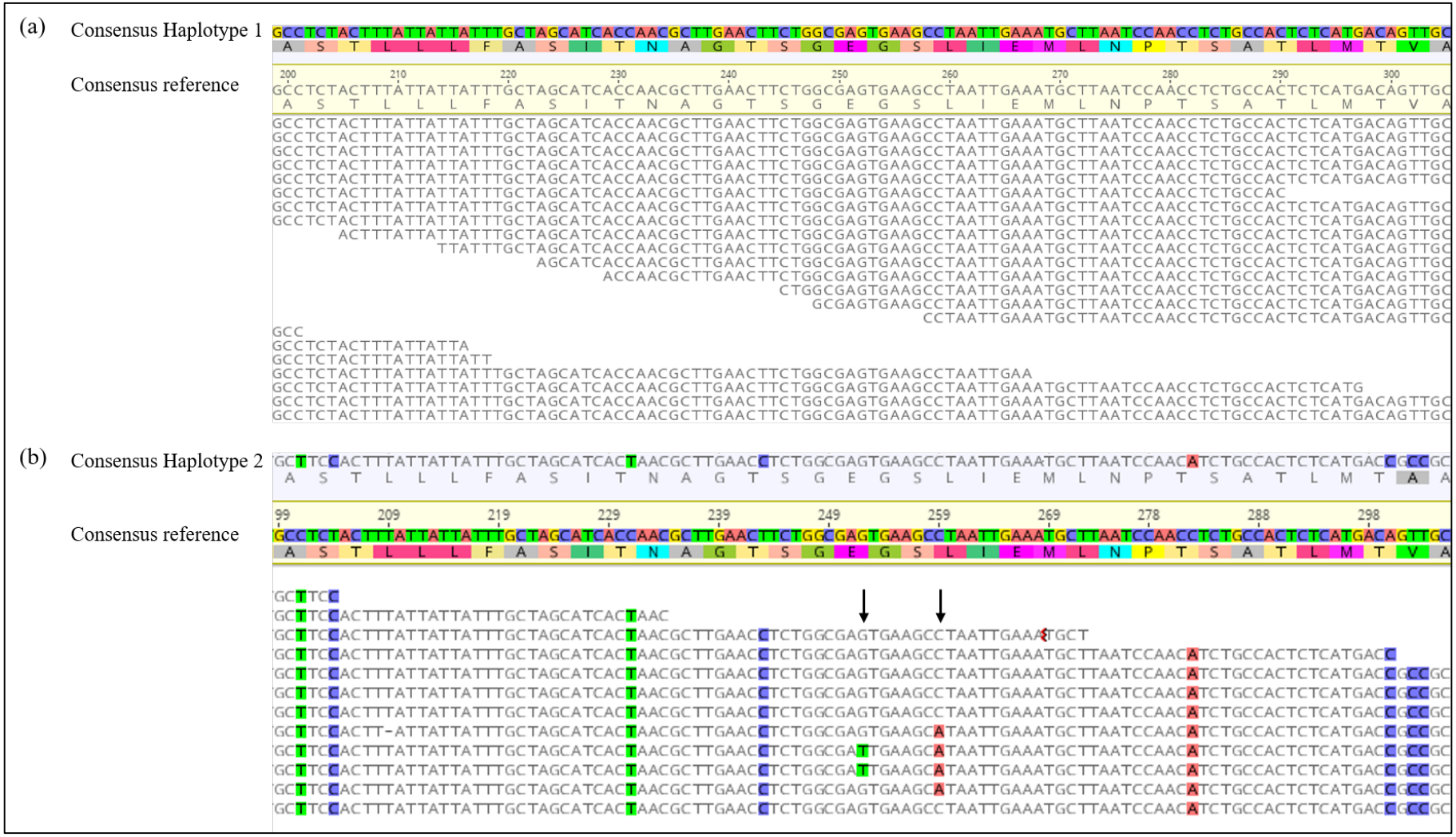


Figure 2.3 Assemblies representing Haplotype 1 and Haplotype 2 for the puffadder shyshark *Haploblepharus edwardsii*. (a) Assembly depicting reads re-mapped to the draft assembly consensus sequence, representing Haplotype 1. (b) Assembly of extracted reads mapped to the consensus with nucleotide substitutions highlighted, the black arrows indicate potential variants that did not meet the 5% frequency threshold, and were thus dismissed in the final Haplotype calling.

2.2.6 Phylogenetic reconstruction

To determine the phylogenetic position of the newly sequenced scyliorhinids within the order Carcharhiniformes, phylogenetic analyses were performed including 37 mitogenomes (**Table A2.1**) representing all families except Leptochariidae Gray 1851. Additionally, the mitogenome from the salmon shark *Lamna ditropis* Hubbs and Follett 1947, representing the order Lamniformes, was included as an outgroup sequence. A MUSCLE alignment with eight iterations was generated in Geneious, excluding tRNAs, rRNAs, and the control region. For this alignment, the complementary strand sequences were used for the *ND6* gene; furthermore, the *ND4* gene region was excluded due to the presence of a premature stop codon in Haplotype 2 for the *Haploblepharus* species. Overlapping gene regions between *ATP8*/*ATP6* and *ND5*/*ND6* were duplicated, thus giving these positions double the weight of others. All incomplete stop codons and ambiguous positions, as identified in GBlocks v0.91b (Castresana 2000) with default settings, were removed from the alignment; resulting in a final alignment of 9 999 bp in length. PartitionFinder v2.1.1 (Lanfear *et al*. 2012, 2016) was used to determine the optimal partition strategy and model of evolution for each partition using the following settings: greedy algorithm, unlinked branch lengths and model selection based on the Bayesian Information Criterion (BIC). A Bayesian tree was generated in MrBayes v3.2.6 (Huelsenbeck and Ronquist 2001) using the best-fit substitution model (GTR + I + G) and three partitions corresponding to the first, second and third nucleotide positions within codons. The Bayesian analysis using the Markov Chain Monte Carlo (MCMC) method was run for 1 000 000 generations, sampled every 1 000 generations and the first 25% of trees were omitted as burn-in with the remaining trees used to calculate the posterior probabilities. The phylogenetic tree was viewed and edited through FigTree v1.4.3 (Rambaut 2016).

2.3 Results

2.3.1 Mitochondrial genome assembly and annotation

The resulting Sanger sequences generated for the four specimens were used to confirm species identification for *H. natalensis* and *P. pantherinum*. Due to the lack of genetic differentiation between *Haploblepharus* species and the presence of ambiguous sites (Gledhill *et al*. submitted), genus-level identification could only be obtained for the two *Haploblepharus* specimens. Consequently, species identification for *Haploblepharus* relied on morphological identification based on taxonomic keys (Human 2007*a*).

The two high-throughput sequencing runs of the barcoded libraries generated 30 GB of quality filtered data with the number of sequences generated per sample ranging from 5 699 873 to 7 341 754 (**Table 2.1**). For each sample the number of sequences identified as belonging to the mitochondrial genome are shown in **Table 2.1**. The lowest mean coverage was ~39 reads and for each assembly at least 85% of the data had a phred quality score of ≥ 20.

Table 2.1 High-throughput sequencing and mitochondrial genome assembly results for four scyliorhinid species: *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN) and *Poroderma pantherinum* (PP).

	Number of sequences	mtDNA sequences	Mean length	Q ≥ 20	Mean coverage
HE	7 341 754	7 525	345.3	87.1%	144.1
HP	6 372 953	3 347	342.8	88.4%	64.3
HN	5 846 001	2 592	412.0	85.6%	65.1
PP	5 699 873	1 561	411.3	85.7%	38.8

Abbreviations: mtDNA (mitochondrial DNA), phred quality score (Q).

The complete and partial mitogenome lengths, along with nucleotide compositions for each respective species are shown in **Table 2.2**. As reported for most elasmobranchs (Yang *et al*. 2014; Chen *et al*. 2016; Ruck *et al*. 2017), the nucleotide base composition was AT-rich with a bias against G. The mitogenomes were similar in size to two of the three publicly available scyliorhinid mitogenomes, *Cephaloscyllium umbratile* Jordan and Fowler 1903 (16 698 bp; Chen *et al*. 2015) and *Scyliorhinus canicula* (16 697 bp; Delarbre *et al*. 1998); while also similar to the mitogenome lengths reported for the majority of Carcharhiniformes (16 691 – 16 754 bp; **Table A2.1**). For the third scyliorhinid, *Halaelurus buergeri*, Chen *et al*. (2016) described a mitogenome longer than in most elasmobranchs (19 100 bp); attributed to the longest reported control region (3 481 bp) due to a 60 bp and 47 bp tandem repeat motif. The complete mitogenome of the leopard catshark *P. pantherinum* comprises 13 protein-coding, 22 tRNA, two rRNA genes, and one non-coding control region (**Table 2.3**). The mitogenomes of *H. natalensis* (**Table 2.4**) and *Haploblepharus* species (**Table 2.5** and **2.6**) are reported as partial mitogenomes as the nucleotide sequences for the respective control regions were not confidently recovered; however, the gene content and organisation were still found to be the same as those

recovered for *P. pantherinum*. Noteworthy are the two possible mitogenome sequences recovered for each of the *Haploblepharus* species, with only one premature stop codon detected in the *ND4* gene region of Haplotype 2 due to a 14 bp frameshift deletion (**Table 2.5** and **2.6**). Gene organisation was in a similar fashion to most other vertebrate taxa (**Figure 2.4**, **2.5**, **2.6** and **2.7**); with all genes starting with the standard ATG codon, except *COI*, which started with the alternate GTG codon. The majority of protein-coding genes terminated with the TAA stop codon, while alternate genes ended with an incomplete stop codon (TA–/T–); which are presumably completed as termination codons (TAA) upon post-transcriptional polyadenylation (Anderson *et al*. 1981; Hou *et al*. 2006; Ki *et al*. 2010). All genes were encoded on the H-strand except for 8 tRNA genes and *ND6*. The usual overlapping gene regions were identified between *ATP8*/*ATP6*, *ND4L*/*ND4* and *ND5*/*ND6* in *P. pantherinum* and *H. natalensis*; additionally, for *Haploblepharus* species $tRNA^{Gln}$ and $tRNA^{Met}$ overlapped by a single nucleotide (**Table 2.5** and **2.6**).

Table 2.2 Mitochondrial genome sizes and nucleotide compositions for each of the four respective study species; only data for *Poroderma pantherinum* represents a complete mitochondrial genome, while the data for *Haploblepharus* and *Halaelurus* represent partial mitochondrial genomes (excluding control regions).

	Size (bp)	A	T	C	G	GC
Haploblepharus edwardsii Haplotype 1	15 628	31.7	31.2	23.4	13.7	37.1
Haploblepharus edwardsii Haplotype 2	15 588	31.6	31.0	23.6	13.9	37.4
Haploblepharus pictus Haplotype 1	15 626	31.7	31.2	23.4	13.7	37.1
Haploblepharus pictus Haplotype 2	15 586	31.6	31.0	23.6	13.9	37.5
Halaelurus natalensis	15 635	31.0	30.7	24.2	14.1	38.3
Poroderma pantherinum	16 686	31.0	30.1	25.0	13.9	38.9

Abbreviations: base pairs (bp).

Table 2.3 Characteristics of the complete mitochondrial genome sequence for the leopard catshark *Poroderma pantherinum*.

Poroderma pantherinum				Codon			
Gene	From (bp)	To (bp)	Size (bp)	Start	Stop	Intergenic nucleotides	Strand
tRNA^{Phe}	1	69	69			0	H
12S rRNA	70	1 024	955			0	H
tRNA^{Val}	1 025	1 096	72			0	H
16S rRNA	1 097	2 763	1 667			0	H
tRNA^{Leu}(UAA)	2 764	2 838	75			0	H
ND1	2 839	3 813	975	ATG	TAA	3	H
tRNA^{Ile}	3 817	3 885	69			1	H
tRNA^{Gln}	3 887	3 958	72			0	L
tRNA^{Met}	3 959	4 028	70			0	H
ND2	4 029	5 074	1 046	ATG	TA–	0	H
tRNA^{Trp}	5 075	5 143	69			1	H
tRNA^{Ala}	5 145	5 213	69			0	L
tRNA^{Asn}	5 214	5 286	73			35	L
tRNA^{Cys}	5 322	5 388	67			1	L
tRNA^{Tyr}	5 390	5 459	70			1	L
COI	5 461	7 014	1 554	GTG	TAA	0	H
tRNA^{Ser}(UGA)	7 015	7 085	71			4	L
tRNA^{Asp}	7 090	7 159	70			4	H
COII	7 164	7 854	691	ATG	T– –	0	H
tRNA^{Lys}	7 855	7 928	74			1	H
ATP8	7 930	8 097	168	ATG	TAA	–10	H
ATP6	8 088	8 770	683	ATG	TA–	0	H
COIII	8 771	9 556	786	ATG	TAA	2	H
tRNA^{Gly}	9 559	9 628	70			0	H
ND3	9 629	9 977	349	ATG	T– –	0	H
tRNA^{Arg}	9 978	10 047	70			0	H
ND4L	10 048	11 344	297	ATG	TAA	–7	H
ND4	10 338	11 718	1 381	ATG	T– –	0	H
tRNA^{His}	11 719	11 787	69			0	H
tRNA^{Ser}(GCU)	11 788	11 854	67			0	H
tRNA^{Leu}(UAG)	11 855	11 926	72			0	H
ND5	11 927	13 756	1 830	ATG	TAA	–4	H
ND6	13 753	14 271	519	ATG	TAA	0	L
tRNA^{Glu}	14 272	14 341	70			2	L
cytb	14 344	15 487	1 144	ATG	T– –	0	H
tRNA^{Thr}	15 488	15 558	71			0	H
tRNA^{Pro}	15 559	15 627	69			0	L
D-loop	15 628	16 686	1 059			0	H

Abbreviations: base pairs (bp).

Table 2.4 Characteristics of the partial mitochondrial genome sequence for the tiger catshark *Halaelurus natalensis*.

Halaelurus natalensis				Codon			
Gene	From (bp)	To (bp)	Size (bp)	Start	Stop	Intergenic nucleotides	Strand
tRNA^{Phe}	1	70	70			0	H
12S rRNA	71	1 031	961			0	H
tRNA^{Val}	1 032	1103	72			0	H
16S rRNA	1 104	2 774	1 671			0	H
tRNA^{Leu}(UAA)	2 775	2 849	75			0	H
ND1	2 850	3 824	975	ATG	TAA	2	H
tRNA^{Ile}	3 827	3 895	69			1	H
tRNA^{Gln}	3 897	3 968	72			0	L
tRNA^{Met}	3 969	4 037	69			0	H
ND2	4 038	5 082	1 045	ATG	T– –	0	H
tRNA^{Trp}	5 083	5 153	71			1	H
tRNA^{Ala}	5 155	5 223	69			0	L
tRNA^{Asn}	5 224	5 296	73			33	L
tRNA^{Cys}	5 330	5 394	65			0	L
tRNA^{Tyr}	5 395	5 463	69			1	L
COI	5 465	7 021	1 557	GTG	TAA	1	H
tRNA^{Ser}(UGA)	7 023	7 093	71			4	L
tRNA^{Asp}	7 098	7 167	70			2	H
COII	7 170	7 860	691	ATG	T– –	0	H
tRNA^{Lys}	7 861	7 934	74			1	H
ATP8	7 936	8 103	168	ATG	TAA	–10	H
ATP6	8 094	8 776	683	ATG	TA–	0	H
COIII	8 777	9 562	786	ATG	TAA	2	H
tRNA^{Gly}	9 565	9 634	70			0	H
ND3	9 635	9 983	349	ATG	T– –	0	H
tRNA^{Arg}	9 984	10 053	70			0	H
ND4L	10 054	10 350	297	ATG	TAA	–7	H
ND4	10 344	11 724	1 381	ATG	T– –	0	H
tRNA^{His}	11 725	11 793	69			0	H
tRNA^{Ser}(GCU)	11 794	11 860	67			0	H
tRNA^{Leu}(UAG)	11 861	11 932	72			0	H
ND5	11 933	13 762	1 830	ATG	TAA	–8	H
ND6	13 755	14 279	525	ATG	TAA	0	L
tRNA^{Glu}	14 280	14 349	70			1	L
cytb	14 351	15 494	1 144	ATG	T– –	0	H
tRNA^{Thr}	15 495	15 565	71			0	H
tRNA^{Pro}	15 566	15 635	70			0	L
D-loop	–	–	–				

Abbreviations: base pairs (bp).

Table 2.5 Characteristics of the two partial mitochondrial genome haplotypes recovered for the puffadder shyshark *Haploblepharus edwardsii*. Stop codons and intergenic spaces are only shown for Haplotype 2 if they differed from those stated for Haplotype 1.

Haploblepharus edwardsii										
	Haplotype 1			Haplotype 2			Codon			
	From (bp)	To (bp)	Size (bp)	From (bp)	To (bp)	Size (bp)	Start	Stop	Intergenic nucleotides	Strand
tRNA^{Phe}	1	70	70	1	70	70			0	H
12S rRNA	71	1 026	956	71	1 026	956			0	H
tRNA^{Val}	1 027	1 098	72	1 027	1 098	72			0	H
16S rRNA	1 099	2 765	1 667	1 099	2 741	1 643			0	H
tRNA^{Leu}(UAA)	2 766	2 840	75	2 742	2 816	75			0	H
ND1	2 841	3 815	975	2 817	3 791	975	ATG	TAA	1	H
tRNA^{Ile}	3 819	3 885	69	3 793	3 861	69			1	H
tRNA^{Gln}	3 887	3 957	71	3 863	3 933	71			–1	L
tRNA^{Met}	3 957	4 026	69	3 933	4 001	69			0	H
ND2	4 026	5 070	1 045	4 002	5 046	1 045	ATG	T– –	0	H
tRNA^{Trp}	5 071	5 141	71	5 047	5 117	71			1	H
tRNA^{Ala}	5 143	5 211	69	5 119	5 187	69			0	L
tRNA^{Asn}	5 212	5 284	73	5 188	5 260	73			34	L
tRNA^{Cys}	5 319	5 384	66	5 295	5 360	66			0	L
tRNA^{Tyr}	5 385	5 453	69	5 361	5 429	69			1	L
COI	5 455	7 011	1 557	5 431	6 987	1 557	GTG	TAA	1	H
tRNA^{Ser}(UGA)	7 013	7 083	71	6 989	7 059	71			4	L
tRNA^{Asp}	7 088	7 157	70	7 064	7 133	70			2	H
COII	7 160	7 850	691	7 136	7 826	691	ATG	T– –	0	H
tRNA^{Lys}	7 851	7 924	74	7 827	7 900	74			1	H
ATP8	7 926	8 093	168	7 902	8 067	166	ATG	TAA	T– – –10	–8 H
ATP6	8 084	8 766	683	8 060	8 742	683	ATG	TA–	0	H
COIII	8 767	9 552	786	8 743	9 528	786	ATG	TAA	T– – 2	0 H

Abbreviations: base pairs (bp).

Table 2.5 Continued.

Haploblepharus edwardsii											
	Haplotype 1			Haplotype 2			Codon				
	From (bp)	To (bp)	Size (bp)	From (bp)	To (bp)	Size (bp)	Start	Stop		Intergenic nucleotides	Strand
tRNA^{Gly}	9 555	9 624	70	9 529	9 598	70				0	H
ND3	9 625	9 973	349	9 599	9 947	349	ATG	T– –		0	H
tRNA^{Arg}	9 974	10 043	70	9 948	10 017	70				0	H
ND4L	10 044	10 340	297	10 018	10 314	297	ATG	TAA		–7	H
ND4	10 334	11 714	1 381	10 308	11 288	981	ATG	T– –	TAA	0 386	H
tRNA^{His}	11 715	11 783	69	11 675	11 743	69				0	H
tRNA$^{Ser(GCU)}$	11 784	11 851	68	11 744	11 811	68				0	H
tRNA$^{Leu(UAG)}$	11 852	11 923	72	11 812	11 883	72				0	H
ND5	11 924	13 753	1 830	11 884	13 713	1 830	ATG	TAA		–8	H
ND6	13 746	14 270	525	13 706	14 230	525	ATG	TAA		0	L
tRNA^{Glu}	14 271	14 340	70	14 231	14 300	70				1	L
cytb	14 342	15 485	1 144	14 302	15 445	1 144	ATG	T– –		0	H
tRNA^{Thr}	15 486	15 556	71	15 446	15 516	71				2	H
tRNA^{Pro}	15 559	15 628	70	15 519	15 588	70				0	L
D-loop	–	–	–	–	–	–					

Abbreviations: base pairs (bp).

Table 2.6 Characteristics of the two partial mitochondrial genome haplotypes recovered for the dark shyshark *Haploblepharus pictus*. Stop codons and intergenic spaces are only shown for Haplotype 2 if they differed from those stated for Haplotype 1.

Haploblepharus pictus											
	Haplotype 1			Haplotype 2			Codon				
	From (bp)	To (bp)	Size (bp)	From (bp)	To (bp)	Size (bp)	Start	Stop		Intergenic nucleotides	Strand
tRNA^{Phe}	1	70	70	1	70	70				0	H
12S rRNA	71	1 024	954	71	1 024	954				0	H
tRNA^{Val}	1 025	1 096	72	1 025	1 096	72				0	H
16S rRNA	1 097	2 763	1 667	1 097	2 739	1 643				0	H
tRNA^{Leu}(UAA)	2 764	2 838	75	2 740	2 814	75				0	H
ND1	2 839	3 813	975	2 815	3 789	975	ATG	TAA		1	H
tRNA^{Ile}	3 815	3 883	69	3 791	3 859	69				1	H
tRNA^{Gln}	3 885	3 955	71	3 861	3 931	71				–1	L
tRNA^{Met}	3 955	4 023	69	3 931	3 999	69				0	H
ND2	4 024	5 068	1 045	4 000	5 044	1 045	ATG	T– –		0	H
tRNA^{Trp}	5 069	5 139	71	5 045	5 115	71				1	H
tRNA^{Ala}	5 141	5 209	69	5 117	5 185	69				0	L
tRNA^{Asn}	5 210	5 282	73	5 186	5 258	73				34	L
tRNA^{Cys}	5 317	5 382	66	5 293	5 358	66				0	L
tRNA^{Tyr}	5 383	5 451	69	5 359	5 427	69				1	L
COI	5 453	7 009	1 557	5 429	6 985	1 557	GTG	TAA		1	H
tRNA^{Ser}(UGA)	7 011	7 081	71	6 987	7 057	71				4	L
tRNA^{Asp}	7 086	7 155	70	7 062	7 131	70				2	H
COII	7 158	7 848	691	7 134	7 824	691	ATG	T– –		0	H
tRNA^{Lys}	7 849	7 922	74	7 825	7 898	74				1	H
ATP8	7 924	8 091	168	7 900	8 065	166	ATG	TAA	T– –	–10	–8 H
ATP6	8 082	8 764	683	8 058	8 740	683	ATG	TA–		0	H
COIII	8 765	9 550	786	8 741	9 526	786	ATG	TAA	T– –	2	0 H

Abbreviations: base pairs (bp).

Table 2.6 Continued.

Haploblepharus pictus											
	Haplotype 1			Haplotype 2			Codon				
	From (bp)	To (bp)	Size (bp)	From (bp)	To (bp)	Size (bp)	Start	Stop		Intergenic nucleotides	Strand
tRNA^{Gly}	9 553	9 622	70	9 527	9 596	70				0	H
ND3	9 623	9 971	349	9 597	9 945	349	ATG	T– –		0	H
tRNA^{Arg}	9 972	10 041	70	9 946	10 015	70				0	H
ND4L	10 042	10 338	297	10 016	10 312	297	ATG	TAA		–7	H
ND4	10 332	11 712	1 381	10 306	11 286	981	ATG	T– –	TAA	0 386	H
tRNA^{His}	11 713	11 781	69	11 673	11 741	69				0	H
tRNA^{Ser}(GCU)	11 782	11 849	68	11 742	11 809	68				0	H
tRNA^{Leu}(UAG)	11 850	11 921	72	11 810	11 881	72				0	H
ND5	11 922	13 751	1 830	11 882	13 711	1 830	ATG	TAA		–8	H
ND6	13 744	14 268	525	13 704	14 228	525	ATG	TAA		0	L
tRNA^{Glu}	14 269	14 338	70	14 229	14 298	70				1	L
cytb	14 340	15 483	1 144	14 300	15 443	1 144	ATG	T– –		0	H
tRNA^{Thr}	15 484	15 554	71	15 444	15 514	71				2	H
tRNA^{Pro}	15 557	15 626	70	15 517	15 586	70				0	L
D-loop	–	–	–	–	–	–					

Abbreviations: base pairs (bp).

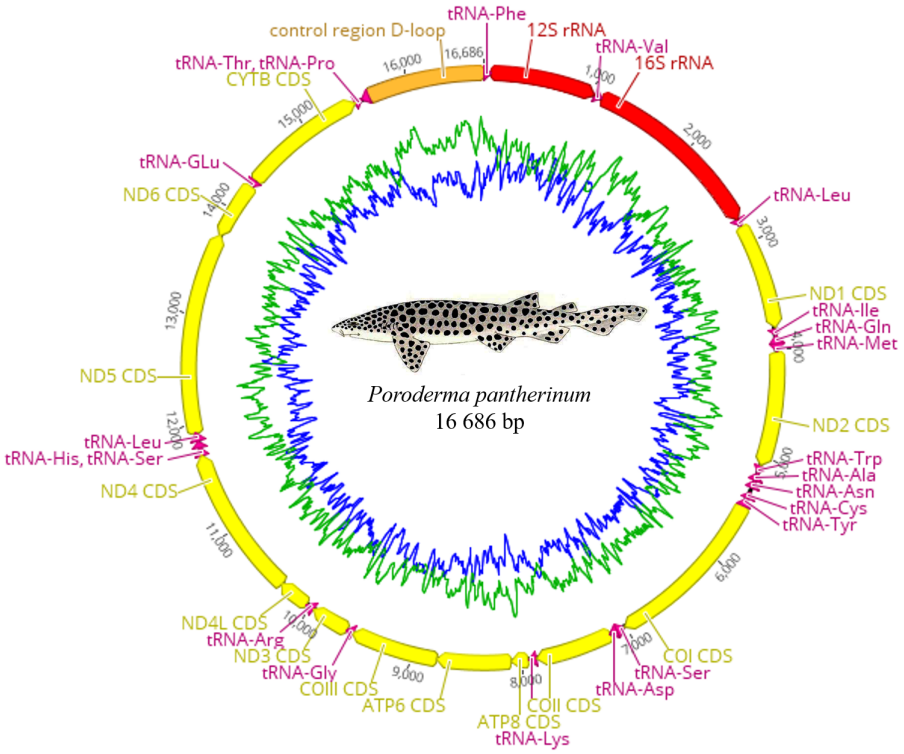


Figure 2.4 Mitochondrial genome organisation for the leopard catshark *Poroderma pantherinum*. The GC content and AT content are plotted in blue and green, respectively.

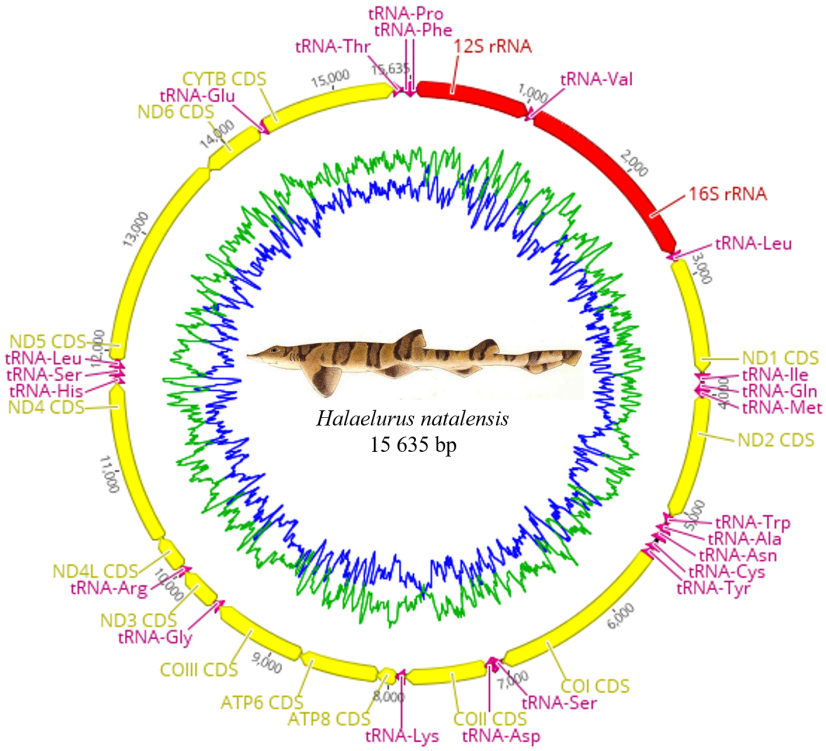


Figure 2.5 Mitochondrial genome organisation for the tiger catshark *Halaelurus natalensis*. The GC content and AT content are plotted in blue and green, respectively.

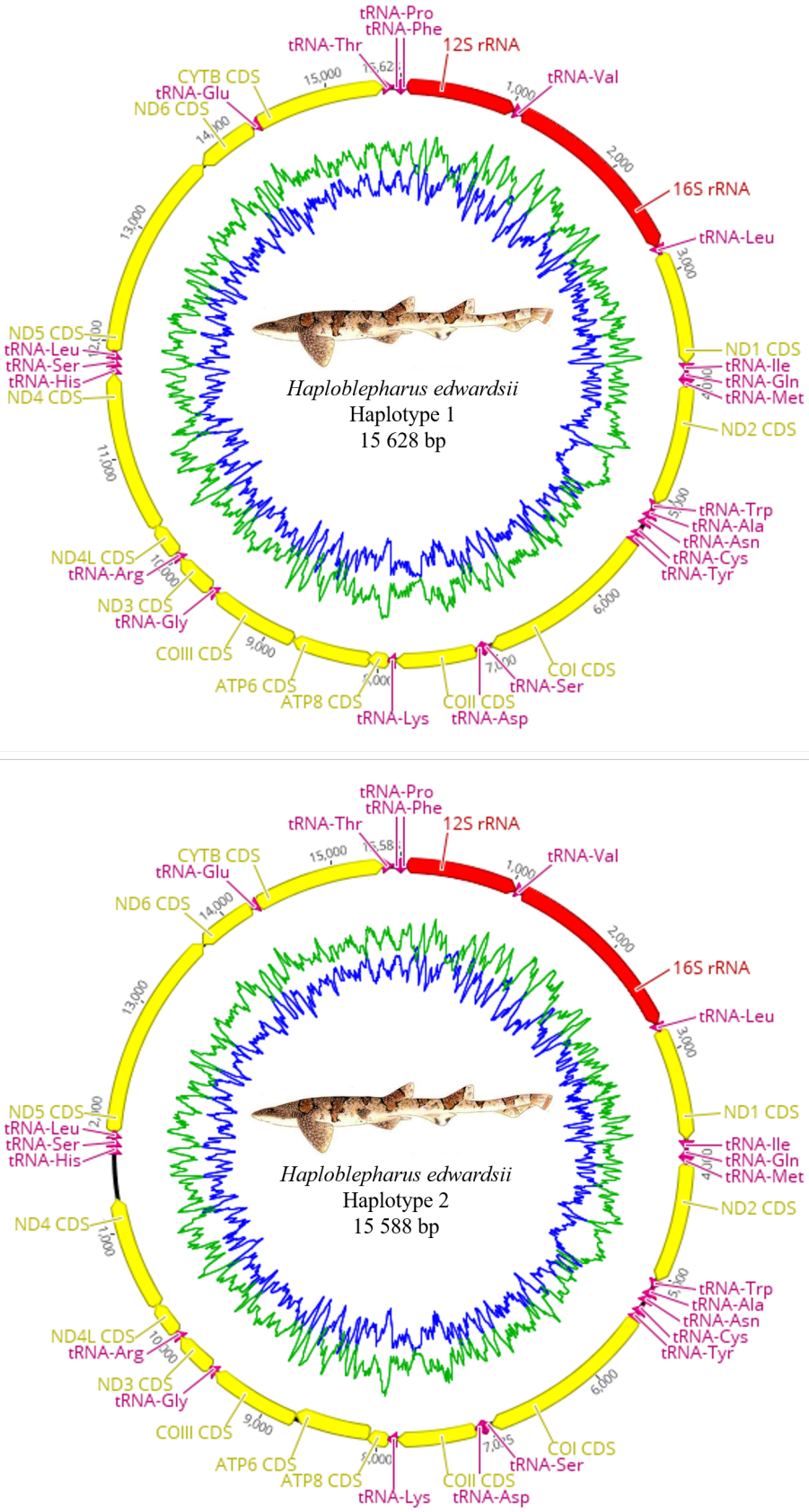


Figure 2.6 Mitochondrial genome organisation for Haplotype 1 and Haplotype 2 for the puffadder shyshark *Haploblepharus edwardsii*. The GC content and AT content are plotted in blue and green, respectively.

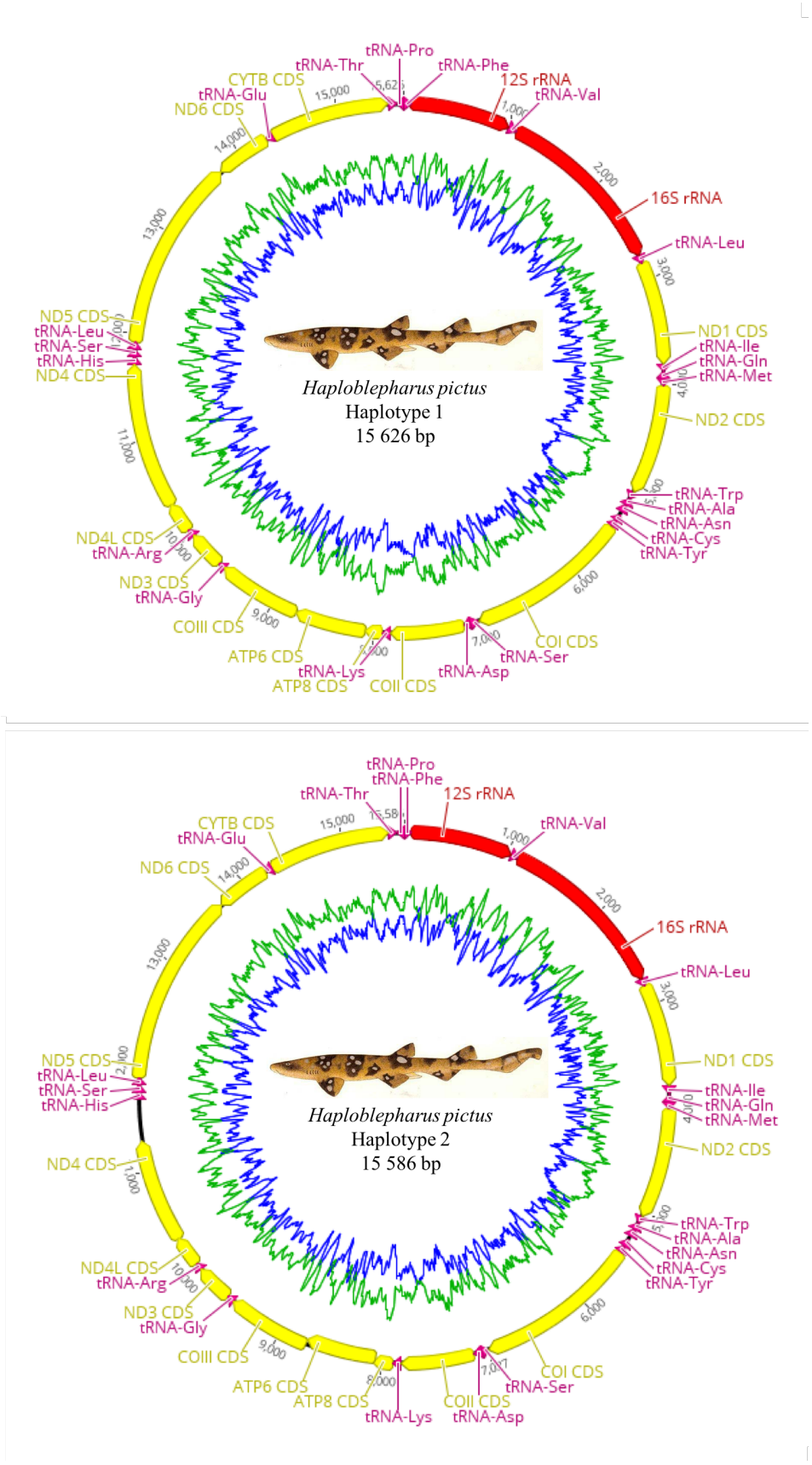


Figure 2.7 Mitochondrial genome organisation for Haplotype 1 and Haplotype 2 for the dark shyshark *Haploblepharus pictus*. The GC content and AT content are plotted in blue and green, respectively.

2.3.2 Haplotype separation for Haploblepharus species

At least two different mitogenome templates were evident in the mitogenome assemblies for *H. edwardsii* and *H. pictus*. Over 900 single nucleotide polymorphism (SNP) variants were identified throughout each mitogenome using the 'Find Variations/SNPs' feature. While the presence of more than two mitogenome templates is probable, this study only investigated the two most frequent haplotypes. Accordingly, following haplotype separation approximately 400 SNPs remained in the mitogenome assemblies for *H. edwardsii* and *H. pictus*.

To exclude cross-contamination due to human error as a possible explanation for the presence of mtDNA SNPs, the newly separated haplotypes were compared to previously generated *COI* Sanger sequences (**Figure 2.8**; Gledhill *et al*. submitted). The double peaks present in numerous Sanger sequences corresponded to SNPs identified in the mitogenome assemblies. Notably, Sanger sequences were generated from samples collected from various different geographic locations along the South African coastline; additionally, DNA was extracted and samples sequenced in different batches, with some sequences generated in 2016 and others only generated in 2018. Interestingly, at position 210 and 237, only HP10 displayed a C/T transition which was confirmed by both Sanger and high-throughput sequencing data (**Figure 2.8**). Similarly, position 287 and 603 showed possible nucleotide differences to the haplotypes recovered in this study (**Figure 2.8**).

As suggested by Dudu *et al*. (2012), the possible presence of NUMTs was acknowledged and haplotype calling was performed with caution. The 12S rRNA region of the mitogenome assembly displayed what is characteristic of the presence of a NUMT (Richly and Leister 2004; White *et al*. 2008). While the presence of SNPs was detected in both *Haploblepharus* species, upon haplotype separation these sites were found on sequence reads that displayed misaligned 5′ and/or 3′ regions (**Figure A2.1**). The length between the misaligned regions is ~600 bp, which is within the range of sizes previously reported for vertebrate NUMTs (Zhang and Hewitt 1996). Following this, only a single haplotype was recovered for the 12S rRNA gene for both *Haploblepharus* species.

To exclude NUMTs as a possible explanation for ambiguous sites identified in protein-coding sequences, all sequences were translated into amino acid sequences using the vertebrate genetic code. While both synonymous and nonsynonymous variants were present, only one premature stop codon was detected in the *ND4* gene region due to a frameshift deletion. In the *ND2* gene region, only six of 43 SNPs were nonsynonymous, while only two of 48 and five of the 34 were nonsynonymous for the *COI* and *cytb* gene regions, respectively (**Table A2.2**). The comparison of protein sequences with other publically available sequences (**Table A2.1**) revealed that both putative amino acids were present, thus confirming the viability and functionality of the protein. As shown for the three gene regions (**Table A2.2**), Haplotype 1 was detected at a higher frequency in *H. edwardsii* than in *H. pictus*, while the converse is true for Haplotype 2. Additionally, average phred quality scores ranged from 23 to 27, further supporting the presence of sequencing errors to be of minimal concern.

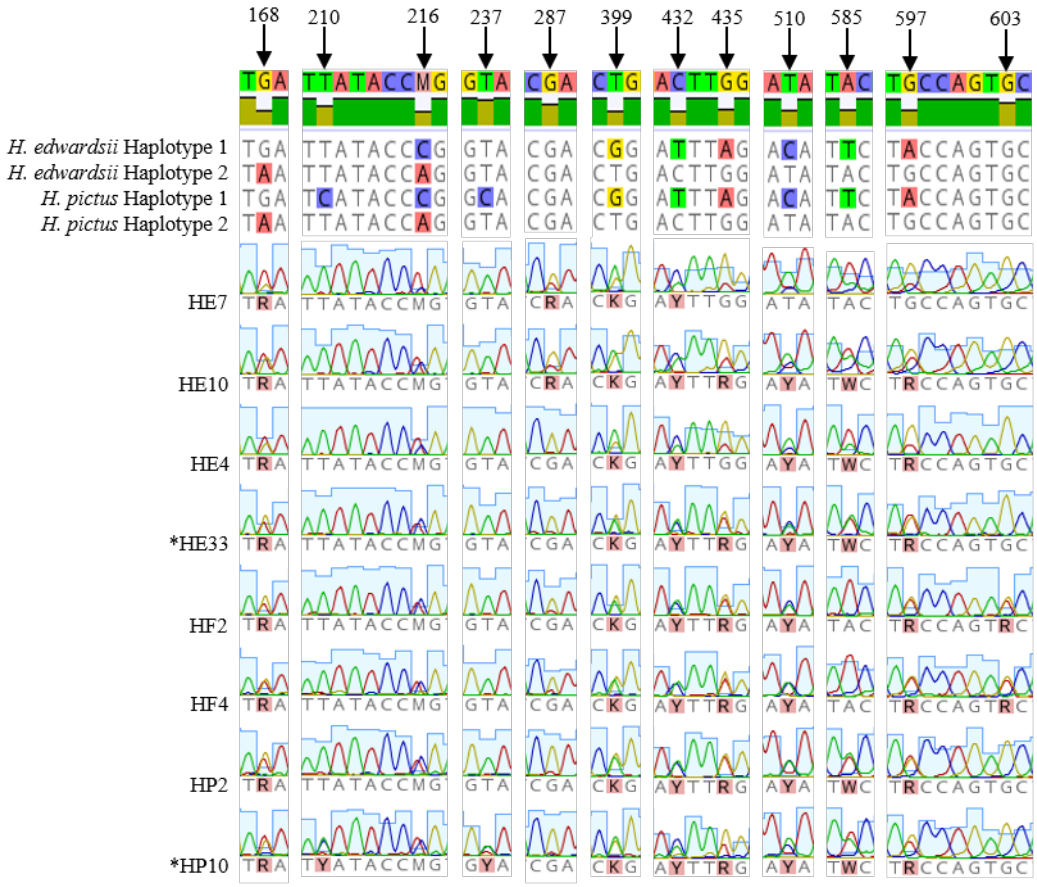


Figure 2.8 Partial consensus sequences and sequence electropherograms of cytochrome *c* oxidase subunit I (*COI*) in three *Haploblepharus* species, including: *H. edwardsii* (HE), *H. fuscus* (HF), *H. pictus* (HP); illustrating the presence of multiple mitochondrial DNA haplotypes. Samples denoted with an asterisk were used in high-throughput sequencing.

2.3.3 Sequence divergence

The degree of interspecific genetic differentiation was estimated for each protein-coding gene region (**Table 2.7**, **2.8**, **2.9** and **A2.3** – **A2.14**). All gene regions indicated that *P. pantherinum* displayed the greatest genetic distance from all other study species, ranging from 13.89 to 23.51% (**Table A2.5** and **Table A2.11**), with *ND4* providing the greatest genetic distance estimate. Genetic divergence estimates between *Haploblepharus* species and *H. natalensis* ranged from 5.34 to 13.44% depending on the gene region assessed. Interestingly, very little genetic differentiation was detected between *H. edwardsii* Haplotype 1 and *H. pictus* Haplotype 1, as well as *H. edwardsii* Haplotype 2 and *H. pictus* Haplotype 2, for all gene regions assessed (0.00 to 0.38%). In contrast, a greater genetic distance was recorded for each gene region between *H. edwardsii* Haplotype 1 and 2, and *H. pictus* Haplotype 1 and 2 (0.34 to 4.11%), with *ND2* providing the greatest genetic distance estimate (**Table 2.8**). The DNA barcoding region displayed relatively low levels of genetic distance between Haplotype 1 and Haplotype 2 (< 2.0%; **Table 2.7**). Also, *cytb* sequences obtained from Human (2003) displayed little genetic distance from Haplotype 2 (0.15 to 0.60%; **Table 2.9**).

Table 2.7 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for the 655 bp DNA barcoding region (cytochrome *c* oxidase subunit I).

			COI			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.47	0.22	0.47	1.57	1.99
HE_2	1.54		0.52	0.00	1.57	1.98
HP_1	0.31	1.86		0.52	1.61	1.98
HP_2	1.54	0.00	1.86		1.57	1.98
HN	13.54	13.52	13.94	13.52		1.88
PP	20.47	20.24	20.47	20.24	20.61	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table 2.8 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 1 046 bp of the nicotinamide adenine dehydrogenase subunit 2 (*ND2*) gene region.

			ND2			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.62	0.00	0.62	1.03	1.26
HE_2	4.11		0.62	0.00	1.06	1.27
HP_1	0.00	4.11		0.62	1.03	1.26
HP_2	4.11	0.00	4.11		1.06	1.27
HN	11.39	11.67	11.39	11.67		1.20
PP	21.82	21.53	21.82	21.53	20.86	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table 2.9 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 644 bp of the cytochrome subunit *b* (*cytb*) gene region. Sequences retrieved from Human (2003) are indicated by an asterisk (*).

					cytb						
	HE_1	HE_2	HP_1	HP_2	HE*	HF*	HP*	HN	HN*	PP	PP*
HE_1		0.63	0.00	0.63	0.65	0.65	0.67	1.16	1.20	1.55	1.56
HE_2	3.01		0.63	0.00	0.15	0.15	0.29	1.19	1.21	1.56	1.58
HP_1	0.00	3.01		0.63	0.65	0.65	0.67	1.16	1.20	1.55	1.56
HP_2	3.01	0.00	3.01		0.15	0.15	0.29	1.19	1.21	1.56	1.58
HE*	3.16	0.15	3.16	0.15		0.00	0.25	1.20	1.22	1.56	1.57
HF*	3.16	0.15	3.16	0.15	0.00		0.25	1.20	1.22	1.56	1.57
HP*	3.61	0.60	3.61	0.60	0.45	0.45		1.22	1.24	1.55	1.57
HN	10.69	11.90	10.69	11.90	12.05	12.05	12.50		0.43	1.51	1.52
HN*	11.45	12.35	11.45	12.35	12.50	12.50	12.95	1.36		1.52	1.53
PP	20.78	20.78	20.78	20.78	20.63	20.63	20.63	20.48	21.08		0.21
PP*	21.08	21.08	21.08	21.08	20.93	20.93	20.93	20.78	21.39	0.30	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

2.3.4 Phylogenetic reconstruction

Protein-coding sequences, excluding *ND4*, from 37 elasmobranch mitogenomes were included in the phylogenetic analyses, providing a final dataset of 9 999 bp in length. Only two nodes had less than 80% support based on this dataset. Within Carcharhiniformes, Scyliorhinidae proved to be paraphyletic; with the subfamily Scyliorhininae Gill 1862 forming a clade basal to the rest of the Carcharhiniformes. The Bayesian tree (**Figure 2.9**) illustrated that *P. pantherinum* clusters with two other scyliorhinid species (*Cephaloscyllium umbratile* and *Scyliorhinus canicula*) representing the subfamily Scyliorhininae with 100% support; while *H. natalensis* as well as the haplotypes recovered for *Haploblepharus* species clustered with an alternate catshark species, *Halaelurus buergeri,* representing the subfamily Pentanchinae Smith and Radcliffe 1912.

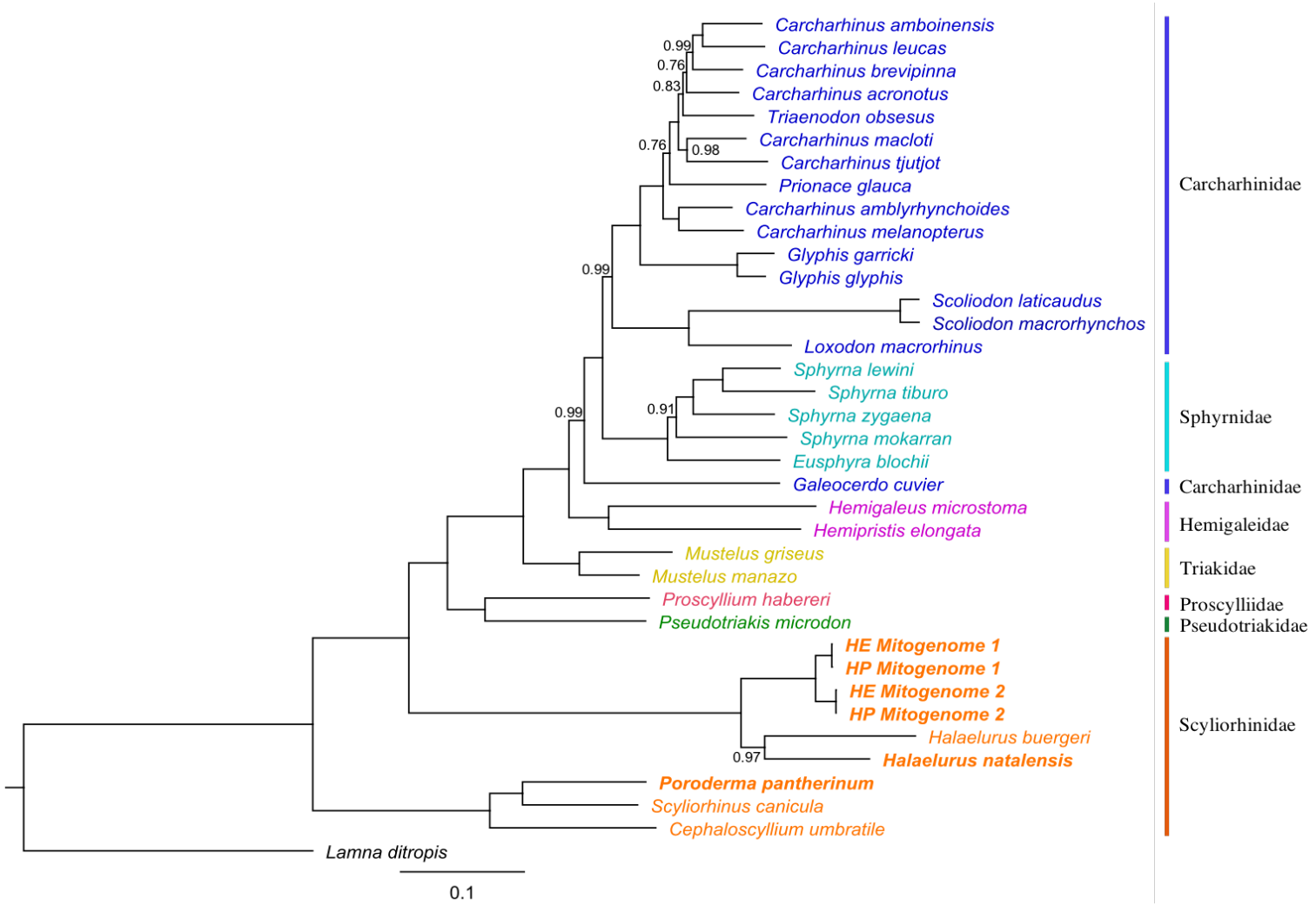


Figure 2.9 Bayesian tree depicting the phylogenetic positions of southern African endemic scyliorhinids, indicated in bold, within the order Carcharhiniformes (see **Table A2.1** for accession numbers), based on the protein-coding sequences (excluding *ND4*) of 37 mitochondrial genomes, using *Lamna ditropis* as an outgroup. Both mitochondrial genome haplotypes recovered for *Haploblepharus edwardsii* (HE) and *Haploblepharus pictus* (HP) are included. Posterior probabilities only displayed if below 1.0.

2.4 Discussion

This study reports the first complete mitogenome sequence for a South African endemic catshark, *Poroderma pantherinum*. Additionally, the partial mitogenomes for *Halaelurus natalensis*, *Haploblepharus edwardsii* and *Haploblepharus pictus* were recovered from high-throughput sequencing data. Mitogenome assemblies for *H. edwardsii* and *H. pictus* contained single nucleotide polymorphism (SNP) sequence variants in various mitochondrial genes, including both synonymous and nonsynonymous variants. Following mtDNA haplotype separation, interspecific sequence divergence was assessed for each protein-coding gene; with genetic and evolutionary distances being congruent among all genes assessed. Interestingly, divergence estimates between the mtDNA haplotypes recovered from a single *Haploblepharus* specimen met previously proposed species discrimination thresholds (> 3.5%; Zemlak *et al*. 2009). Accordingly, this is the first study to describe mtDNA heteroplasmy in elasmobranchs, with evidence hinting at interspecific hybridisation and paternal leakage as possible factors responsible for the phenomenon; however, alternate hypotheses are also discussed. Finally, the phylogenetic reconstruction performed in this study confirmed that Scyliorhinidae, as presently recognised, is paraphyletic.

Recent advances in high-throughput sequencing technologies have provided an efficient method for the recovery of whole mitogenomes at a significantly reduced cost (Feutry *et al*. 2014); leading to a notable increase in the availability of whole mitogenome data (Díaz-Jaimes *et al*. 2016). While the majority of molecular phylogenies are based on single genes, the increased availability of mitogenome data and consequent analyses of mitogenomes are providing new insights into intra- and interspecific relationships (Arnason *et al*. 2008; Morin *et al*. 2010; Feutry *et al*. 2014; Qin *et al*. 2015). In this study, the use of sequencing data generated on the Ion Torrent S5™ allowed for the efficient recovery of mitogenome sequences for four southern African endemic scyliorhinid species; however, some factors such as read length, precision of the assemblies, and haplotype separation can be improved. The control region sequences for *H. natalensis* and *Haploblepharus* species were not confidently recovered owing to poor mapping qualities; presumably due to the presence of repeat regions which is typical in the control region for elasmobranchs (Castro *et al*. 2007; Poorvliet and Hoarau 2013; Chen *et al*. 2016). Consequently, only partial mitogenomes are reported for *H. natalensis*, *H. edwardsii* and *H. pictus*; future work is required to confidently recover the control region sequences for these species.

In general, the mitochondrial genome is a relatively conserved molecule for the majority of animal taxa (Sederoff 1984; Gissi *et al*. 2008); comprising 13 intronless, protein-coding genes, all of which are involved in a process known as aerobic respiration (Ladoukakis and Zouros 2017). Moreover, mitogenome characteristics such as GC content, codon usage, gene organisation and mitogenome size have been shown to be similar in various elasmobranch species (Yang *et al*. 2014; Díaz-Jaimes *et al*. 2016; Ruck *et al*. 2017). Accordingly, the characteristics of the mitogenomes recovered in this study were all within the range of what is expected for species within the order Carcharhiniformes. Díaz-

Jaimes *et al*. (2016) reported that one of the main differences between two genera within Carcharhiniformes was a difference in mitogenome size, due to the typical high occurrence of tandem repeats in the control region (Castro *et al*. 2007; Poorvliet and Hoarau 2013; Chen *et al*. 2016). One of the largest reported mitogenomes for elasmobranchs belongs to the blackspotted catshark *Halaelurus buergeri*. Chen *et al*. (2016) attributed this to the longest reported control region (3 481 bp) which contains both a 60 bp and 47 bp tandem repeat motif. The retrieval and comparison of control region sequences from *H. natalensis* and *Haploblepharus* species could provide insight into whether the presence of an unusually long control region is conserved within congeneric species or within the tribe Halaelurini; alternatively, highlighting a valuable difference between genera within a genetically and morphologically conserved tribe.

For both *H. edwardsii* and *H. pictus*, the draft mitogenome assemblies indicated the presence of SNP sequence variants within a single individual. Possible cross-contamination between samples due to human error was ruled out as double peaks corresponding to the SNP sites were identified in numerous Sanger sequences generated for other *Haploblepharus* specimens (Gledhill *et al*. submitted). Dudu *et al*. (2012) cautioned that the possible presence of NUMTs should be accounted for when detecting SNP sites in mtDNA. While the presence of NUMTs cannot be completely dismissed, especially in non-coding gene regions, only a single region (12S rRNA) in the *Haploblepharus* mitogenomes displayed characteristics attributed to the presence of a NUMT. Previous studies revealed nucleotide substitution frequencies between closely related species to be 8 to 30 times more frequent for synonymous sites in comparison to nonsynonymous sites (Pesole *et al*. 1999; Doiron *et al*. 2002; Shigenbou *et al*. 2005). However, nonsynonymous SNPs have been reported in the *ND4, ND5* and *cytb* gene regions for fish species (Shigenbou *et al*. 2005; Dudu *et al*. 2012). Nonsynonymous variants detected in this study were assessed through the comparison of elasmobranch amino acid sequences and all putative amino acids were identified in alternate species, illustrating the viability and functionality of the proteins. Since there is no meiotic control in mtDNA replication, defected molecules such as the haplotype containing the truncated *ND4* gene can increase in frequency stochastically or due to faster replication attributed to its smaller size (Just *et al*. 2015). The presence of the truncated *ND4* gene may remain for a number of generations, as a functional *ND4* protein can be transcribed from the alternate haplotype. Therefore, this study is the first to describe the presence of SNP sequence variants from a dataset comprising two recognised elasmobranch species.

Overall, all gene regions used to assess interspecific genetic differentiation were congruent; however, the degree of genetic differentiation differed depending on the gene region assessed. As expected, *P. pantherinum* displayed the greatest genetic divergence from all other study species. Interestingly, a low level of genetic distance was evident between Haplotype 1 and Haplotype 2 within each *Haploblepharus* species; comparable to intraspecific genetic distances previously reported for sharks (Naylor *et al*. 2012; Steinke *et al*. 2016; Gledhill *et al*. submitted). In contrast, higher levels of

genetic distances were apparent between intraspecific Haplotype 1 and Haplotype 2. While the genetic distance evident among haplotypes for the DNA barcoding region do not meet previously suggested species delineation thresholds (> 2.0%); similar patterns of interspecific divergences have been reported for two closely related *Poroderma* species (Naylor *et al*. 2012; Steinke *et al*. 2016; Gledhill *et al*. submitted). Moreover, the genetic distance among haplotypes exceeded the 3.5% species delineation threshold suggested for the *ND2* gene region (Zemlak *et al*. 2009). Considering that the genetic distance between haplotypes within a single species is comparable to interspecific levels of genetic differentiation, the presence of mitogenome haplotypes is attributed to the occurrence of mtDNA heteroplasmy. Although relatively rare, the occurrence of point heteroplasmy has previously been reported in animals (Dudu *et al*. 2012).

It is widely accepted that mtDNA is maternally inherited, non-recombining and displays an elevated mutation rate in comparison to the nuclear genome (Dudu *et al*. 2012; Ladoukakis and Zouros 2017); making the use of mtDNA as a molecular tool appealing for evolutionary and conservation biology (Just *et al*. 2015). However, the general assumption of uniparental inheritance and homoplasmy is contrasted by accumulating evidence of exceptions to the rules; demonstrated by a growing list of taxa that display mtDNA biparental inheritance, heteroplasmy and mtDNA recombination (Kmiec *et al*. 2006; Levsen *et al*. 2016). Mitochondrial DNA heteroplasmy refers to the presence of multiple mitochondrial genomes within a single individual or tissue (Just *et al*. 2015). Length heteroplasmy occurs when individuals possess mtDNA molecules that differ in length, most commonly attributed to the poor replication fidelity within the hypervariable regions of the D-loop (Dudu *et al*. 2012). A less frequent phenomenon, known as point heteroplasmy, occurs due to the presence of various mtDNA molecules that differ from one another at a given nucleotide position (Dudu *et al*. 2012). Point heteroplasmy is considered to be a transient state (Santos *et al*. 2005, 2008; Irwin *et al*. 2009), owing to the germ-line bottleneck in oogenesis which can lead to complete homoplasmic shifts between generations (Levsen *et al*. 2016). In cows, mice and humans point heteroplasmy has been shown to generally resolve to homoplasmy within a few generations (Cree *et al*. 2008; Khrapko 2008). The presence of heteroplasmy at variable frequencies may be attributed to stochastic losses or due to selection pressures (Levsen *et al*. 2016). While mtDNA heteroplasmy has frequently been reported in teleost species (Arnason and Rand 1992; Broughton and Dowling 1994; Shigenbou *et al*. 2005; Dudu *et al*. 2012), to date no studies have described heteroplasmy in elasmobranchs (Marino *et al*. 2017).

Kmiec *et al*. (2006) suggested that mtDNA heteroplasmy could arise through two main processes: mutation or paternal leakage, further explaining that paternal leakage is the favoured process in flowering plants due to the low mtDNA mutation rate. Moreover, Dudu *et al*. (2012) stated that while various factors could lead to the presence of heteroplasmy, biparental inheritance of mtDNA had been reported in numerous fish species (Magoulas and Zouros 1993; Guo *et al*. 2006). Paternal leakage is a phenomenon predicted to occur due to the probable breakdown of mechanisms that recognise and

remove paternal mtDNA from the oocyte during interspecific hybridisation (White *et al*. 2008). Hybridisation among *Haploblepharus* species was previously hypothesised due to the high occurrence of species misidentification and overlap in morphological characters (Human 2003). Given the sympatric distribution of recently diverged *Haploblepharus* species and that interspecific hybridisation between closely related elasmobranch species has been described (Morgan *et al*. 2012; Cruz *et al*. 2015; Marino *et al*. 2015; Corrigan *et al*. 2017); hybridisation among *Haploblepharus* species is highly possible. Under the given assumption of interspecific hybridisation, the presence of heteroplasmy is possibly a consequence of mtDNA introgression.

The topology of the Bayesian tree generated in this study was largely congruent with previous phylogenies reported for elasmobranchs using individual mtDNA and/or nuclear genes (Human *et al*. 2006; Naylor *et al*. 2012; Chen *et al*. 2016; van Staden *et al*. 2018; Gledhill *et al*. submitted). However, the paraphyly of Scyliorhinidae contrasts the morphological conservatism displayed by the members of this family (Compagno 1988; Human *et al*. 2006). Numerous anatomical features have been described that group scyliorhinid taxa morphologically, including: posteriorly placed dorsal fins, rounded dorsal and pectoral fins, enlarged anterior nasal flaps, and clasper morphology (Compagno 1988). Conversely, molecular data has supported the presence of two subfamilies, namely, Scyliorhininae and Pentanchinae. The subfamily Scyliorhininae includes the genera *Cephaloscyllium*, *Scyliorhinus* and *Poroderma*; while Pentanchinae comprises 10 genera (Compagno 1988), represented in this study by *Halaelurus* and *Haploblepharus*. This study is the first to assess and confirm the monophyly of Scyliorhininae using representative species from all of the respective genera. However, whole mitogenome data has not been generated for the majority of other scyliorhinid genera and thus the presence of Pentanchinae is only supported by the monophyletic relationship observed among the genera *Halaelurus* and *Haploblepharus*. The *Halaelurus* and *Haploblepharus* clade was previously reported as unlikely due to contrasting results in the morphological phylogeny; future research requires the inclusion of genetic data from a representative of *Holohalaelurus* to confidently explore these interrelationships.

The Bayesian analysis illustrated the relatively low levels of interspecific genetic divergence between *Haploblepharus* Haplotype 1 and Haplotype 2; supporting the presence of recently diverged mitogenomes (Boomer *et al*. 2012; DiBattista *et al*. 2016). While the paraphyletic relationship within Scyliorhinidae is gaining support, the clustering of co-distributed southern African endemic scyliorhinids with alternate scyliorhinid species (*H. buergeri* and *C. umbratile*) displaying distributions endemic to the northern Pacific provides valuable insight into the origin of these species. Anderson (1994) suggested that the dispersal of species across biogeographical barriers could result in sympatric species that originated from different areas of endemism. Accordingly, the observed genetic divergence between *P. pantherinum* and the rest of the study species (*H. natalensis*, *H. edwardsii* and *H. pictus*) suggests the occurrence of two separate colonisation events of the southern African coastline.

2.5 Conclusion

In summary, this chapter reports on the first complete mitochondrial genome sequence for a South African endemic catshark, *Poroderma pantherinum*; as well as the partial mitogenomes for *Halaelurus natalensis*, *Haploblepharus edwardsii* and *Haploblepharus pictus*. The presence of single nucleotide polymorphism sequence variants detected for *H. edwardsii* and *H. pictus* was assessed, and a case of heteroplasmy possibly due to paternal leakage was described. Furthermore, interspecific sequence divergence was assessed for all protein-coding genes and evolutionary distances were congruent across all gene regions assessed. Finally, the phylogenetic reconstruction performed in this study confirmed that Scyliorhinidae, as presently recognised, is paraphyletic; providing valuable insight into the genetic relationships among southern African scyliorhinids.

2.6 Appendix

Table A2.1 Mitochondrial genome size in base pairs (bp) and accession numbers for publically available elasmobranch sequences assessed in this study.

Family	Scientific name	Size (bp)	Accession number
Carcharhinidae	*Carcharhinus acronotus*	16 719	NC_024055.1
	Carcharhinus amblyrhynchoides	16 705	NC_023948.1
	Carcharhinus amboinensis	16 704	NC_026696.1
	Carcharhinus brevipinna	16 706	KM244770.1
	Carcharhinus leucas	16 704	KF646785.1
	Carcharhinus macloti	16 701	NC_024862.1
	Carcharhinus melanopterus	16 706	NC_024284.1
	Carcharhinus tjutjot	16 705	KP091436.1
	Galeocerdo cuvier	16 703	NC_022193.1
	Glyphis garricki	16 702	KF646786.1
	Glyphis glyphis	16 701	NC_021768.2
	Loxodon macrorhinus	16 702	KT347599.1
	Prionace glauca	16 705	NC_022819.1
	Scoliodon laticaudus	16 695	KP336547.1
	Scoliodon macrorhynchos	16 693	NC_018052.1
	Triaenodon obsesus	16 700	KJ748376.1
Hemigaleidae	*Hemigaleus microstoma*	16 701	KT003687.1
	Hemipristis elongata	16 691	KU508621.1
Proscylliidae	*Proscyllium habereri*	16 708	KU721838.1
Pseudotriakidae	*Pseudotriakis microdon*	16 700	NC_022735.1
Scyliorhinidae	*Cephaloscyllium umbratile*	16 698	NC_029399.1
	Halaelurus buergeri	19 100	NC_0311811.1
	Halaelurus natalensis		This study
	Haploblepharus edwardsii		This study
	Haploblepharus pictus		This study
	Poroderma pantherinum	16 686	This study
	Scyliorhinus canicula	16 697	NC_001950.1
Sphyrnidae	*Eusphyra blochii*	16 727	NC_031812.1
	Sphyrna lewini	16 726	NC_022679.1
	Sphyrna mokarran	16 719	KY464952.1
	Sphyrna tiburo	16 723	KM453976.1
	Sphyrna zygaena	16 731	NC_025778.1
Triakidae	*Mustelus griseus*	16 754	NC_023527.1
	Mustelus manazo	16 707	NC_000890.1
Lamniformes	*Lamna ditropis* (outgroup)	16 699	KF962053.1

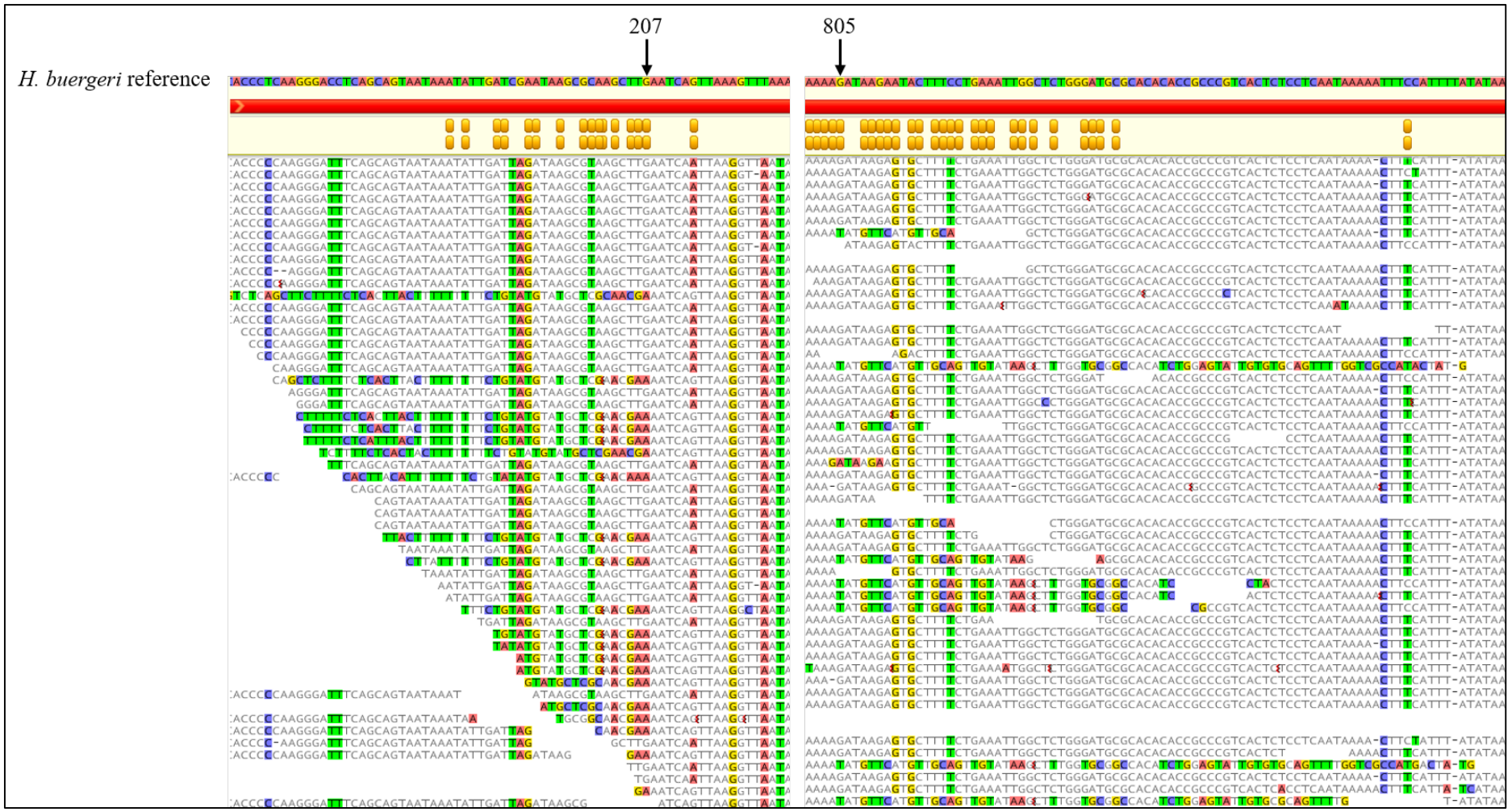


Figure A2.1 Draft assembly for the puffadder shyshark *Haploblepharus edwardsii*, depicting the possible presence of an approximately 600 bp nuclear mitochondrial DNA segment (NUMT) in the 12S rRNA gene region. Sequence reads displayed relatively few nucleotide changes to the consensus between 207 bp and 805 bp in the assembly, however, the 5′ and/or 3′ regions of these sequence reads are misaligned (possibly nuclear DNA). Abbreviations: base pairs (bp), five prime (5′), three prime (3′).

Table A2.2 Heteroplasmic sites identified using high-throughput sequencing data from two morphologically similar scyliorhinids, *Haploblepharus edwardsii* and *Haploblepharus pictus*. Details include: gene region, position of the site within the gene region, depth of coverage at the respective site, nucleotides at Haplotype 1 and Haplotype 2, nucleotide frequencies, phred quality scores (Q), the position of the site within a codon, and the respective amino acid change.

		Haploblepharus edwardsii							*Haploblepharus pictus*								
		Haplotype 1				Haplotype 2			Haplotype 1				Haplotype 2				
	Position	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Codon position	Amino acid change
ND2	19	246	A	82.1	17	T	17.5	30	85	A	71.8	18	T	27.1	28	1	Thr/Ser
	27	248	T	81.0	16	C	17.7	29	84	T	72.6	16	C	26.2	27	3	
	37	244	C	75.0	14	T	18.9	16	85	C	70.6	13	T	25.9	17	1	
	44	242	T	80.6	25	G	18.6	27	86	T	74.4	25	G	24.4	28	2	Leu/Arg
	69	236	A	81.4	29	C	18.2	28	87	A	75.9	29	C	23.0	29	3	
	81	240	T	81.7	18	C	18.3	17	85	T	74.1	18	C	24.7	18	3	
	177	213	T	83.1	15	C	16.9	29	66	T	78.8	11	C	21.2	29	3	
	198	200	C	83.0	16	T	16.0	17	64	C	71.9	16	T	25.0	14	3	
	201	201	T	83.1	29	C	16.9	17	65	T	76.9	29	C	23.1	14	3	
	228	197	C	82.2	25	T	16.8	31	67	C	79.1	17	T	20.9	29	3	
	240	190	T	83.2	25	C	16.2	26	64	T	79.7	18	C	20.3	17	3	
	279	181	C	85.3	24	A	14.7	29	67	C	82.1	17	A	17.9	31	3	
	297	172	A	84.6	30	C	15.4	23	64	A	81.3	29	C	18.8	16	3	
	299	167	T	84.7	25	C	15.3	25	63	T	82.5	17	C	17.5	18	2	Val/Ala
	300	166	T	84.6	26	C	15.4	24	64	T	82.8	18	C	17.2	16	3	
	321	165	A	74.4	26	C	15.4	26	61	A	77.2	27	C	16.4	25	3	
	339	160	T	87.6	29	C	12.4	33	64	T	87.5	30	C	12.5	34	3	
	375	150	A	91.3	25	C	8.7	24	52	A	90.4	26	C	9.6	32	3	
	384	148	C	91.9	29	T	8.1	23	50	C	90.0	30	T	10.0	24	3	
	387	147	C	91.8	29	T	8.2	26	50	C	90.0	31	T	10.0	29	3	

Table A2.2 Continued.

	Haploblepharus edwardsii							*Haploblepharus pictus*								
	Haplotype 1				Haplotype 2			Haplotype 1				Haplotype 2				
Position	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Codon position	Amino acid change
429	135	T	87.4	25	C	10.4	21	47	T	87.2	26	C	10.6	23	3	
459	133	G	88.0	27	T	10.5	27	47	G	89.4	27	T	8.5	24	3	
463	130	T	89.2	21	C	10.0	26	46	T	93.5	21	C	6.5	29	1	
508	105	T	91.4	30	C	8.6	31	40	T	87.5	31	C	12.5	28	1	
525	106	A	92.5	27	G	6.6	31	41	A	87.8	29	G	12.2	27	3	
564	98	A	92.9	30	T	6.1	27	37	A	89.2	30	T	8.1	20	3	
571	100	A	93.0	26	T	7.0	23	37	A	89.2	27	T	8.1	17	1	Ile/Phe
609	94	A	93.6	22	G	6.4	30	35	A	88.6	23	G	8.6	26	3	
654	92	A	92.7	19	C	6.5	28	33	A	90.9	28	C	9.1	27	3	
735	89	T	91.4	23	A	6.7	30	32	T	90.6	28	A	6.3	18	3	
762	80	C	92.5	11	A	6.3	26	34	C	91.2	34	A	8.8	26	3	
798	92	C	93.5	25	T	6.5	28	35	C	91.4	26	T	8.6	30	3	
831	102	T	89.2	25	C	10.8	27	34	T	91.2	27	C	8.8	24	3	
861	107	A	86.0	29	G	14.0	28	37	A	89.2	30	G	10.8	31	3	
891	112	T	85.7	25	C	14.3	27	41	T	90.2	19	C	9.8	31	3	
894	112	T	84.8	28	C	15.2	29	43	T	88.4	30	C	9.3	38	3	
927	122	C	83.6	29	T	15.6	25	45	C	88.9	31	T	11.1	30	3	
935	126	T	83.3	25	C	15.9	29	48	T	89.6	18	C	8.3	33	2	Leu/Ser
939	126	A	85.7	28	G	14.3	29	49	A	89.8	31	G	10.2	30	3	
942	126	C	84.1	26	T	14.3	27	50	C	90.0	27	T	10.0	29	3	
958	134	T	84.3	24	C	14.9	31	51	T	86.3	17	C	13.7	32	1	Tyr/His

Table A2.2 Continued.

		Haploblepharus edwardsii							*Haploblepharus pictus*								
		Haplotype 1				Haplotype 2			Haplotype 1				Haplotype 2				
	Position	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Codon position	Amino acid change
	984	146	A	86.3	27	G	13.7	26	51	A	86.3	30	G	13.7	29	3	
	990	149	T	86.6	27	C	13.4	23	49	T	85.7	29	C	14.3	29	3	
Mean				86.3	24		12.8	26			84.7	25		14.4	26		
COI	168	167	G	90.4	29	A	9.0	25	65	G	89.2	30	A	10.8	25	3	
	210	167	T	91.1	17	T	8.9	25	63	C	87.3	28	T	11.1	24	3	
	216	165	C	90.3	11	A	9.1	29	64	C	87.5	11	A	10.9	28	3	
	237	169	T	91.2	15	T	9.5	26	68	C	86.8	29	T	11.8	26	3	
	399	174	G	90.2	10	T	9.8	29	67	G	88.1	11	T	11.9	28	3	
	432	167	T	92.2	11	C	7.8	29	69	T	88.4	11	C	11.6	28	3	
	435	163	A	92.0	30	G	8.0	22	69	A	88.4	30	G	11.6	22	3	
	510	173	C	90.8	27	T	9.2	24	60	C	85.0	28	T	15.0	27	3	
	585	187	T	89.3	16	A	8.0	31	66	T	81.8	17	A	18.2	31	3	
	597	188	A	91.5	29	G	8.5	29	65	A	81.5	30	G	16.9	30	3	
	663	179	T	91.6	28	C	8.4	21	64	T	84.4	28	C	15.6	28	3	
	678	179	G	91.6	28	A	8.4	25	59	G	84.7	28	A	15.3	25	3	
	717	190	A	94.7	19	A	5.3	17	62	G	85.5	15	A	14.5	27	3	
	720	192	T	91.7	29	C	8.3	19	62	T	85.5	30	C	14.5	24	3	
	756	190	G	93.2	21	A	6.8	24	60	G	86.7	23	A	13.3	29	3	
	816	181	T	92.3	26	A	7.2	23	65	T	85.7	27	A	14.3	23	3	
	861	180	T	92.2	30	A	7.8	32	65	T	86.2	30	A	13.8	31	3	

Table A2.2 Continued.

	Haploblepharus edwardsii							*Haploblepharus pictus*								
	Haplotype 1				Haplotype 2			Haplotype 1				Haplotype 2				
Position	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Codon position	Amino acid change
870	182	C	92.9	18	T	7.1	29	64	C	85.9	18	T	14.1	28	3	
885	185	T	92.4	26	G	7.0	23	63	T	84.1	27	G	12.7	23	3	
921	183	T	93.4	31	C	6.6	28	63	T	87.3	30	C	11.1	22	3	
933	178	C	93.8	28	T	4.5	29	63	C	85.7	28	T	14.3	28	3	
945	179	C	93.9	11	T	4.5	29	63	C	84.1	12	T	14.3	28	3	
975	194	T	93.8	29	C	6.2	25	72	T	84.7	30	C	12.5	27	3	
1014	198	A	93.5	27	T	5.7	23	74	A	85.1	30	T	13.5	19	3	
1017	200	G	94.0	29	A	4.5	29	73	G	84.9	29	A	13.7	34	3	
1026	199	A	93.5	28	G	6.0	24	73	A	84.9	29	G	13.7	25	3	
1053	194	A	94.3	27	G	5.2	26	69	A	85.5	29	G	14.5	30	3	
1065	202	G	92.6	23	A	5.4	21	68	G	86.8	24	A	11.8	27	3	
1080	200	C	93.0	29	T	7.0	27	71	C	85.9	30	T	14.1	27	3	
1098	195	T	91.8	18	C	7.7	18	75	T	86.7	19	C	13.3	19	3	
1158	178	C	92.1	29	A	7.3	28	69	C	88.4	31	A	11.6	30	3	
1176	184	C	92.9	28	T	7.1	20	67	C	88.1	30	T	11.9	18	3	
1182	184	T	91.8	26	C	8.2	27	68	T	88.2	27	C	11.8	31	3	
1194	180	A	92.8	28	C	7.2	22	67	A	88.1	29	C	11.9	23	3	
1255	163	A	92.0	27	G	8.0	30	59	A	88.1	29	G	11.9	30	1	Ile/Val
1260	164	A	90.9	28	G	9.1	25	58	A	89.7	31	G	10.3	30	3	
1287	158	T	91.8	11	C	7.6	32	58	T	87.9	12	C	12.1	31	3	
1320	156	T	91.7	17	C	8.3	31	55	T	85.5	19	C	12.7	31	3	

Table A2.2 Continued.

		Haploblepharus edwardsii							*Haploblepharus pictus*								
		Haplotype 1				Haplotype 2			Haplotype 1				Haplotype 2				
	Position	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Codon position	Amino acid change
	1338	149	C	84.6	14	A	6.7	32	51	C	74.7	14	A	15.7	35	3	
	1371	136	T	91.9	17	C	8.1	32	44	T	84.1	18	C	15.9	33	3	
	1380	132	C	92.4	27	T	6.8	34	45	C	84.4	26	T	13.3	32	3	
	1384	130	T	92.3	24	C	7.7	22	44	T	84.1	25	C	15.9	23	1	
	1440	126	A	88.9	25	G	11.1	29	44	A	86.4	25	G	13.6	35	3	
	1447	125	C	92.0	25	T	8.0	33	46	C	87.0	27	T	13.0	35	1	
	1465	127	T	92.9	28	C	5.5	34	47	T	87.2	28	C	10.6	33	1	Tyr/His
	1488	118	C	92.4	29	T	7.6	30	46	C	87.0	30	T	13.0	33	3	
	1530	117	C	90.6	27	T	8.5	21	46	C	87.0	29	T	13.0	26	3	
Mean				90.1	23		7.3	26			84.2	24		13.0	27		
cytb	52	122	G	90.2	27	A	9.8	20	47	G	85.1	23	A	14.9	21	1	Ala/Thr
	112	135	C	91.1	30	T	8.1	21	53	C	86.8	54	T	13.2	20	3	
	123	138	C	91.3	26	T	8.0	26	53	C	86.8	29	T	13.2	19	3	
	127	138	A	90.6	28	G	9.4	20	53	A	84.9	19	G	13.2	32	1	Ile/Val
	138	131	C	88.5	31	T	11.5	25	53	C	86.8	35	T	13.2	19	3	
	171	128	C	93.0	30	T	7.0	28	54	C	87.0	31	T	13.0	32	3	
	195	128	A	89.1	30	G	10.2	21	53	A	88.7	32	G	11.3	30	3	
	201	129	T	88.4	26	C	10.1	25	53	T	88.7	19	C	11.3	33	3	
	231	125	T	88.8	25	C	11.2	27	55	T	87.3	18	C	12.7	32	3	
	268	117	T	85.5	26	C	14.5	24	48	T	79.2	18	C	20.8	21	1	

Table A2.2 Continued.

	Haploblepharus edwardsii							*Haploblepharus pictus*								
	Haplotype 1				Haplotype 2			Haplotype 1				Haplotype 2				
Position	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Codon position	Amino acid change
297	110	T	87.3	24	C	11.8	28	45	T	88.9	16	C	11.1	34	3	
315	94	C	90.4	28	T	9.6	23	46	C	89.1	26	T	10.9	30	3	
330	96	C	87.5	28	T	11.5	26	47	C	76.6	30	T	21.3	25	3	
367	94	T	90.4	28	C	9.6	29	46	T	82.6	20	C	17.4	31	1	
378	92	T	87.0	29	C	13.0	26	44	T	75.0	31	C	25.0	19	3	
534	84	C	85.7	28	T	13.1	22	36	C	86.2	27	T	13.8	23	3	
546	91	C	84.6	25	T	15.4	21	36	C	86.2	29	T	13.8	30	3	
594	104	A	88.5	29	C	10.6	28	43	A	83.7	30	C	14.0	30	3	
633	113	T	86.7	27	C	9.7	24	40	T	82.5	27	C	17.5	29	3	
660	124	T	91.1	21	C	8.9	21	42	T	85.7	18	C	14.3	17	3	
675	132	C	88.6	30	T	11.4	25	42	C	83.3	55	T	16.7	19	3	
693	134	A	88.1	27	C	11.2	27	41	A	82.9	28	C	14.6	26	3	
739	141	T	92.2	27	A	7.8	33	48	T	89.6	19	A	10.4	34	1	Leu/Met
747	144	T	91.0	29	C	8.3	24	49	T	87.8	20	C	12.2	23	3	
771	151	C	94.0	23	T	6.0	22	55	C	87.3	19	T	12.7	25	3	
792	147	A	89.8	23	G	6.8	28	54	A	83.3	16	G	16.7	25	3	
816	155	G	92.3	29	A	7.7	27	60	G	81.7	30	A	18.3	19	3	
843	166	T	89.2	29	C	10.8	31	59	T	78.0	29	C	22.0	29	3	
861	170	T	90.6	28	C	7.6	28	63	T	77.8	29	C	20.6	30	3	
865	169	C	89.3	29	T	8.3	25	64	C	75.0	31	T	21.9	18	1	
928	165	G	87.9	28	A	11.5	29	69	G	75.4	29	A	24.6	31	1	Val/Met

Table A2.2 Continued.

	Haploblepharus edwardsii								*Haploblepharus pictus*								
	Haplotype 1					Haplotype 2			Haplotype 1					Haplotype 2			
	Position	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Coverage	Nucleotide	Frequency	Q	Variant	Frequency	Q	Codon position	Amino acid change
	996	164	T	89.6	24	C	10.4	27	70	T	78.6	18	C	21.4	30	3	
	1051	153	G	90.8	24	A	8.5	28	74	G	78.4	18	A	21.6	31	1	Val/Ile
	1074	138	T	88.4	24	C	11.6	26	68	T	73.5	17	C	26.5	18	3	
Mean				89.3	27		10.0	25			83.2	26		16.4	26		

Table A2.3 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 975 bp of the nicotinamide adenine dehydrogenase subunit 1 (*ND1*) gene region.

			ND1			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.54	0.00	0.54	0.96	1.17
HE_2	3.08		0.54	0.00	1.01	1.21
HP_1	0.00	3.08		0.54	0.96	1.17
HP_2	3.08	0.00	3.08		1.01	1.21
HN	10.36	11.49	10.36	11.49		1.15
PP	17.23	18.05	17.23	18.05	19.18	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.4 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 1 557 bp of the cytochrome *c* oxidase subunit I (*COI*) gene region. Uncorrected *p*-distances and K2P distances are displayed, with the latter indicated in parentheses.

			COI			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.43 (0.44)	0.12 (0.11)	0.43 (0.44)	0.80 (0.97)	0.95 (1.29)
HE_2	2.83 (2.90)		0.45 (0.45)	0.00 (0.00)	0.76 (0.91)	0.94 (1.23)
HP_1	0.19 (0.19)	3.02 (3.10)		0.45 (0.45)	0.80 (0.98)	0.93 (1.27)
HP_2	2.83 (2.90)	0.00 (0.00)	3.02 (3.10)		0.76 (0.91)	0.94 (1.23)
HN	10.94 (12.09)	9.97 (10.91)	11.07 (12.25)	9.97 (10.91)		0.91 (1.18)
PP	17.05 (19.70)	16.09 (18.37)	17.05 (19.70)	16.09 (18.37)	15.44 (17.48)	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.5 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 691 bp of the cytochrome *c* oxidase subunit II (*COII*) gene region.

			COII			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.42	0.00	0.42	0.92	1.28
HE_2	1.16		0.42	0.00	0.96	1.31
HP_1	0.00	1.16		0.42	0.92	1.28
HP_2	1.16	0.00	1.16		0.96	1.31
HN	6.95	7.24	6.95	7.24		1.28
PP	13.89	14.18	13.89	14.18	14.62	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.6 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 168 bp of the ATP synthase subunit 8 (*ATP8*) gene region.

	ATP8					
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.81	0.00	0.81	2.32	2.83
HE_2	1.19		0.81	0.00	2.29	2.83
HP_1	0.00	1.19		0.81	2.32	2.83
HP_2	1.19	0.00	1.19		2.29	2.83
HN	9.52	9.52	9.52	9.52		2.72
PP	17.86	17.86	17.86	17.86	17.26	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.7 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 683 bp of the ATP synthase subunit 6 (*ATP6*) gene region.

	ATP6					
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.57	0.00	0.57	0.96	1.35
HE_2	2.49		0.57	0.00	1.02	1.41
HP_1	0.00	2.49		0.57	0.96	1.35
HP_2	2.49	0.00	2.49		1.02	1.41
HN	7.47	8.78	7.47	8.78		1.40
PP	16.25	17.57	16.25	17.57	16.84	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.8 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 786 bp of the cytochrome *c* oxidase subunit III (*COIII*) gene region.

	COIII					
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.59	0.00	0.59	1.06	1.20
HE_2	2.81		0.59	0.00	1.10	1.28
HP_1	0.00	2.81		0.59	1.06	1.20
HP_2	2.81	0.00	2.81		1.10	1.28
HN	9.92	10.18	9.92	10.18		1.29
PP	15.78	17.18	15.78	17.18	17.30	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.9 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 349 bp of the nicotinamide adenine dehydrogenase subunit 3 (*ND3*) gene region.

			ND3			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.68	0.00	0.68	1.58	2.05
HE_2	1.72		0.68	0.00	15.4	2.01
HP_1	0.00	1.72		0.68	1.58	2.05
HP_2	1.72	0.00	1.72		1.54	2.01
HN	10.60	9.46	10.60	9.46		1.97
PP	19.20	18.62	19.20	18.62	18.34	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.10 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 297 bp of the nicotinamide adenine dehydrogenase subunit 4L (*ND4L*) gene region.

			ND4L			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.32	0.00	0.32	1.29	2.28
HE_2	0.34		0.32	0.00	1.32	2.28
HP_1	0.00	0.34		0.32	1.29	2.28
HP_2	0.34	0.00	0.34		1.32	2.28
HN	5.39	5.72	5.39	5.72		2.18
PP	21.21	21.55	21.21	21.55	20.20	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.11 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 1 367 bp and 889 bp, the latter indicated in parentheses, of the nicotinamide adenine dehydrogenase subunit 4 (*ND4*) gene region.

			ND4			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.50 (0.57)	0.07 (0.11)	0.50 (0.57)	0.93 (1.00)	1.09 (1.31)
HE_2	3.29 (3.04)		0.50 (0.59)	0.00 (0.00)	0.98 (1.01)	1.09 (1.29)
HP_1	0.07 (0.11)	3.37 (3.15)		0.50 (0.59)	0.92 (0.99)	1.09 (1.32)
HP_2	3.29 (3.04)	0.00 (0.00)	3.37 (3.15)		0.98 (1.01)	1.09 (1.29)
HN	12.00 (11.47)	12.51 (12.04)	11.92 (11.36)	12.51 (12.04)		1.18 (1.40)
PP	21.21 (21.26)	21.14 (21.26)	21.29 (22.16)	21.14 (21.26)	22.97 (23.51)	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.12 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 1 830 bp of the nicotinamide adenine dehydrogenase subunit 5 (*ND5*) gene region.

			ND5			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.32	0.00	0.32	0.71	0.84
HE_2	1.97		0.32	0.00	0.70	0.85
HP_1	0.00	1.97		0.32	0.71	0.84
HP_2	1.97	0.00	0.197		0.70	0.85
HN	11.15	10.33	11.15	10.66		0.87
PP	20.77	20.55	20.77	20.55	21.64	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.13 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 525 bp of the nicotinamide adenine dehydrogenase subunit 6 (*ND6*) gene region.

			ND6			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.77	0.19	0.72	1.40	1.68
HE_2	3.62		0.76	0.26	1.42	1.69
HP_1	0.19	3.43		0.70	1.40	1.68
HP_2	3.24	0.38	3.05		1.40	1.68
HN	12.52	13.10	12.33	12.72		1.71
PP	21.77	22.54	21.58	22.16	21.19	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

Table A2.14 Sequence divergence values below the diagonal and standard deviations above the diagonal between four scyliorhinid species, including Haplotype 1 and 2 for *Haploblepharus* species, for 1 144 bp of the cytochrome subunit *b* (*cytb*) gene region.

			cytb			
	HE_1	HE_2	HP_1	HP_2	HN	PP
HE_1		0.49	0.00	0.49	0.90	1.06
HE_2	2.97		0.49	0.00	0.90	1.06
HP_1	0.00	2.97		0.49	0.90	1.06
HP_2	2.97	0.00	2.97		0.90	1.06
HN	11.10	11.54	11.10	11.54		1.14
PP	21.94	21.85	21.94	21.85	22.55	

Abbreviations: base pair (bp), *Haploblepharus edwardsii* (HE), *Haploblepharus pictus* (HP), *Halaelurus natalensis* (HN), *Poroderma pantherinum* (PP).

CHAPTER 3

Detecting signatures of admixture between three sympatric *Haploblepharus* species using novel microsatellite markers

Abstract

The genus *Haploblepharus* is an understudied group comprising four recognised scyliorhinid species endemic to southern African waters: *H. edwardsii*, *H. fuscus*, *H. kistnasamyi* and *H. pictus*. Species identification between these co-distributed species has historically been problematic due to the high degree of morphological conservatism between congeners, further complicated by the possibility of interspecific hybridisation. This study constructed two microsatellite assays comprising 10 polymorphic markers developed for the puffadder shyshark *H. edwardsii*. These assays were characterised in 35 *H. edwardsii* individuals from four sampling locations along the South African coastline and tested for cross-species utility in *H. fuscus*, *H. pictus* and *Halaelurus natalensis*. Genetic diversity estimates (N_A, A_R, H_O and H_E) and genetic differentiation (F_{ST}) were assessed for each species. Furthermore, the genetic differentiation between *Haploblepharus* species, using *H. natalensis* as an outgroup, was examined to infer the potential use of these markers in species assignment as well as for detecting signatures of admixture. All microsatellite markers were polymorphic in each of the four study species, with an A_R, H_E and *PIC* as high as 5.22, 0.82 and 0.78, respectively. The null hypothesis of panmixia was rejected in only one of the study species, *H. pictus*, where genetic discontinuity was evident due to geographic distance. Interspecific genetic differentiation (F_{ST} = 0.091 to 0.384, $P < 0.05$) was statistically significant between all species; however, the level of differentiation between *H. fuscus* and *H. pictus* (F_{ST} = 0.091) was low in comparison and seems to be at a population level rather than at a species level. Species assignment using Bayesian clustering analysis (STRUCTURE) and multivariate analyses (DAPC and PCoA) illustrated the presence of overlapping genetic clusters and signatures of admixture between *Haploblepharus* taxa.

3.1 Introduction

Accurate species identification is required prior to addressing any ecological or evolutionary questions (Dudgeon *et al*. 2012; White and Last 2012); moreover, it is paramount for the effective implementation of conservation and management plans. Despite the fact that one quarter of chondrichthyans are threatened with an elevated risk of extinction (Dulvy *et al*. 2014), there is a lack of adequate scientific information on chondrichthyan population structure (Dudgeon *et al*. 2012) and few molecular markers are available for the group, especially for southern African endemics (Bester-van der Merwe and Gledhill 2015). In recent years, the combination of overfishing and other anthropogenic effects have drastically altered marine ecosystems (Cortés 2000; Stevens *et al*. 2000; Dudley and Simpfendorfer 2006; Ferretti *et al.* 2010; Dulvy *et al*. 2017). In addition to negative ecological impacts (Ferretti *et al*. 2010; Price *et al*. 2015), population declines in the wild can alter the levels and distribution of genetic diversity among populations (Dudgeon *et al*. 2012). Molecular approaches have proven useful for characterising genetic variability and defining reproductively isolated stocks in marine organisms, which aids in elucidating historical and contemporary processes responsible for the observed patterns of spatial genetic differentiation (Veríssimo *et al*. 2010; Vignaud *et al*. 2013; Dudgeon and Ovenden 2015; Kousteni *et al*. 2015; Maduna *et al*. 2017).

The general lack of molecular markers for many elasmobranch species has previously delayed the study of population genetic structure; further impeding conservation action. However, recent advances in high-throughput sequencing technologies have allowed the accurate and efficient recovery of microsatellite loci from non-model organisms (Boomer and Stow 2010; Chabot and Nigenda 2011; Pirog *et al*. 2015; Maduna *et al*. 2017, 2018). Furthermore, owing to highly conserved microsatellite flanking sequences (Martin *et al*. 2002), cross-species amplification is an effective alternate approach to *de novo* development and has been tested in elasmobranchs with relatively high success rates (Griffiths *et al*. 2011; Maduna *et al*. 2014; Blower *et al*. 2015; Pirog *et al*. 2015). The use of cross-amplified markers in a standardised panel allows for molecular species identification and comparative population genetics (Maduna *et al*. 2014, 2017; Marino *et al*. 2014; Giresi *et al*. 2015). Previous studies on chondrichthyans indicated that intraspecific differentiation appears to be correlated to the respective species' dispersal ability, which in turn is dependent on maximum body size and habitat preference (Veríssimo *et al*. 2010). Numerous barriers to gene flow exist, such as hydrodynamic barriers (Feldheim *et al*. 2001; Veríssimo *et al*. 2010; Karl *et al*. 2012; Kousteni *et al*. 2015), thermal barriers (Veríssimo *et al*. 2010) and biogeographic barriers (Whitney *et al*. 2012; Kousteni *et al*. 2015); with the potential to overcome the majority of these barriers requiring actively swimming adults (Musick *et al*. 2004). Despite the progress of sequencing technologies, species-specific microsatellite markers have only been developed for two scyliorhinid species: the swellshark *Cephaloscyllium ventriosum* (Garman 1880) (Feldheim *et al*. 2016) and the yellow-spotted catshark *Scyliorhinus canicula* (Linnaeus 1758) (Griffiths *et al*. 2011); with a population genetics assessment only conducted on the latter of the two species

(Kousteni *et al*. 2015). As expected for coastal and demersal small-sized chondrichthyans, high levels of genetic differentiation were evident among *S. canicula* populations in both mitochondrial and nuclear markers; attributed to the species' limited dispersal ability and the presence of hydrodynamic barriers (Kousteni *et al*. 2015).

Southern Africa has been identified as a biodiversity hotspot for chondrichthyans, with over 200 different species occurring in the surrounding waters (Compagno 1999; Ebert and van Hees 2015). Additionally, southern African waters have been identified as a conservation priority due to the threats to sharks (Dulvy *et al*. 2014; Davidson and Dulvy 2017; Stein *et al*. 2018). The need for conservation action focused on imperilled endemics, a large proportion of which comprises scyliorhinids (Human 2003; Human 2007*a*; Ebert and van Hees 2015), has recently been highlighted (Davidson and Dulvy 2017; Stein *et al*. 2018). The rich and diverse marine fauna present can partly be attributed to the variety of interconnected habitats along the coastline (Griffiths *et al*. 2010; Teske *et al*. 2011). Additionally, South Africa is located in the transition zone between two major oceanic currents: the warm Agulhas Current on the east coast and the cold Benguela Current on the west coast (Griffiths *et al.* 2010; Briggs and Bowen 2012). This transition zone has been well-documented as a phylogeographic break separating distinct populations of many marine taxa, with some populations endemic to the transition zone (Teske *et al*. 2006; Teske *et al*. 2011). However, only a few studies have investigated the impacts of these oceanographic features on gene flow between elasmobranch populations; with some studies demonstrating a lack of genetic structuring across the phylogeographic break (Benavides *et al*. 2011), while others described cases of restricted gene flow (Bitalo *et al*. 2015; Maduna *et al*. 2016, 2017; Soekoe 2016; Bester-van der Merwe *et al*. 2017).

The understudied southern African endemic catshark genus *Haploblepharus* Garman 1913 comprises four recognised species: *H. edwardsii* (Schinz 1822), *H. fuscus* Smith 1950, *H. kistnasamyi* Human and Compagno 2006 and *H. pictus* (Müller and Henle 1838) (Human 2007*a*). Although not targeted by fisheries, scyliorhinids are vulnerable to trawl fishing as they inhabit continental shelves (Shepherd and Meyers 2005). Consequently, a high number of scyliorhinids are taken as bycatch by numerous fisheries (Human 2003; da Silva *et al*. 2015). Due to suspected population declines, the International Union for the Conservation of Nature (IUCN) has assessed three of the *Haploblepharus* species (*H. edwardsii*, *H. fuscus* and *H. kistnasamyi*) in threatened categories according to Red List criteria (Human 2009*a*, *b*, *c*). Alternatively, due to the apparent abundance and lack of threats, *H. pictus* has been assessed as Least Concern (Human 2009*d*); however, the population trends of *Haploblepharus* remain largely unknown (Human 2007*a*). Species identification within this genus has historically been problematic, stemming from the use of colour patterns and unreliable morphological characters in species identification keys (Human and Compagno 2006; Human 2007*a*). A further complication to accurate species identification is the possibility of interspecific hybridisation, which was raised by Human (2007*a*) due to the difficulty in classifying some specimens that shared morphological and/or

colour patterns of different *Haploblepharus* taxa. A taxonomic revision of *Haploblepharus* revealed that the described distributions of some species had included those of other *Haploblepharus* taxa, leading to the over- or underestimation of both distribution ranges and abundances of these species (Human 2003; Human 2007*a*). Therefore, owing to the frequent occurrence of species misidentification, the distribution ranges of these species are not yet resolved.

There is limited knowledge on the biology of *Haploblepharus*, especially for *H. fuscus* and *H. kistnasamyi* (Human 2003; Human 2007*a*). Through internal fertilisation and oviparity, both *H. edwardsii* and *H. pictus* produce egg cases with no apparent breeding seasons (Von Bonde 1945; Bass *et al*. 1975; Dainty 2002). This reproduction strategy is assumed for *H. fuscus* and *H. kistnasamyi*, however, the juveniles of these species are scarce and the habitat for egg laying remains unknown (Human 2003; Human 2007*a*). In an attempt to alleviate species misidentification, a revised taxonomic dichotomous key was described for *Haploblepharus* (Human 2007*a*); however, misidentification between *Haploblepharus* species remains widespread (Gledhill *et al*. submitted). Some of the most distinguishable characteristics between *H. edwardsii* and *H. pictus* are size at sexual maturity and species-specific egg case colouration (Bertolini 1993; Branch *et al*. 1994; Dainty 2002). Human (2007*b*) performed multivariate analyses on morphometric and meristic data to examine species group clustering and discrimination in *Haploblepharus*. The analyses were unable to accurately classify any of the species to their assigned group with a 100% success rate, with the number of correctly assigned specimens per species ranging from as low as 38.5% to ~85% (Human 2007*b*). A more recent study was unable to accurately differentiate between three *Haploblepharus* species (*H. edwardsii*, *H. fuscus* and *H. pictus*) based on three mitochondrial gene regions (cytochrome *c* oxidase subunit I, cytochrome *b* and nicotinamide adenine dehydrogenase subunit 2) as well as the internal transcribed spacer 2 (*ITS2*) nuclear gene (Gledhill *et al*. submitted). Both of the aforementioned studies were also performed for *Poroderma* Smith 1837, another South African endemic catshark genus (Human 2003; Ebert and van Hees 2015). The shape variation analyses were able to classify *Poroderma* specimens into their assigned species groups with a 100% success rate (Human 2006). Although genetic distances between *P. africanum* (Gmelin 1789) and *P. pantherinum* (Smith *in* Müller and Henle 1838) were low, the taxa were accurately identified based on fixed nucleotide differences at all four molecular markers (Gledhill *et al*. submitted). The ability of these analyses to accurately distinguish between the two closely related *Poroderma* species illustrates and supports the utility of these methods for species discrimination.

Given the above, a unique opportunity to contribute to scientific knowledge on southern African endemic scyliorhinids is presented. This chapter reports on the *de novo* development of species-specific microsatellite markers, the utility of these markers in cross-species amplification and preliminary genetic diversity indices. Furthermore, the newly developed markers were used to estimate interspecific differentiation and assess the presence of admixture between *Haploblepharus* species.

3.2 Materials and Methods

3.2.1 Sample collection and DNA extraction

A total of 88 tissue samples (fin clips) were collected from four scyliorhinid species (*Haploblepharus edwardsii*, *H. fuscus*, *H. pictus* and *Halaelurus natalensis* Regan 1904) that are well-represented in southern African waters (**Table 3.1**). These samples were collected from eight different geographic locations along the South African coastline (**Figure 3.1**), including: False Bay, Hermanus, Gansbaai, Agulhas Bank, Mossel Bay, Riet River West, Kleinemonde West and Kleinemonde East. The majority of inshore specimens were caught by hand during snorkelling surveys or collected by recreational anglers while line fishing. Offshore specimens were sampled by scientists on-board the research vessel *Africana* during demersal trawl surveys. Species identification was performed in field by trained shark biologists and specimens were identified to the species level based on external morphology and colour patterns according to taxonomic keys described by Human (2007*a*) for *Haploblepharus* species and Ebert *et al*. (2013) for *H. natalensis*. The sex, total length (TL) and pre-caudal length (PCL) were recorded for all specimens. Additionally, all specimens were photographed for voucher purposes (**Figure A3.1**). Only specimens unambiguously assigned to taxonomic groups were selected for genotyping: *H. edwardsii* displaying brilliant orange saddles with dark borders, a slender body and pointed snout (**Figure A3.1a**, **b**, **c**, **d**, **e** and **f**), *H. pictus* displaying dark colouration with a robust body and rounded snout (**Figure A3.1g** and **h**), and *H. fuscus* with a uniform chocolate brown colouration, stocky body and broadly rounded snout (**Figure A3.1i**) (Human 2007*a*). Where possible, samples were selected to represent the widest geographic range; however, due to opportunistic sampling this did not encompass the entire distribution ranges of the respective species.

Fin clips were stored in 90% ethanol at room temperature. Total genomic DNA (gDNA) was extracted using a standard cetyltrimethylammonium bromide (CTAB) extraction protocol (Sambrook and Russell 2001). The concentration and quality of extracted DNA was quantified by measuring its optical density at 230 nm (A_{230}), 260 nm (A_{260}) and 280 nm (A_{280}) using a NanoDrop™ ND 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA; www.thermofisher.com). DNA stock solutions were diluted to working stock concentrations of ~50 ng/µL and stored at -20℃ until further use.

Table 3.1 Geographic co-ordinates of sampling locations along the South African coastline and sample sizes for four scyliorhinid species: *Haploblepharus edwardsii*, *H. fuscus*, *H. pictus* and *Halaelurus natalensis*.

Species	Sampling location	Geographic co-ordinates	*N*
Haploblepharus edwardsii	False Bay	34˚11´S, 18˚26´E	16
(*N* = 35)	Hermanus	34˚24´S, 19˚15´E	3
	Gansbaai	34˚35´S, 19˚20´E	7
	Agulhas Bank	35˚30´S, 21˚00´E	9
Haploblepharus fuscus	Riet River West	33˚34´S, 26˚59´E	5
(*N* = 16)	Kleinemonde West	33˚33´S, 27˚02´E	4
	Kleinemonde East	33˚31´S, 27˚05´E	7
Haploblepharus pictus	False Bay	34˚11´S, 18˚26´E	7
(*N* = 31)	Hermanus	34˚24´S, 19˚15´E	6
	Gansbaai	34˚35´S, 19˚20´E	8
	Mossel Bay	34˚10´S, 22˚09´E	10
Halaelurus natalensis	Mossel Bay	34˚10´S, 22˚09´E	6

Abbreviations: Number of individuals (*N*).

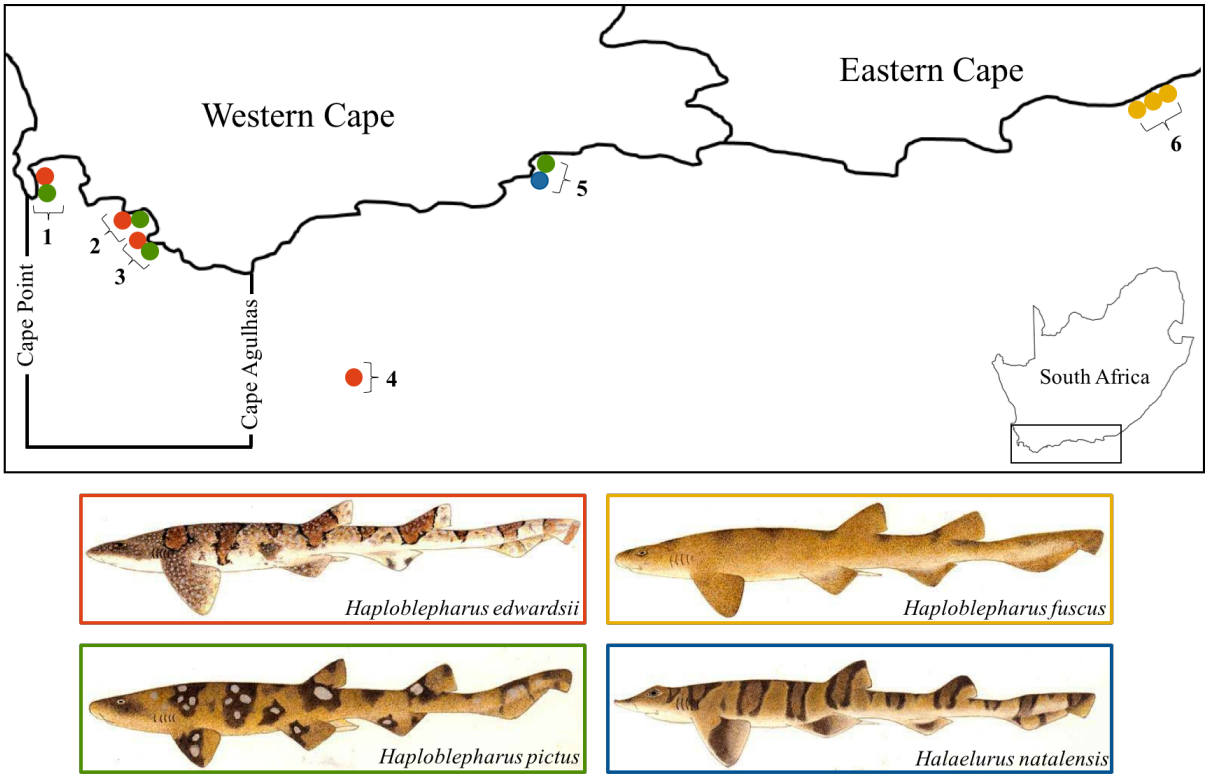


Figure 3.1 Sampling locations along the South African coastline for four scyliorhinid species; with red representing *Haploblepharus edwardsii*, yellow representing *Haploblepharus fuscus*, green representing *Haploblepharus pictus* and blue representing *Halaelurus natalensis*. Locations: 1 – False Bay, 2 – Hermanus, 3 – Gansbaai, 4 – Agulhas Bank, 5 – Mossel Bay and 6 – Port Alfred (including Riet River West, Kleinemonde West and Kleinemonde East). The recognised phylogeographic break, known as the transition zone, that occurs between Cape Point and Cape Agulhas is also shown.

3.2.2 Microsatellite development and validation

The quality filtered sequence reads generated for *Haploblepharus edwardsii* and *H. pictus*, as described in section 2.2.3 in Chapter 2, were used for species-specific microsatellite development. Contigs were constructed using SPAdes v3.10.1 (Nurk *et al*. 2013) and those larger than 1000 bp were selected for microsatellite identification in BatchPrimer3 v1.0 (You *et al*. 2008) using the standard settings. Microsatellite loci identified with ≥ 6 uninterrupted repeat motifs were selected for a Basic Local Alignment Search Tool (BLAST) search using the BLASTn (query nucleotide against nucleotide database) function against the National Center for Biotechnology Information (NCBI) database to highlight contigs containing hits with known microsatellites of other elasmobranch or teleost species. In an attempt to develop novel microsatellite markers, sequences that contained hits as well as those with no significant hits were selected for primer design using BatchPrimer3 (**Table 3.2**).

Polymerase chain reaction (PCR) was performed on a GeneAmp® PCR System 2700 in a 10 µL reaction volume. Reactions included 50 ng of template DNA, 1X PCR Buffer, 2.5 mM $MgCl_2$, 200 µM of deoxynucleotide triphosphates (dNTPs), 0.2 µM of each primer and 0.25 U of GoTaq® DNA polymerase. The PCR cycling conditions consisted of an initial denaturation step of one cycle at 94˚C for 3 min, followed by 35 cycles of denaturation at 94˚C for 30 s, optimised annealing temperature (T_A) for 30 s, elongation at 72˚C for 30 s, and a final elongation step of one cycle at 72˚C for 10 min. Thereafter, PCR products were stored at 4˚C. Optimum annealing temperature was determined for each of the primer pairs when a single band was present per sample on an agarose gel (**Table 3.2**). Levels of polymorphism were assessed by subjecting successfully amplified products from a panel of eight individuals of *H. edwardsii* or *H. pictus* to gel electrophoresis on a 3% agarose gel. Microsatellites were considered polymorphic when two bands were distinguishable in a single individual (i.e. heterozygote).

Polymorphic microsatellites were selected, and primers fluorescently labelled using one of the following dyes: FAM, VIC, PET or NED (**Table 3.3**). Labelled primers were then used in the optimisation of two multiplex panels (Haplo_MP1 and Haplo_MP2). A panel of 35 *H. edwardsii* specimens from four different geographic locations were genotyped for marker characterisation. Multiplex PCR was conducted using the Qiagen Multiplex PCR Kit according to the manufacturer's protocol with a T_A of 57˚C for both multiplex panels. PCR products were diluted with distilled water for fragment analysis performed on an ABI 3730XL DNA Analyser using the LIZ600 internal size standard. Individuals were genotyped based on fragment sizes via the Microsatellite Plugin v1.4.4 (Biomatters Ltd.) in Geneious® v10.2.3 (Kearse *et al*. 2012).

To determine the transferability and utility of these markers for future studies, the 10 microsatellite loci were also tested in *Haploblepharus fuscus*, *Haploblepharus pictus* and *Halaelurus natalensis* using the previously described PCR conditions and microsatellite genotyping methods.

Table 3.2 Details of 22 microsatellite loci primer pairs, of which 19 were developed for *Haploblepharus edwardsii* (HE) and three for *Haploblepharus pictus* (HP), indicating the expected PCR product size in base pairs (bp).

Locus	Primer sequence (5'-3')	Motif	PCR product size	T_A	BLAST result	Gel result
HE1	F: TGGAATGTGAATAATGGTTGG R: TCTCATGTGGGTTATTGATGG	$(GT)_{11}$	253	55	*Leucoraja erinacea*	Polymorphic
HE2	F: ACAGATGCGGCTGTTCAGAA R: ACCACGTCCTATCCTGGTCA	$(TG)_{11}$	364	55	*Ginglymostoma cirratum*	Polymorphic
HE3	F: AATAATGGTGCGGCTATATGA R: AAAGAACTTTGCCTTGGAAGA	$(CT)_{11}$	244	55	*Carcharhinus plumbeus*	Polymorphic
HE4	F: CGCTCACGTGACTATTAGCAT R: ATTCATAGGGGAGAGACCAGA	$(CA)_{11}$	300	55	No significant hits	Polymorphic
HE5	F: GGAACAGTAGTGGCCTCAGAT R: CACCACCTATAACATCACCAC	$(AG)_{10}$	253	55	*Prionace glauca*	Polymorphic
HE6	F: GCAGCAGTAAGTACGCTGGA R: GAGGACTCTCAGGGCAACAC	$(CT)_{12}$	320	–	*Carcharhinus isodon*	–
HE7	F: TCCCTTAGAATGCATAGGTCA R: ATGGGGAAATTGACTGGCACT	$(CTAAT)_6$	348	57	*Triakis scyllium*	Polymorphic
HE8	F: TCTTCTCATGTTTCCCTGTGA R: CAATGGAACTTTGGATTGTTG	$(CT)_{12}$	353	57	*Scyliorhinus canicula*	Monomorphic
HE9	F: CCCCTACCCCGAGTGAACGGT R: CGTGGCTTTAAATTGAGGGGG	$(AG)_{12}$	336	55	No significant hits	Monomorphic
HE10	F: GGAACACAACATTCTGCACTC R: GTCCCAAGAAGGTTAACATAA	$(CA)_{12}$	217	–	No significant hits	–
HE11	F: TATCTTTGCCATTCTGCTTTG R: AGGAAACATTTTGTGCATTGA	$(AG)_{11}$	253	55	No significant hits	Polymorphic
HE12	F: GGCTCCAAAGTGGAAGTTAATC R: TCTGTGAAAATTGCCTAGTCG	$(AG)_{10}$	263	55	*Leucoraja erinacea*	Polymorphic

Abbreviations: Forward primer (F), reverse primer (R), annealing temperature in ˚C (T_A).

Table 3.2 Continued.

Locus	Primer sequence (5'-3')	Motif	PCR product size	T_A	BLAST result	Gel result
HE13	F: CTTCCCTTCCATGATTTGACT R: ACTCTCTTCGTCGTTGTCCTT	$(ATCAA)_6$	250	56	No significant hits	Polymorphic
HE14	F: ATCCATGCTTACAATGGAGGT R: TTTTGCTCAAAATGTTGTGGT	$(TA)_{10}$	235	55	No significant hits	Monomorphic
HE15	F: ACGAAGGTAAGTGGAACATCC R: ATTCTCGCAATGTATGTCTGC	$(CA)_{10}$	243	55	No significant hits	Polymorphic
HE16	F: AATTCCTATGCCCTCATTACG R: TATTCAGGGTGTTTCCATTCC	$(CT)_{10}$	249	55	No significant hits	Polymorphic
HE17	F: TAATGTGACATGAAGCCGATT R: CAGCATTTGAAGCTAAGCAGA	$(AG)_{10}$	251	56	*Galeocerdo cuvier*	Monomorphic
HE18	F: TAGTGGCATGGAGCAAAATTA R: GTTTCCATGCAGTGAACCTAA	$(GT)_{10}$	247	55	No significant hits	Polymorphic
HE19	F: GTTCCTGGAAATGAGCAAAAT R: AACGGGCAGGTTTATAACAGT	$(GA)_{10}$	284	55	No significant hits	Monomorphic
HP1	F: TAGCTGGGAGAGTGTTCAATG R: CACCACACCCTCTGATTTTAA	$(CA)_{10}$	364	55	*Mustelus canis*	Monomorphic
HP2	F: CTTGCTCATAGGGTGGAATCT R: GCATAGGTCGGATGGATTAGT	$(AT)_{12}$	242	56	*Leucoraja erinacea*	Polymorphic
HP3	F: CAGCTCTTCATATCAGCAAGG R: ATTGGGAATGGTGTCTGTTTT	$(TC)_{11}$	262	55	*Squalus acanthias*	Monomorphic

Abbreviations: Forward primer (F), reverse primer (R), annealing temperature in ˚C (T_A).

3.2.3 Microsatellite characterisation

For the four study species, samples sharing identical multilocus genotypes were identified using the Microsatellite Excel Toolkit v1.0 (MSATTOOLS; Park 2001) and samples with ≥ 95% matching alleles were excluded from further analyses. All loci were assessed for scoring errors due to the presence of allelic dropout, stuttering or null alleles using MICRO-CHECKER v2.2.3 (Van Oosterhout *et al*. 2004). Additionally, the frequency of null alleles was estimated using FreeNA (Chapuis and Estoup 2007), following the Expectation Maximisation (EM) method described by Dempster *et al*. (1977). The exact probability test in GENEPOP v4.2 (Rousset 2008) was used to detect any deviations from Hardy-Weinberg equilibrium (HWE) based on 10 000 iterations (10 000 dememorisations, 500 batches). The presence of linkage disequilibrium among loci was also assessed using an exact test, as implemented in GENEPOP based on 10 000 iterations. A test to identify any candidate loci under selection was performed in ARLEQUIN v3.5.2.2 (Excoffier and Lischer 2010) using 20 000 simulations. To minimise type 1 errors, a false discovery rate (FDR; Benjamini and Hochberg 1995) control was implemented to adjust *P*-values for multiple tests.

For each species and sampling site, the number of alleles per locus (N_A), allelic richness (A_R) standardised for small sample size, observed heterozygosity (H_O) and expected heterozygosity (H_E) under Hardy-Weinberg equilibrium conditions were calculated using the DIVERSITY package (Keenan *et al*. 2013) for R (R Development Core Team 2015). The polymorphic information content (*PIC*) was calculated in MSATTOOLS according to the equation described in Botstein *et al*. (1980). The inbreeding coefficient (F_{IS}) was calculated and tested for deviations from zero using a permutation test (1 000 permutations) in ARLEQUIN, with significance values adjusted for multiple tests using the FDR correction.

3.2.4 Intra- and interspecific population genetic analyses

To test for genetic homogeneity across sampling sites within species, an analysis of molecular variance (AMOVA) was calculated in ARLEQUIN based on genetic distance matrices estimated by pairwise differences. Furthermore, a hierarchical AMOVA was performed for the entire dataset to estimate genetic subdivision between species. Significance levels for variance components and fixation indices were based on 20 000 permutations. Intra- and interspecific pairwise F_{ST} (Weir and Cockerham 1984) was estimated in ARLEQUIN using 20 000 permutations. To account for the study sampling strategy, measures of genetic differentiation were considered significant if the lower limit of the 95% confidence interval (CI) was > 0, and *P*-values were < 0.05 following the FDR correction. To visualise population and species distinctness, discriminant analysis of principal components (DAPC) was performed, which maximises between group variance while minimising within group variance (Jombart 2008; Jombart *et al*. 2010). DAPC plots for clusters defined by sampling locations were generated using the ADEGENET (Jombart 2008) package for R. Subsequently, the *find.clusters* function was used to estimate the number of genetic clusters present. This function runs successive *K*-means clustering with

increasing number of clusters (*k*; Jombart *et al*. 2010). For selecting the optimal *k*, the Bayesian Information Criterion (BIC) for assessing the best supported model, and therefore the number and nature of the clusters, was applied as recommended by Jombart *et al*. (2010). Moreover, the pattern of allelic distribution among species was visualised by generating a Principal Co-ordinate Analysis (PCoA) in GenAlEx v6.5 (Peakall and Smouse 2012) based on calculated genetic distances.

Finally, to detect genetic structure and individual ancestries within and among species, a Bayesian clustering model-based method was implemented in STRUCTURE v2.3.4 (Pritchard *et al*. 2000, 2010). The most probable number of genetic clusters present was determined using an admixture model with correlated allele frequencies. The model was applied for 20 iterations across $K = 1$ to $K = 6$ with each iteration consisting of 1 000 000 Markov Chain Monte Carlo (MCMC) generations and an initial burn-in phase of 100 000 generations; performed both with and without prior population information. The commonly used statistic ΔK described in Evanno *et al*. (2005) was implemented in STRUCTURE SELECTOR (Li and Liu 2018) to identify the most probable number of genetic clusters (*K*) present when assessing the entire dataset. Furthermore, the four estimators of Puechmaille (Puechmaille 2016) were calculated in STRUCTURE SELECTOR to identify the number of clusters present for each species. The Puechmaille estimates are reported as a more accurate method of determining *K* when sampling sizes are uneven; however, they are only applicable when prior knowledge on sampling location is known (Puechmaille 2016). For the selected *K* value, the average coefficient of membership (*Qi*) for each species as well as the individual membership (*qi*) to the inferred clusters was assessed. The index of admixture in Bayesian analysis has frequently been used to identify introgression (Barilani *et al*. 2006; Sanz *et al*. 2008; Scandura *et al*. 2009; Khosravi *et al*. 2013; Ito *et al*. 2015). Moreover, it has been used to detect hybrid individuals by defining a threshold value of *qi* (Randi 2008). To confidently identify pure individuals in this study, a stringent *qi* value threshold (> 0.95) was implemented; while a less stringent threshold (< 0.80) was used to confidently identify admixed individuals. Graphical representations of the STRUCTURE results were generated using CLUMPAK (Kopelman *et al*. 2015).

3.3 Results

3.3.1 Microsatellite validation and characterisation

The high-throughput sequencing run of the two 400 bp libraries for *H. edwardsii* and *H. pictus* generated 8.5 GB and 7.3 GB of quality filtered sequence data, respectively. The *de novo* assembly of the quality filtered reads recovered a total of 82 656 and 77 079 contigs for *H. edwardsii* and *H. pictus*, respectively. In total, 33 462 contigs longer than 1 000 bp were recovered from both datasets. A total of 22 contigs, 19 from *H. edwardsii* and three from *H. pictus*, identified as containing a repeat motif were selected for primer design with an expected PCR product size ranging between 217 and 364 bp. Of the 22 loci tested, two were not successfully amplified while only 13 were determined to be

polymorphic based on initial screening *via* agarose gel electrophoresis. Primers for 10 polymorphic loci were fluorescently labelled and used to create two multiplex assays (Haplo_MP1 and Haplo_MP2; **Table 3.3**). The two assays were validated and characterised in 35 *H. edwardsii* individuals from four different geographic locations (**Table 3.1** and **Figure 3.1**). The genetic diversity statistics for both multiplex assays are summarised in **Table 3.3**. All fluorescently labelled primers were successfully amplified and produced a total of 51 alleles. No evidence for the presence of scoring errors or null alleles was detected by MICRO-CHECKER. Moreover, the absence of null alleles was supported by the relatively low frequencies estimated in FreeNa (**Table 3.3**). After correcting for multiple tests, all loci conformed to HWE and linkage disequilibrium was not detected between any of the pairs of loci tested. All loci were found to conform to selective neutrality, as determined by the F_{ST}-outlier test in ARLEQUIN. The *PIC* ranged from 0.35 to 0.74, additionally, the H_O and H_E ranged from 0.22 to 1.00 and 0.44 to 0.78, respectively. The F_{IS} value ranged from -0.505 to 0.543. Following this, all 10 loci were included in subsequent assessments of population genetic structure.

3.3.2 Cross-species transferability

To assess the utility of the two multiplex assays in cross-species amplification, the assays were tested on *Haploblepharus fuscus* and *H. pictus* as well as a more distantly related scyliorhinid species, *Halaelurus natalensis*. The cross-species amplification rate of success was 100% in all three species (**Table 3.4**). To validate the potential use of these markers for future population genetic analyses, genetic diversity statistics for each of the respective species were inferred using samples from the aforementioned geographic locations (**Table 3.1** and **Figure 3.1**). A summary of the genetic diversity statistics for each species is shown in **Table 3.5** (for details, see **Table A3.1**, **A3.2** and **A3.3**). Although the sample size for *H. natalensis* is too small to obtain comparative diversity estimates, the results are still presented here. For each species, no evidence for scoring errors or null alleles was detected by MICRO-CHECKER. After correcting for multiple tests, all loci in each species conformed to HWE and no evidence for linkage disequilibrium between any of the loci pairs tested was found. A single locus (HE1) was found to be under putative directional selection in *Haploblepharus pictus*, and thus deviated from selective neutrality; while all other loci for each of the respective species conformed to selective neutrality. All 10 microsatellites were variable in each species despite small sample sizes, with an A_R, H_E and *PIC* as high as 5.22, 0.82 and 0.78, respectively (**Table 3.5**).

Table 3.3 Basic genetic diversity statistics as characterised by two polymorphic microsatellite multiplex assays for the puffadder shyshark *Haploblepharus edwardsii* based on four sampling locations in South Africa: False Bay, Hermanus, Gansbaai and Agulhas Bank.

Locus	Dye	Site	*N*	Size range (bp)	N_A	A_R	H_O	H_E	*PIC*	F_{IS}	P_{HW}	Fr_{NULL}
HE1	PET	False Bay	16	225 – 247	5	3.53	1.00	0.75	0.71	-0.308	0.088	0.000
		Hermanus	3		2	–	1.00	0.78	0.74	-0.091	1.000	0.000
		Gansbaai	7		4	3.22	0.86	0.69	0.63	-0.161	0.415	0.000
		Agulhas Bank	9		5	3.40	0.89	0.71	0.66	-0.196	0.948	0.000
HE2	FAM	False Bay	16	352 – 366	4	2.92	1.00	0.65	0.59	-0.505	0.013	0.000
		Hermanus	3		3	–	0.67	0.67	0.59	0.200	0.466	0.001
		Gansbaai	7		5	3.79	1.00	0.76	0.72	-0.254	0.839	0.000
		Agulhas Bank	9		4	3.01	0.89	0.65	0.59	-0.320	0.905	0.000
HE4	NED	False Bay	16	254 – 270	4	3.12	0.75	0.70	0.64	-0.043	0.062	0.000
		Hermanus	3		3	–	0.67	0.61	0.54	0.111	1.000	0.000
		Gansbaai	6		4	3.23	1.00	0.68	0.62	-0.395	0.353	0.000
		Agulhas Bank	6		5	3.41	0.67	0.67	0.62	0.091	0.516	0.000
HE5	FAM	False Bay	16	246 – 256	6	3.37	1.00	0.69	0.66	-0.424	0.112	0.000
		Hermanus	3		2	–	1.00	0.50	0.38	-1.000	0.400	0.000
		Gansbaai	7		5	3.61	1.00	0.73	0.69	-0.292	1.000	0.000
		Agulhas Bank	9		4	3.09	1.00	0.67	0.61	-0.440	0.340	0.000
HE13	VIC	False Bay	16	238 – 248	3	2.43	0.56	0.53	0.47	-0.027	1.000	0.000
		Hermanus	3		2	–	0.33	0.50	0.38	0.500	1.000	0.111
		Gansbaai	7		3	2.42	0.57	0.56	0.47	0.059	1.000	0.000
		Agulhas Bank	9		3	2.48	0.78	0.54	0.47	-0.400	0.335	0.000
Haplo_MP1 (mean)					3.80	3.14	0.83	0.65	0.59	-0.195	–	0.006

Abbreviations: Number of individuals (*N*), base pairs (bp), number of alleles per locus (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (*PIC*), inbreeding coefficient (F_{IS}), probability of conforming to Hardy-Weinberg expectations (P_{HW}), frequency of null alleles (Fr_{NULL}).

Table 3.3 Continued.

Locus	Dye	Site	*N*	Size range (bp)	N_A	A_R	H_O	H_E	*PIC*	F_{IS}	P_{HW}	Fr_{NULL}
HE7	FAM	False Bay	16	303 – 328	2	1.98	0.63	0.49	0.37	-0.240	0.609	0.000
		Hermanus	3		3	–	1.00	0.61	0.54	-0.500	1.000	0.000
		Gansbaai	7		3	2.54	0.71	0.52	0.46	-0.304	1.000	0.000
		Agulhas Bank	9		2	1.95	0.22	0.44	0.35	0.543	0.167	0.161
HE12	PET	False Bay	16	256 – 268	5	3.12	1.00	0.68	0.63	-0.450	0.006	0.000
		Hermanus	3		4	–	0.67	0.67	0.62	0.200	0.599	0.000
		Gansbaai	7		5	3.61	1.00	0.73	0.69	-0.292	0.650	0.000
		Agulhas Bank	8		5	3.56	0.88	0.72	0.68	-0.153	1.000	0.000
HE15	VIC	False Bay	16	248 – 254	2	1.98	0.25	0.49	0.37	0.516	0.056	0.163
		Hermanus	3		3	–	0.67	0.50	0.45	-0.143	1.000	0.000
		Gansbaai	7		3	2.36	0.71	0.50	0.43	-0.364	1.000	0.000
		Agulhas Bank	9		3	2.52	0.44	0.57	0.49	0.273	0.381	0.074
HE16	NED	False Bay	16	231 – 249	6	3.19	0.94	0.67	0.63	-0.368	0.207	0.000
		Hermanus	3		3	–	0.67	0.50	0.45	-0.143	1.000	0.000
		Gansbaai	7		4	3.08	1.00	0.66	0.60	-0.448	0.201	0.000
		Agulhas Bank	9		5	3.52	0.89	0.73	0.69	-0.164	0.824	0.000
HE18	FAM	False Bay	16	237 – 247	5	3.38	0.56	0.73	0.68	0.256	0.232	0.089
		Hermanus	3		3	–	0.67	0.61	0.54	0.111	1.000	0.000
		Gansbaai	7		5	3.47	0.57	0.70	0.66	0.262	0.110	0.039
		Agulhas Bank	9		4	3.29	0.56	0.71	0.66	0.273	0.049	0.113
Haplo_MP2 (mean)					3.75	2.90	0.70	0.61	0.54	-0.058	–	0.032
Overall (mean)					3.78	3.02	0.77	0.63	0.57	-0.126	–	0.019

Abbreviations: Number of individuals (*N*), base pairs (bp), number of alleles per locus (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (*PIC*), inbreeding coefficient (F_{IS}), probability of conforming to Hardy-Weinberg expectations (P_{HW}), frequency of null alleles (Fr_{NULL}).

Table 3.4 Cross-species transferability results of 10 microsatellite loci developed for *Haploblepharus edwardsii* and tested in *H. fuscus*, *H. pictus* and *Halaelurus natalensis*; where + indicates the presence of solid PCR bands with sufficient intensity for scoring alleles, with the number of alleles indicated in parenthesis.

Locus / Species	*Haploblepharus fuscus* (*N* = 16)	*Haploblepharus pictus* (*N* = 31)	*Halaelurus natalensis* (*N* = 6)
HE1	+ (6)	+ (7)	+ (5)
HE2	+ (6)	+ (6)	+ (5)
HE4	+ (4)	+ (4)	+ (2)
HE5	+ (4)	+ (4)	+ (3)
HE7	+ (2)	+ (5)	+ (3)
HE12	+ (3)	+ (5)	+ (2)
HE13	+ (3)	+ (3)	+ (2)
HE15	+ (2)	+ (3)	+ (3)
HE16	+ (4)	+ (7)	+ (1)
HE18	+ (6)	+ (7)	+ (3)

Abbreviations: Number of individuals (*N*).

Table 3.5 Summary of the mean basic genetic diversity statistics for the cross-species amplification of 10 microsatellite loci in *Haploblepharus fuscus*, *H. pictus* and *Halaelurus natalensis*, with ranges of statistics indicated in parenthesis.

Species	*N*	N_A	A_R	H_O	H_E	*PIC*
Haploblepharus fuscus	16	4 (2 – 6)	3.10 (2.00 – 5.22)	0.74 (0.38 – 1.00)	0.58 (0.40 – 0.82)	0.50 (0.33 – 0.78)
Haploblepharus pictus	31	5.10 (2 – 7)	3.40 (2.03 – 4.60)	0.71 (0.29 – 1.00)	0.60 (0.26 – 0.76)	0.54 (0.24 – 0.71)
Halaelurus natalensis	6	2.90 (1 – 5)	–	0.53 (0.00 – 1.00)	0.54 (0.00 – 0.82)	0.43 (0.00 – 0.70)

Abbreviations: Number of individuals (*N*), number of alleles per locus (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (*PIC*).

3.3.3 Intraspecific genetic differentiation

3.3.3.1 Puffadder shyshark *Haploblepharus edwardsii*

The single level AMOVA (F_{ST} = 0.026; **Table A3.4**) indicated that no statistically significant genetic differentiation was present among sampling locations. Moreover, pairwise F_{ST} values ranged from 0.000 to 0.057 with no statistical significance following the FDR correction (**Table A3.5**). The lowest genetic differentiation occurred between Gansbaai and Hermanus, and the highest genetic differentiation was detected between Agulhas Bank and Hermanus. The DAPC analysis including location prior revealed four genetic clusters corresponding to sampling locations (**Figure 3.2a**). Alternatively, when excluding location prior by using the *find.clusters* function, the DAPC analysis identified the presence of three genetic clusters (k = 3) based on the BIC score (**Figure 3.2b** and **A3.2a**). However, the four estimators of Puechmaille (2016) illustrated the presence of one to two genetic clusters (K = 1 to 2) within the dataset based on Bayesian analyses (**Figure A3.3a** and **A3.4a**).

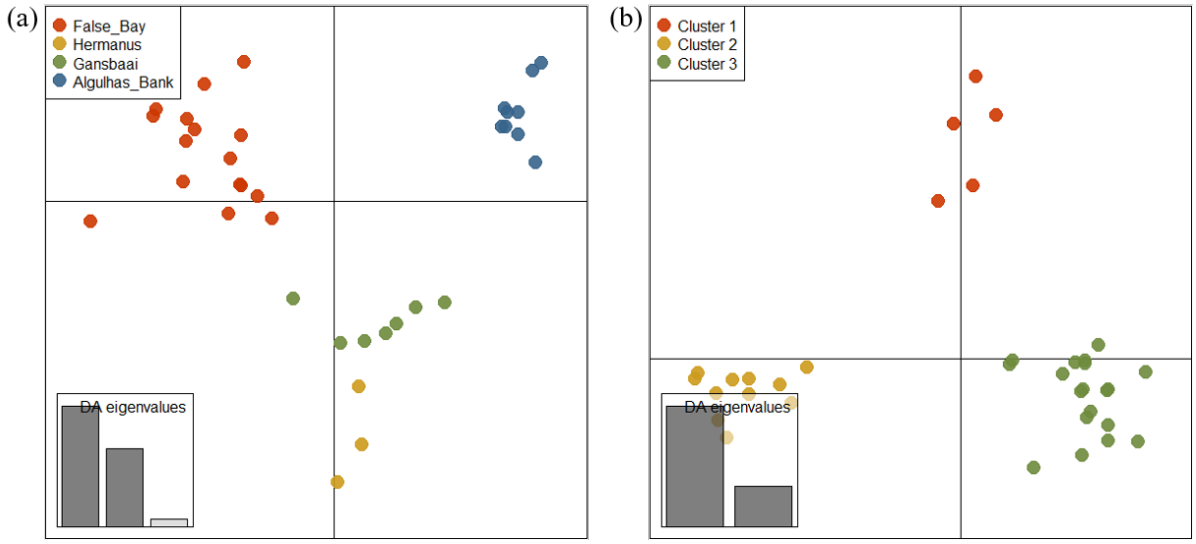


Figure 3.2 Scatter-plots generated by the discriminant analysis of principal components (DAPC), displaying population differentiation among four geographic sampling sites along the South African coastline for the puffadder shyshark *Haploblepharus edwardsii*. (a) DAPC plot for clusters defined by sampling location. (b) DAPC plot for analysis excluding location prior, indicating the presence of three genetic clusters.

3.3.3.2 Brown shyshark *Haploblepharus fuscus*

The AMOVA showed high molecular variation within individuals, with no statistically significant differentiation between sampling locations (F_{ST} = 0.005; **Table A3.4**). Moreover, no pairwise F_{ST} values were statistically significant following the FDR correction (**Table A3.6**). The DAPC analysis including location prior revealed three genetic clusters corresponding to sampling locations (**Figure 3.3**). The *find.clusters* function was unable to accurately detect the number of genetic clusters present, presumably due to the small sample size. The four estimators of Puechmaille (2016) illustrated the presence of one to two genetic clusters (K = 1 to 2) within the dataset based on Bayesian analyses (**Figure A3.3b** and **A3.4b**).

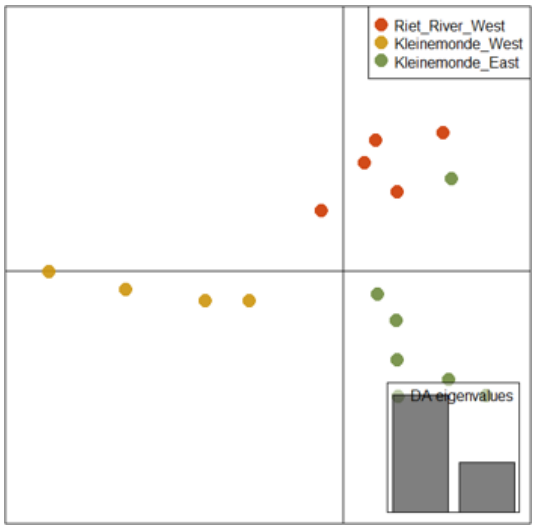


Figure 3.3 Scatter-plot generated by the discriminant analysis of principal components (DAPC), displaying population differentiation among three sampling locations along the South African coastline for the brown shyshark *Haploblepharus fuscus*.

3.3.3.3 Dark shyshark *Haploblepharus pictus*

The presence of population differentiation between sampling locations was indicated by the single level AMOVA as little variation was attributed to the within population level of subdivision, while a significant level of variation amongst populations existed (F_{ST} = 0.069, lower 95% CI > 0 and P < 0.05 following the FDR correction; **Table A3.4**). Pairwise differentiation tests indicated statistically significant genetic differentiation between False Bay and Gansbaai (F_{ST} = 0.050), as well as between each sampling location and Mossel Bay (lower 95% CI > 0 and P < 0.05 following the FDR correction; **Table 3.6**). The DAPC analysis including location prior revealed four genetic clusters corresponding to sampling locations (**Figure 3.4a**). Alternatively, when excluding location prior by using the *find.clusters* function, the DAPC analysis identified the presence of three genetic clusters (k = 3) based on the BIC score (**Figure 3.4b** and **A3.2b**). However, the four estimators of Puechmaille (2016) illustrated the presence of two genetic clusters (K = 2) within the dataset (**Figure A3.3c** and **A3.4c**). The Bayesian analysis illustrated the presence of a genetic cline, with each extreme represented by the most western (False Bay) and eastern (Mossel Bay) sampling locations, respectively (**Figure A3.3c**).

Table 3.6 Pairwise F_{ST} values (below the diagonal) and corresponding P-values (above the diagonal) between four sampling locations for the dark shyshark *Haploblepharus pictus* along the South African coastline, with significant P-values indicated in bold.

	False Bay	Hermanus	Gansbaai	Mossel Bay
False Bay		0.042	**0.001**	**0.001**
Hermanus	0.026		0.123	**0.000**
Gansbaai	0.050	0.000		**0.000**
Mossel Bay	0.080	0.072	0.053	

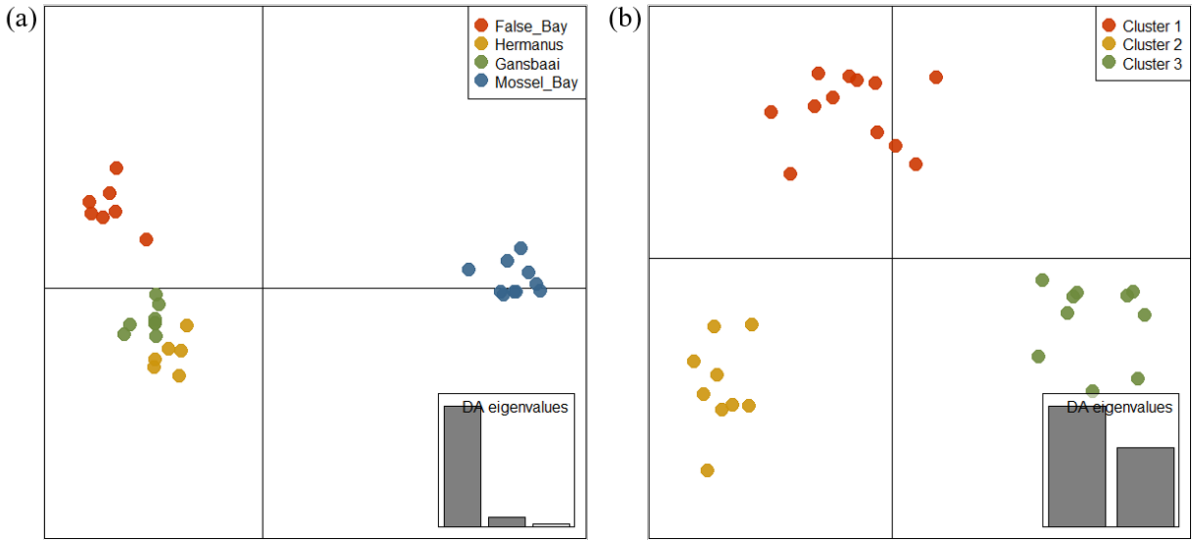


Figure 3.4 Scatter-plots generated by the discriminant analysis of principal components (DAPC), illustrating population differentiation among four geographic sampling sites along the South African coastline for the dark shyshark *Haploblepharus pictus*. (a) DAPC plot for clusters defined by sampling location. (b) DAPC plot for analysis excluding location prior, indicating the presence of three genetic clusters.

3.3.4 Interspecific genetic differentiation

The hierarchical AMOVA indicated that a significant level of genetic variation was attributed to the among species and among populations within species subdivisions (F_{CT} = 0.151, F_{SC} = 0.040, lower 95% CI > 0 and P < 0.05 following the FDR correction; **Table A3.4**). Pairwise differentiation tests indicated that all species were significantly differentiated with F_{ST} values ranging from 0.091 to 0.382 (**Table 3.7**). The lowest genetic differentiation was found between *H. fuscus* and *H. pictus*, while *H. edwardsii* was equally differentiated from *H. fuscus* and *H. pictus*. The highest genetic differentiation was found between *H. fuscus* and the outgroup *Halaelurus natalensis*. The DAPC analysis including prior information on species assignment revealed clear clustering of *H. natalensis* from the three *Haploblepharus* species (**Figure 3.5a**). Removal of the outgroup species from the DAPC analysis allowed the visualisation of three *Haploblepharus* clusters (**Figure 3.5b**). Subsequently, when excluding prior information on species assignment, DAPC analysis identified four genetic clusters (k = 4) based on the BIC score (**Figure 3.5c** and **A3.2c**). Two of the clusters consisted of individuals from a single species while the other two clusters were shared among individuals from all three species (**Figure 3.5d**). The PCoA illustrated overlapping genetic clusters for the three *Haploblepharus* species, further supporting the relatively low genetic differentiation between *H. fuscus* and *H. pictus* (**Figure 3.6**). Percentages of variation explained by the first two axes were 16.36% and 11.28%, respectively. Based on the STRUCTURE results, the ΔK estimate indicated that the most probable number of clusters present in the dataset was four genetic clusters (K = 4; **Figure 3.7**). STRUCTURE membership results for population average (Qi) ranged from 0.655 to 0.982, with the lowest membership resulting from *H. pictus* (**Table 3.8**). Furthermore, individual assignment (qi) scores ranged from 0.004 to 0.988 for *H.*

edwardsii, 0.004 to 0.986 for *H. fuscus*, 0.002 to 0.984 for *H. pictus* and 0.003 to 0.992 for *H. natalensis*; with *qi* values only displayed for individuals identified as possible hybrids using a stringent threshold (*qi* < 0.95) in **Table 3.9**.

Table 3.7 Interspecific pairwise F_{ST} values (below the diagonal) and corresponding *P*-values (above the diagonal) between four scyliorhinid species distributed along the South African coastline, with significant *P*-values indicated in bold.

	H. edwardsii	*H. fuscus*	*H. pictus*	*H. natalensis*
H. edwardsii		**0.000**	**0.000**	**0.000**
H. fuscus	0.113		**0.000**	**0.000**
H. pictus	0.118	0.091		**0.000**
H. natalensis	0.302	0.382	0.346	

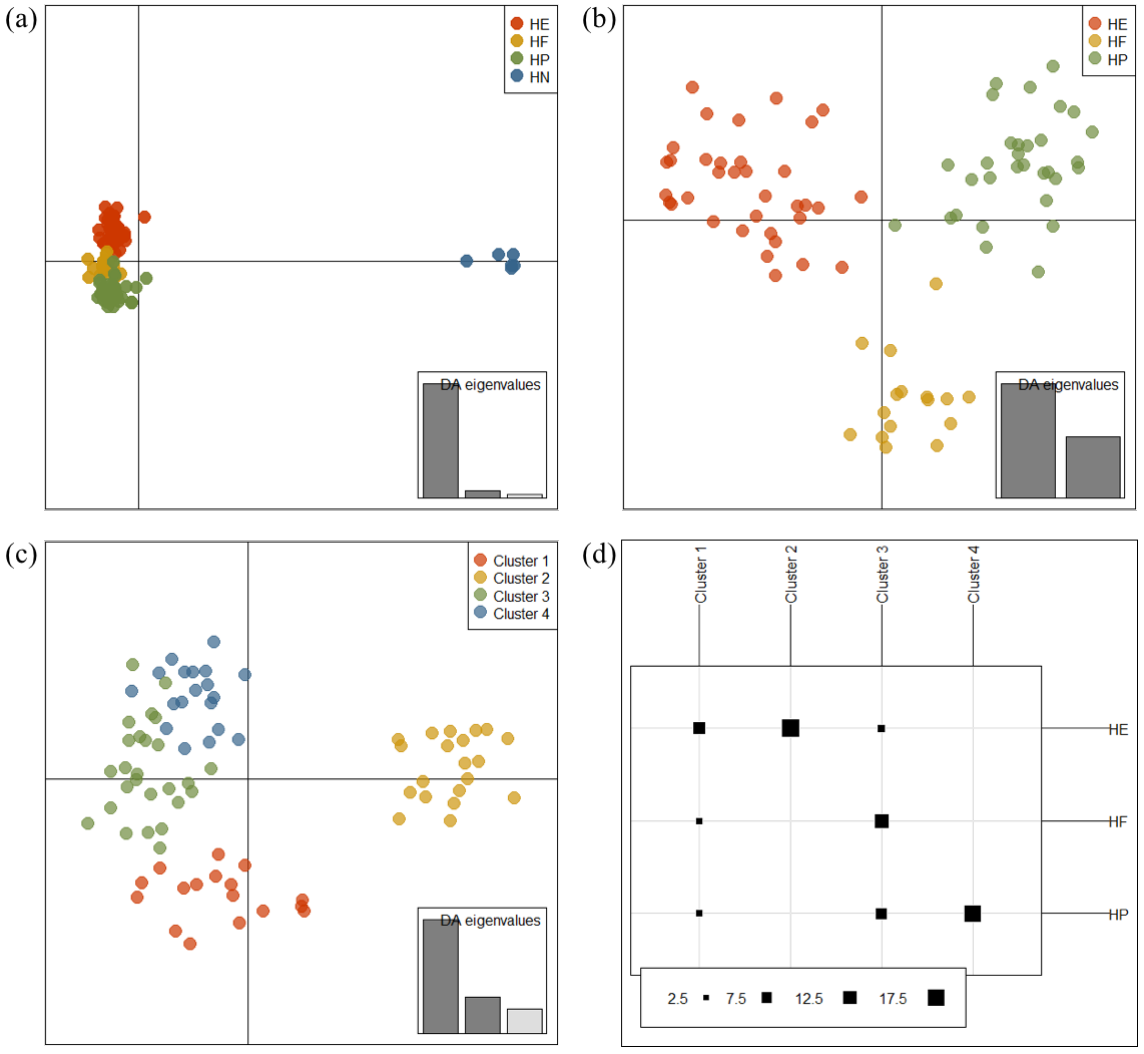


Figure 3.5 Discriminant analysis of principal components (DAPC) results recovered for four scyliorhinid species: *Haploblepharus edwardsii* (HE), *Haploblepharus fuscus* (HF), *Haploblepharus pictus* (HP) and *Halaelurus natalensis* (HN). (a) DAPC plot for clusters defined by taxonomic assignment. (b) DAPC plot for clusters defined by taxonomic assignment, excluding the outgroup species *Halaelurus natalensis*. (c) DAPC plot for analysis excluding prior information on taxonomic assignment. (d) Cluster table displaying the assignment of each species to four genetic clusters ($k = 4$).

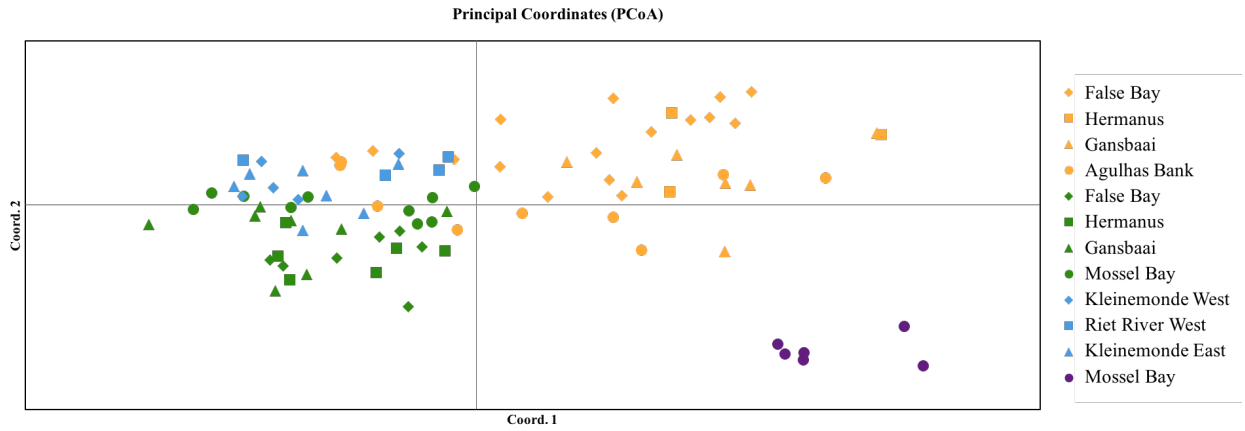


Figure 3.6 First and second co-ordinates for the principal co-ordinate analysis using 10 microsatellite markers for 88 scyliorhinid specimens; with orange representing *Haploblepharus edwardsii*, green representing *Haploblepharus pictus*, blue representing *Haploblepharus fuscus* and purple representing *Halaelurus natalensis*. Percentages of variation explained by the first and second axes were 16.36% and 11.28%, respectively.

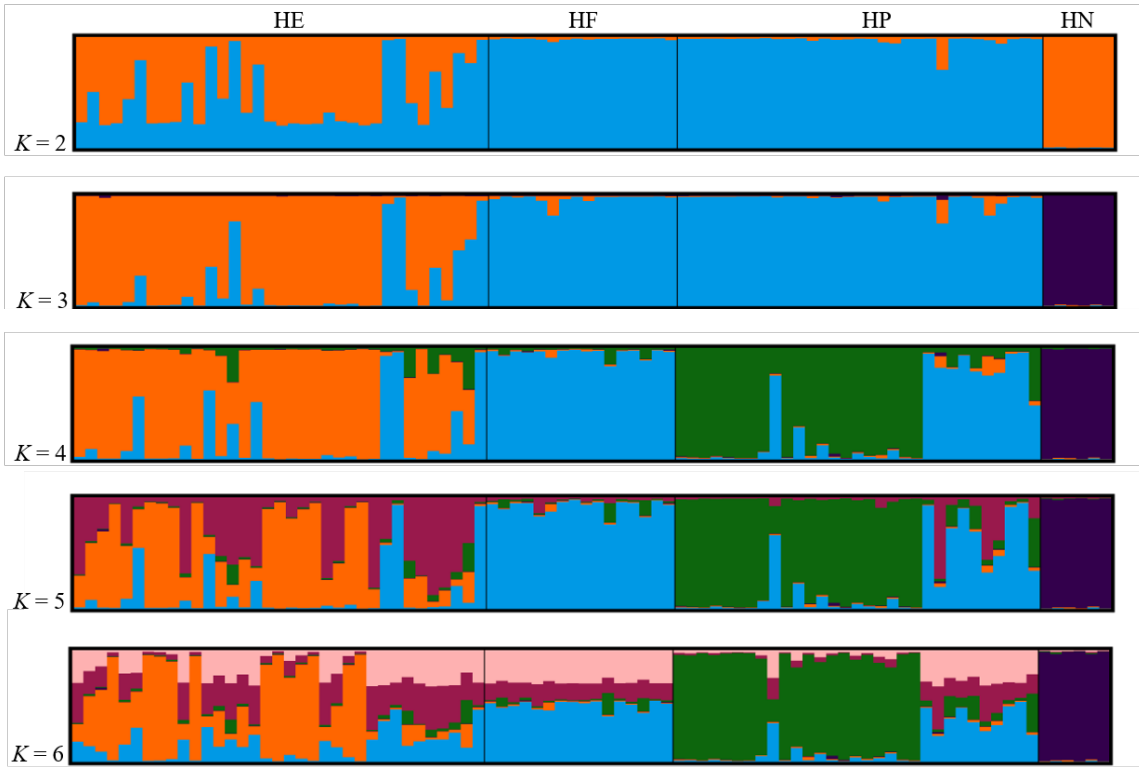


Figure 3.7 Individual cluster assignments for four scyliorhinid species, including: *Haploblepharus edwardsii* (HE), *Haploblepharus fuscus* (HF), *Haploblepharus pictus* (HP) and *Halaelurus natalensis* (HN). Cluster assignments based on STRUCTURE results are displayed for $K = 2$ to $K = 6$, where each vertical bar represents a single individual and each colour represents a genetic cluster.

Table 3.8 Average population membership proportion to inferred genetic clusters for each pre-defined taxonomic group, including: *Haploblepharus edwardsii* (HE), *Haploblepharus fuscus* (HF), *Haploblepharus pictus* (HP) and *Halaelurus natalensis* (HN).

	Clusters (K = 4)			
Taxonomic assignment	HE	HF	HP	HN
Haploblepharus edwardsii	0.768	0.176	0.051	0.004
Haploblepharus fuscus	0.014	0.953	0.029	0.004
Haploblepharus pictus	0.023	0.316	0.655	0.005
Halaelurus natalensis	0.007	0.006	0.006	0.982

Table 3.9 Individual membership for *Haploblepharus edwardsii* (HE), *Haploblepharus fuscus* (HF), *Haploblepharus pictus* (HP) and *Halaelurus natalensis* (HN) specimens; only shown for individuals identified as possible hybrids using a stringent threshold ($qi < 0.95$). Individuals identified as hybrids using a less stringent threshold ($qi < 0.80$) are highlighted by *, while individuals assigned to an unsuspected cluster using the less stringent *qi* threshold are highlighted by **.

		Clusters ($K = 4$)				
Sample	Location	HE	HF	HP	HN	Significance
HE23	False Bay	0.890	0.093	0.014	0.003	
HE26		0.903	0.077	0.017	0.002	
HE27		0.412	0.564	0.021	0.004	*
HE31		0.823	0.124	0.050	0.003	
HE56		0.360	0.621	0.017	0.003	*
HE80		0.897	0.031	0.067	0.004	
HE83		0.374	0.313	0.311	0.003	*
HE115		0.473	0.511	0.013	0.003	*
HE12	Gansbaai	0.935	0.041	0.021	0.003	
HE33	Agulhas Bank	0.037	0.930	0.030	0.003	**
HE35		0.011	0.962	0.024	0.003	**
HE38		0.714	0.018	0.263	0.005	*
HE41		0.690	0.080	0.225	0.004	*
HE42		0.887	0.054	0.055	0.004	
HE43		0.439	0.435	0.122	0.004	*
HE47		0.487	0.137	0.369	0.007	*
HF22	Riet River West	0.060	0.930	0.008	0.003	
HF6	Kleinemonde West	0.012	0.912	0.073	0.003	
HF3	Kleinemonde East	0.016	0.835	0.147	0.003	
HF19		0.009	0.887	0.100	0.004	
HP2	Hermanus	0.010	0.057	0.931	0.003	
HP11		0.010	0.083	0.900	0.007	
HP14	Gansbaai	0.004	0.067	0.927	0.002	
HP92		0.016	0.749	0.232	0.003	*
HP95		0.010	0.287	0.700	0.002	*
HP97		0.014	0.130	0.854	0.003	
HP52	Mossel Bay	0.009	0.952	0.036	0.003	**
HP53		0.102	0.823	0.039	0.035	**
HP58		0.015	0.804	0.179	0.003	**
HP61		0.004	0.936	0.057	0.003	**
HP63		0.028	0.789	0.180	0.003	*
HP65		0.173	0.755	0.069	0.004	*
HP69		0.123	0.777	0.092	0.008	*
HP71		0.015	0.953	0.029	0.003	**
HP72		0.006	0.962	0.029	0.003	**
HP73		0.039	0.483	0.474	0.004	*

3.4 Discussion

This study is the first to develop species-specific microsatellite markers for a South African endemic catshark *Haploblepharus edwardsii*. Moreover, the cross-species utility of the markers was assessed in *H. fuscus*, *H. pictus* and a more distantly related scyliorhinid *Halaelurus natalensis*. As a result of the high transferability success and the variability of the markers in all species tested, these markers could prove useful in future population genetic studies for scyliorhinid species. Assessment of the genetic diversity for each of the four study species indicated that these markers are informative for population genetic analyses, species identification, and for assessing signatures of admixture among the closely related *Haploblepharus* species.

Recent advances in high-throughput sequencing technologies have accelerated the mining of species-specific microsatellite loci for sharks (*e.g.,* Boomer and Stow 2010; Chabot and Nigenda 2011; Pirog *et al*. 2015; Maduna *et al*. 2017); leading to significant progress in the understanding of contemporary processes that drive chondrichthyan population structuring patterns (Dudgeon *et al*. 2012). In this study, the use of reduced genome sequencing data generated on the Ion Torrent S5™ allowed for the cost effective and efficient recovery of species-specific microsatellite loci. Furthermore, two multiplex assays comprising 10 polymorphic microsatellites were successfully constructed and optimised for the puffadder shyshark *Haploblepharus edwardsii*. The characterisation of these markers revealed similar genetic diversity indices as those found in previous studies on *Scyliorhinus canicula* (Griffiths *et al*. 2011; Kousteni *et al*. 2015).

The *de novo* development of microsatellite markers has previously been challenging due to the low levels of polymorphism found in elasmobranchs (Dudgeon *et al*. 2012). Cross-species amplification has been an effective alternate approach for marker development owing to the highly conserved sequences flanking microsatellite loci (Martin *et al*. 2002; Boomer and Stow 2010). As described for several other vertebrate taxa (Primmer *et al*. 2005; Carreras-Carbonell *et al*. 2007; Hendrix *et al*. 2010), the cross-species amplification rate of success is often negatively correlated to the evolutionary distance between the focal and target species (Barbara *et al*. 2007; Griffiths *et al*. 2011; Maduna *et al*. 2014). However, the microsatellite flanking sequences in sharks have previously been shown to remain conserved following 250 million years of divergent evolution (Martin *et al*. 2002). The application of cross-species amplification enables the study and comparison of population genetic structure of co-distributed species; allowing the identification of factors influencing gene flow such as oceanographical barriers or life-history differences (*e.g.* dispersal ability) between species (Maduna *et al*. 2017). Furthermore, these markers can be applied to identify different species in taxonomic disputes and to detect introgression (Khosravi *et al*. 2013; Abdul-Muneer 2014; Giresi *et al*. 2015; Ito *et al*. 2015). Accordingly, in this study the cross-species utility of the markers for other scyliorhinid species was assessed in *H. fuscus*, *H. pictus* and *Halaelurus natalensis.* Similar to previous findings reported for shark species (Griffiths *et al*. 2011; Maduna *et al*. 2014; Pirog *et al*. 2015), a high cross-species

amplification rate of success (100%) was observed for the three study species; suggesting that these markers may be useful in future population genetic studies for catsharks which constitute a family of poorly studied species.

Although the direct comparison of population genetic diversity indices among species is potentially affected by ascertainment bias, given that the markers were isolated from *H. edwardsii*, it is interesting to note that similar levels of genetic diversity were obtained for all three *Haploblepharus* species. As reported for other elasmobranchs (Veríssimo *et al*. 2010), the preliminary intraspecific population differentiation estimates hinted at a positive correlation between population differentiation (F_{ST}) and geographic distance among populations. Due to the presumed limited dispersal ability of scyliorhinid species because of their life-history traits, especially the preference for coastal and benthic habitats (Springer 1979), the presence of significant population differentiation would be expected; as reported for *S. canicula* (Kousteni *et al*. 2015). However, in this study, the null hypothesis of panmixia was only rejected for *H. pictus*.

The lack of genetic differentiation among *H. fuscus* populations (F_{ST} = 0.000) can be explained by the close proximity of the sampling locations (< 10km). Surprisingly, very little genetic differentiation (maximum F_{ST} = 0.057) was recorded for *H. edwardsii* between the offshore Agulhas Bank and coastal sampling sites. The apparent lack of genetic differentiation over the ~280km region could possibly be attributed to the Agulhas Bank forming a shallow plateau, providing a relatively continuous habitat for *H. edwardsii*. The multivariate analyses (DAPC) for *H. edwardsii* supported the presence of low levels of genetic differentiation, particularly illustrating the lack of differentiation between Hermanus and Gansbaai individuals. Interestingly, for both *H. edwardsii* and *H. fuscus*, the Bayesian analyses (STRUCTURE) indicated the presence of either one or two genetic clusters based on the four estimators of Puechmaille (Puechmaille 2016). While the Bayesian method applied in STRUCTURE is the most widely utilised method for detecting population genetic structure (Pritchard *et al*. 2000, 2010; Puechmaille 2016), previous studies have demonstrated that the performance and accuracy of the program is affected by uneven sampling schemes (Schwartz and McKelvey 2009; Puechmaille *et al*. 2011; Puechmaille 2016). In such cases, the use of estimates that are less influenced by uneven sampling schemes was suggested (Puechmaille 2016). Of the four estimators of Puechmaille, the median estimators (MedMeaK and MedMedK) are known to be less sensitive while the estimators based on the maximum (MaxMeaK and MaxMedK) are more sensitive (Puechmaille 2016). Accordingly, for the *H. edwardsii* and *H. fuscus* datasets, the presence of one contemporary admixed cluster is probable (as indicated by the median estimates); however, the possibility of two ancestral clusters which have since interbred cannot be completely ruled out (as indicated by the maximum estimates). In contrast, *H. pictus* populations were significantly differentiated once distance between sampling locations was in excess of 100km. The Bayesian analysis illustrated the presence of a genetic cline, supporting the presence of intraspecific spatial variation. The difference in population genetic differentiation estimates between *H.*

edwardsii and *H. pictus* hints at possible life-history differences (*e.g.* mobility). Due to comparable habitat niches and morphology, the dispersal ability of *H. edwardsii* and *H. pictus* is assumed to be similar. However, acoustic tracking for an alternate catshark genus *Poroderma* indicated that movement patterns between the two closely related species (*P. africanum* and *P. pantherinum*) differed substantially (Watson R, perscomm). Although these species are sympatric and have similar morphological characteristics, *P. pantherinum* displays a high site-fidelity while *P. africanum* tends to be mobile within Mossel Bay (Watson R, perscomm). *Haploblepharus* may present a similar case, where *H. edwardsii* is mobile along the shallow Agulhas Bank while *H. pictus* displays a higher site-fidelity; however, conventional or acoustic tracking data is required to elucidate the movement patterns of these species.

Species assignment based on Bayesian (STRUCTURE) and multivariate (PCoA and DAPC) analyses was accurate for the more distantly related species *Halaelurus natalensis*, indicating the utility of these markers for species discrimination. In contrast, some of the *Haploblepharus* specimens presented a more complex case. Interspecific differentiation was explored by estimating pairwise F_{ST} which supported the discrimination of *H. natalensis* (minimum $F_{ST} = 0.302$). Although all interspecific F_{ST} values were statistically significant, the level of genetic differentiation between *H. fuscus* and *H. pictus* ($F_{ST} = 0.091$) is comparable to *H. pictus* intraspecific F_{ST} values (maximum $F_{ST} = 0.080$); possibly indicating that the genetic differentiation present could be at a population level rather than at a species level. This was supported by the PCoA plot which displays overlapping genetic clusters, especially between *H. fuscus* and *H. pictus*. Without any prior information on location sampled or taxonomic species assignment, both DAPC and STRUCTURE revealed the presence of four genetic clusters. Two distinct and two shared genetic clusters were identified by the DAPC analysis. Some of the *H. edwardsii* and *H. pictus* individuals were assigned to distinct genetic clusters while all three *Haploblepharus* taxa were assigned to the remaining two shared clusters. Although four genetic clusters were identified by STRUCTURE, only *H. fuscus* and *H. natalensis* were assigned to single genetic clusters; while signatures of admixture were evident in the *H. edwardsii* and *H. pictus* genetic clusters. The index of admixture in Bayesian analysis has frequently been used to identify introgression (Barilani *et al*. 2006; Sanz *et al*. 2008; Scandura *et al*. 2009; Khosravi *et al*. 2013; Ito *et al*. 2015), and by implementing *qi* thresholds this study was able to accurately identify pure and admixed individuals. Furthermore, some individuals taxonomically identified as *H. edwardsii* and *H. pictus* showed membership to the *H. fuscus* ancestral cluster ($qi > 0.80$), possibly indicating a case of species misidentification.

Species misidentification between closely related species is commonly cited as a possible explanation for the lack of genetic differentiation between species (Gledhill *et al*. submitted), and in this case could explain the unsuspected cluster assignment. While confusion exists between *Haploblepharus* taxa, species identification was performed by trained shark biologists according to taxonomic keys (Human 2007*a*). All specimens were photographed and whole *H. edwardsii* specimens

from the offshore Agulhas Bank sampling location were retained for voucher purposes. Furthermore, genotyping was only performed for specimens unambiguously assigned to a taxonomic group. The majority of unsuspected cluster assignments were found for Agulhas Bank *H. edwardsii* and Mossel Bay *H. pictus* specimens. Comprehensive morphometric and meristic analyses based on morphological data collected from the *H. edwardsii* whole specimens is required to further support the suspected allopatric distribution of *H. edwardsii* in offshore locations. Alternatively, the Agulhas Bank shallow plateau and the eastward range expansion of forest-forming kelp (Bolton *et al*. 2012) may provide a corridor for gene flow between the Atlantic and Indian Oceans; possibly allowing secondary contact between recently diverged species (*H. edwardsii* and *H. fuscus*). In contrast, specimens taxonomically identified as *H. pictus* are assumed to display an allopatric distribution in Mossel Bay (Watson R, perscomm); however, 60% of these individuals assigned to the *H. fuscus* genetic cluster using a *qi* threshold of > 0.80. Additionally, intraspecific F_{ST} values as well as multivariate and Bayesian analyses have indicated that Mossel Bay *H. pictus* specimens display the greatest genetic differentiation in relation to all other sampling locations. Accordingly, the presence of a genetic cline in *H. pictus* may not be solely attributed to intraspecific spatial variation, alternatively it may be attributed to incorrect taxonomic assignment and/or interspecific hybridisation.

While the presence of admixture between *Haploblepharus* taxa is evident, distinct genetic clusters were also present. Approximately 59% of 88 specimens genotyped were unambiguously assigned (*qi* > 0.95) to a distinct genetic cluster that confirmed accurate taxonomic assignment. In closely related species such as *H. edwardsii*, *H. fuscus* and *H. pictus* it is difficult to discern whether the detected admixture is from contemporary hybridisation or a consequence of incomplete lineage sorting due to recent speciation. This topic has received much interest in recent years (Sang and Zhong 2000; Buckley *et al*. 2006; Holland *et al*. 2008), particularly due to the increasing detection of introgression events; however, few effective approaches exist for distinguishing between these two processes (DiBattista *et al*. 2016). Incomplete lineage sorting acts to eliminate ancestral polymorphism over time (Avise 2000). Although not completely ruled out, the presence of distinct species clusters hints at contemporary hybridisation as an explanation for the presence of admixed individuals rather than incomplete lineage sorting. A limitation to microsatellite-based conclusions is the homoplasy of alleles (DiBattista *et al*. 2016): alleles identical in size but not identical by descent. In other marine taxa, studies have yielded ambiguous claims of hybridisation as some alleles 'shared' among species are identical in size but experienced independent evolutionary pathways (Henriques *et al*. 2016). For recently diverged species, introgression appears to be the favoured explanation when mtDNA sequence divergence meets previously suggested divergence thresholds (van Herwerden *et al*. 2006; Yaakub *et al*. 2006; Marie *et al*. 2007; Montanari *et al*. 2014). Given that rates of mitochondrial DNA evolution are slower in elasmobranchs than in other taxa (Martin 1992), the ~1.5% sequence divergence between the two *COI* haplotypes present for *H. edwardsii* and *H. pictus* in Chapter 2 suggests that introgression may be the more favourable explanation.

This study highlights the presence of significant population differentiation among *H. pictus* sampling locations, although additional sampling is required to accurately determine the extent of population delineation. The finding that geographic distance between populations may present a break in the species' genetic connectivity should be accounted for in future conservation plans by defining distinct management units. The lack of genetic structure among *H. edwardsii* sampling locations was surprising; requiring further exploration in terms of an increased number of samples and sampling locations, as well as the use of conventional or acoustic tracking data. Furthermore, the potential presence of hybridisation among *Haploblepharus* species provides an exciting opportunity to study the evolutionary consequences of introgression. As the definition of a 'species' is fluid, the identification of these specimens is largely dependent on the parameters used to define a species (Hinojosa-Alvarez *et al*. 2016). For example, if the suspected hybridisation between *Haploblepharus* taxa is accepted, then the genus cannot be separated into distinct groups of non-interbreeding biological species. Moreover, the morphological conservatism and overlap in anatomical characters, leading to visual misidentification, does not conform to strict morphological species parameters. Accordingly, future research requires an integrative approach, using morphological and meristic data in conjunction with additional diagnostic molecular markers to accurately tease apart the relative contribution of the different factors influencing the signatures of admixture observed in this study.

3.5 Conclusions

In summary, this chapter reports on the development of species-specific microsatellite loci for the puffadder shyshark *Haploblepharus edwardsii*. The cross-species utility of these genetic markers was assessed in three additional southern African endemic scyliorhinids (*H. fuscus*, *H. pictus* and *Halaelurus natalensis*). The potential use of these markers in a biodiversity conservation context was assessed by evaluating the marker utility in population genetic analyses, species identification and the detection of signatures of admixture. These markers proved useful in all applications assessed and owing to the high transferability success, could potentially be used in future population genetic studies for catsharks which constitute a family of poorly studied species.

3.6 Appendix

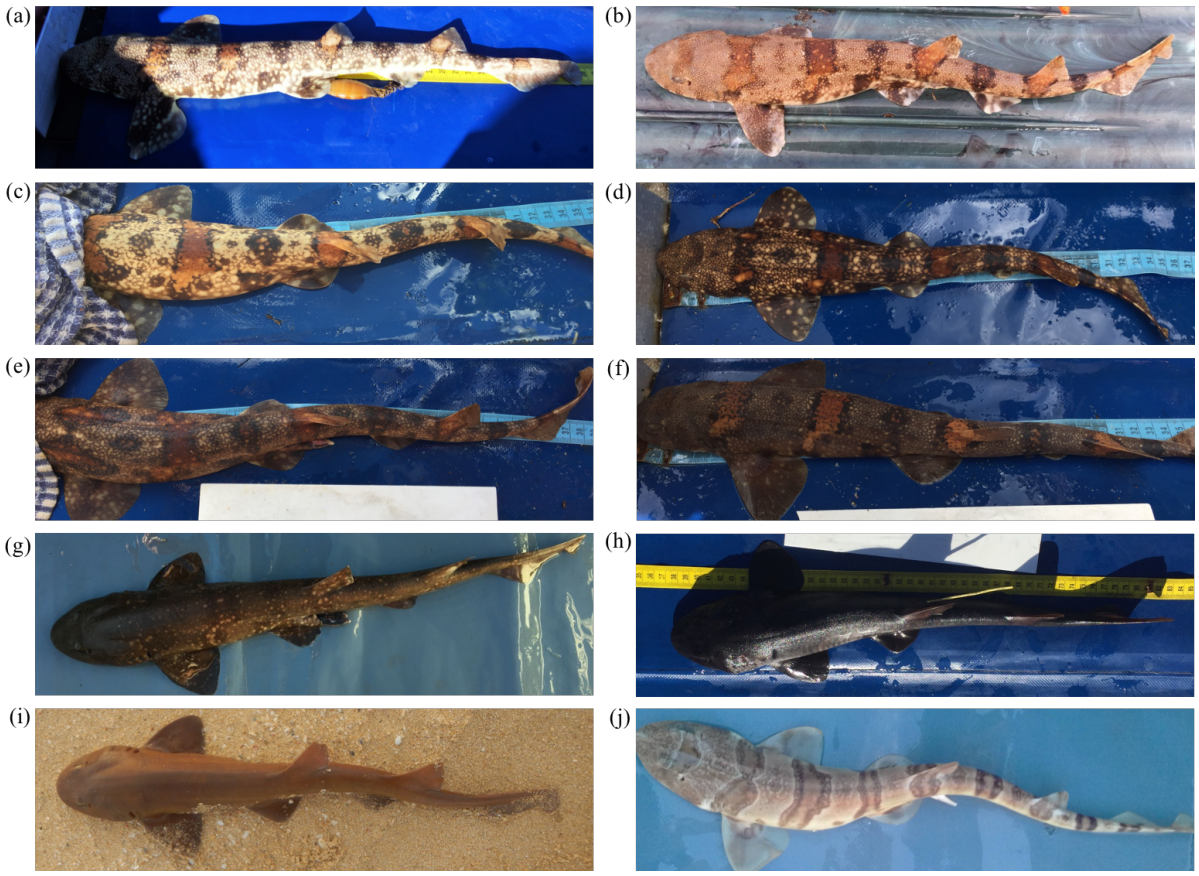


Figure A3.1 Photographs of specimens for voucher purposes illustrating the degree of intraspecific colour variation, particularly for the puffadder shyshark *Haploblepharus edwardsii* and the dark shyshark *Haploblepharus pictus*. (a-f) *H. edwardsii*. (g-h) *H. pictus*. (i) The brown shyshark *Haploblepharus fuscus*. (j) The tiger catshark *Halaelurus natalensis*.

Table A3.1 Basic genetic diversity statistics as characterised by two polymorphic microsatellite multiplex assays for the brown shyshark *Haploblepharus fuscus* based on three sampling locations in Port Alfred, South Africa: Riet River West, Kleinemonde West and Kleinemonde East.

Locus	Dye	Site	*N*	Size range (bp)	N_A	A_R	H_O	H_E	*PIC*	F_{IS}	P_{HW}	Fr_{NULL}
HE1	PET	Riet River West	5	227 – 251	5	4.38	1.00	0.68	0.64	-0.379	1.000	0.000
		Kleinemonde West	4		3	3.00	1.00	0.59	0.51	-0.600	0.314	0.000
		Kleinemonde East	7		5	3.96	1.00	0.70	0.66	-0.355	0.153	0.000
HE2	FAM	Riet River West	5	352 – 368	3	2.80	1.00	0.58	0.49	-0.667	0.176	0.000
		Kleinemonde West	4		3	3.00	1.00	0.59	0.51	-0.600	0.314	0.000
		Kleinemonde East	7		5	3.70	1.00	0.65	0.60	-0.474	0.326	0.000
HE4	NED	Riet River West	5	254 – 266	3	2.78	0.40	0.46	0.41	0.238	0.334	0.000
		Kleinemonde West	4		3	3.00	0.50	0.41	0.37	-0.091	1.000	0.000
		Kleinemonde East	7		2	1.99	0.57	0.41	0.32	-0.333	1.000	0.000
HE5	FAM	Riet River West	5	246 – 254	4	3.60	1.00	0.64	0.58	-0.481	0.428	0.000
		Kleinemonde West	4		3	3.00	1.00	0.59	0.51	-0.600	0.314	0.000
		Kleinemonde East	7		3	2.57	1.00	0.56	0.46	-0.750	0.049	0.000
HE13	VIC	Riet River West	5	238 – 248	3	2.98	1.00	0.62	0.55	-0.538	0.429	0.000
		Kleinemonde West	4		3	3.00	1.00	0.59	0.51	-0.600	0.314	0.000
		Kleinemonde East	7		3	2.93	1.00	0.62	0.55	-0.556	0.133	0.000
Haplo_MP1 (mean)					3.40	3.11	0.90	0.58	0.51	-0.453	–	0.000

Abbreviations: Number of individuals (*N*), base pairs (bp), number of alleles per locus (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (*PIC*), inbreeding coefficient (F_{IS}), probability of conforming to Hardy-Weinberg expectations (P_{HW}), frequency of null alleles (Fr_{NULL}).

Table A3.1 Continued.

Locus	Dye	Site	*N*	Size range (bp)	N_A	A_R	H_O	H_E	*PIC*	F_{IS}	P_{HW}	Fr_{NULL}
HE7	FAM	Riet River West	5	323 – 328	2	1.80	0.20	0.18	0.16	0.000	–	0.000
		Kleinemonde West	4		2	2.00	0.50	0.50	0.38	0.143	1.000	0.001
		Kleinemonde East	7		2	2.00	0.43	0.46	0.35	0.143	1.000	0.022
HE12	PET	Riet River West	5	258 – 266	3	2.78	0.60	0.46	0.41	-0.200	1.000	0.000
		Kleinemonde West	4		2	2.00	0.25	0.22	0.19	0.000	–	0.000
		Kleinemonde East	7		2	1.99	0.57	0.41	0.32	-0.333	1.000	0.000
HE15	VIC	Riet River West	5	250 – 254	2	2.00	0.80	0.48	0.36	-0.600	0.428	0.000
		Kleinemonde West	4		2	2.00	1.00	0.50	0.38	-1.000	0.314	0.000
		Kleinemonde East	7		2	2.00	0.86	0.49	0.37	-0.714	0.161	0.000
HE16	NED	Riet River West	5	237 – 241	2	1.98	0.40	0.32	0.27	-0.143	1.000	0.000
		Kleinemonde West	4		3	3.00	0.75	0.59	0.51	-0.125	1.000	0.000
		Kleinemonde East	7		3	2.52	0.57	0.44	0.39	-0.231	1.000	0.000
HE18	FAM	Riet River West	5	235 – 245	6	5.51	0.60	0.82	0.79	0.368	0.137	0.133
		Kleinemonde West	4		3	3.00	0.50	0.59	0.51	0.294	1.000	0.047
		Kleinemonde East	7		5	3.96	0.57	0.70	0.66	0.262	0.362	0.108
Haplo_MP2 (mean)					2.73	2.57	0.57	0.48	0.40	-0.142	–	0.021
Overall (mean)					3.07	2.84	0.74	0.53	0.46	-0.297	–	0.010

Abbreviations: Number of individuals (*N*), base pairs (bp), number of alleles per locus (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (*PIC*), inbreeding coefficient (F_{IS}), probability of conforming to Hardy-Weinberg expectations (P_{HW}), frequency of null alleles (Fr_{NULL}).

Table A3.2 Basic genetic diversity statistics as characterised by two polymorphic microsatellite multiplex assays for the dark shyshark *Haploblepharus pictus* based on four sampling locations in South Africa: False Bay, Hermanus, Gansbaai and Mossel Bay.

Locus	Dye	Site	*N*	Size range (bp)	N_A	A_R	H_O	H_E	*PIC*	F_{IS}	P_{HW}	Fr_{NULL}
HE1	PET	False Bay	7	225 – 251	2	2.00	1.00	0.50	0.38	-1.000	0.037	0.000
		Hermanus	6		2	2.00	1.00	0.50	0.38	-1.000	0.091	0.000
		Gansbaai	8		2	2.00	1.00	0.50	0.38	-1.000	0.025	0.000
		Mossel Bay	10		5	4.14	1.00	0.66	0.61	-0.475	0.135	0.000
HE2	FAM	False Bay	7	352 – 368	4	3.86	1.00	0.65	0.60	-0.474	0.145	0.000
		Hermanus	6		3	3.00	1.00	0.61	0.54	-0.579	0.204	0.000
		Gansbaai	8		6	5.19	1.00	0.69	0.65	-0.400	0.732	0.000
		Mossel Bay	10		5	4.61	1.00	0.69	0.65	-0.417	0.346	0.000
HE4	NED	False Bay	6	254 – 266	2	2.00	0.33	0.28	0.24	-0.111	1.000	0.000
		Hermanus	6		4	4.00	0.83	0.64	0.57	-0.220	0.654	0.000
		Gansbaai	8		3	2.75	0.75	0.54	0.45	-0.333	0.272	0.000
		Mossel Bay	10		2	2.00	0.50	0.38	0.30	-0.286	1.000	0.000
HE5	FAM	False Bay	7	246 – 254	3	3.00	1.00	0.62	0.55	-0.556	0.132	0.000
		Hermanus	6		3	3.00	1.00	0.63	0.55	-0.538	0.091	0.000
		Gansbaai	8		4	3.74	1.00	0.68	0.62	-0.418	0.520	0.000
		Mossel Bay	10		4	3.45	1.00	0.62	0.54	-0.593	0.034	0.000
HE13	VIC	False Bay	7	238 – 248	2	1.99	0.29	0.24	0.21	-0.091	1.000	0.000
		Hermanus	6		3	3.00	0.83	0.63	0.55	-0.250	0.653	0.000
		Gansbaai	8		3	2.99	0.88	0.62	0.54	-0.361	0.777	0.000
		Mossel Bay	10		3	2.85	0.50	0.54	0.47	0.126	1.000	0.001
Haplo_MP1 (mean)					3.25	3.08	0.85	0.56	0.49	-0.449	–	0.000

Abbreviations: Number of individuals (*N*), base pairs (bp), number of alleles per locus (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (*PIC*), inbreeding coefficient (F_{IS}), probability of conforming to Hardy-Weinberg expectations (P_{HW}), frequency of null alleles (Fr_{NULL}).

Table A3.2 Continued.

Locus	Dye	Site	*N*	Size range (bp)	N_A	A_R	H_O	H_E	*PIC*	F_{IS}	P_{HW}	Fr_{NULL}
HE7	FAM	False Bay	7	303 – 333	2	2.00	0.29	0.41	0.32	0.368	0.441	0.096
		Hermanus	6		4	4.00	0.67	0.68	0.62	0.111	1.000	0.000
		Gansbaai	8		3	2.74	0.50	0.40	0.35	-0.191	1.000	0.000
		Mossel Bay	10		3	2.60	0.40	0.49	0.41	0.226	0.432	0.034
HE12	PET	False Bay	7	258 – 266	4	3.71	1.00	0.61	0.54	-0.585	0.090	0.000
		Hermanus	6		4	4.00	1.00	0.65	0.60	-0.463	0.481	0.000
		Gansbaai	8		4	3.45	0.50	0.41	0.39	-0.143	1.000	0.000
		Mossel Bay	10		4	3.45	0.80	0.57	0.51	-0.371	0.581	0.000
HE15	VIC	False Bay	7	248 – 254	2	1.86	0.14	0.13	0.12	0.000	–	0.000
		Hermanus	6		2	2.00	0.17	0.15	0.14	0.000	–	0.000
		Gansbaai	8		3	2.90	0.50	0.41	0.37	-0.167	1.000	0.000
		Mossel Bay	10		2	1.95	0.30	0.26	0.22	-0.125	1.000	0.000
HE16	NED	False Bay	7	231 – 247	4	3.85	0.86	0.66	0.60	-0.220	0.573	0.000
		Hermanus	6		5	5.00	1.00	0.76	0.73	-0.224	0.558	0.000
		Gansbaai	8		6	5.20	0.88	0.72	0.68	-0.153	0.110	0.000
		Mossel Bay	10		5	4.40	0.90	0.72	0.67	-0.200	0.469	0.000
HE18	FAM	False Bay	7	235 – 247	3	2.86	0.29	0.54	0.45	0.529	0.105	0.163
		Hermanus	6		3	3.00	0.33	0.50	0.45	0.412	0.192	0.143
		Gansbaai	8		3	2.99	0.50	0.59	0.52	0.211	0.731	0.041
		Mossel Bay	10		6	4.75	0.60	0.73	0.68	0.223	0.210	0.033
Haplo_MP2 (mean)					3.60	4.64	0.58	0.54	0.47	-0.038	–	0.026
Overall (mean)					3.43	3.86	0.72	0.55	0.48	-0.244	–	0.013

Abbreviations: Number of individuals (*N*), base pairs (bp), number of alleles per locus (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (*PIC*), inbreeding coefficient (F_{IS}), probability of conforming to Hardy-Weinberg expectations (P_{HW}), frequency of null alleles (Fr_{NULL}).

Table A3.3 Basic genetic diversity statistics as characterised by two polymorphic microsatellite multiplex assays for the tiger catshark *Halaelurus natalensis* based on a single sampling location in South Africa: Mossel Bay.

Locus	Dye	Site	*N*	Size range (bp)	N_A	A_R	H_O	H_E	*PIC*	F_{IS}	P_{HW}	Fr_{NULL}
HE1	PET	Mossel Bay	6	229 – 253	5	–	0.83	0.74	0.64	-0.136	0.516	0.000
HE2	FAM		5	344 – 368	5	–	1.00	0.82	0.70	-0.250	1.000	0.000
HE4	NED		3	252 – 276	2	–	0.00	0.53	0.35	1.000	0.201	0.315
HE5	FAM		6	230 – 252	3	–	1.00	0.62	0.48	-0.714	0.091	0.000
HE13	VIC		6	238 – 253	2	–	0.33	0.30	0.24	-0.111	1.000	0.000
Haplo_MP1 (mean)					3.4	–	–	0.60	0.48	-0.042	–	0.063

Abbreviations: Number of individuals (*N*), base pairs (bp), number of alleles per locus (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (*PIC*), inbreeding coefficient (F_{IS}), probability of conforming to Hardy-Weinberg expectations (P_{HW}), frequency of null alleles (Fr_{NULL}).

Table A3.3 Continued.

Locus	Dye	Site	*N*	Size range (bp)	N_A	A_R	H_O	H_E	*PIC*	F_{IS}	P_{HW}	Fr_{NULL}
HE7	FAM	Mossel Bay	6	303 – 333	3	–	0.33	0.55	0.45	0.412	0.193	0.143
HE12	PET		6	258 – 260	2	–	0.67	0.48	0.35	-0.429	1.000	0.000
HE15	VIC		5	234 – 252	3	–	0.80	0.69	0.55	-0.185	1.000	0.000
HE16	NED		6	235	1	–	0.00	0.00	0.00	–	–	0.001
HE18	FAM		6	215 – 225	3	–	0.33	0.67	0.54	0.524	0.048	0.176
Haplo_MP2 (mean)					2.4	–	–	0.48	0.38	0.081	–	0.064
Overall (mean)					2.9	–	–	0.54	0.43	0.019	–	0.064

Abbreviations: Number of individuals (*N*), base pairs (bp), number of alleles per locus (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (*PIC*), inbreeding coefficient (F_{IS}), probability of conforming to Hardy-Weinberg expectations (P_{HW}), frequency of null alleles (Fr_{NULL}).

Table A3.4 Analysis of molecular variance (AMOVA) for *Haploblepharus edwardsii*, *Haploblepharus fuscus*, *Haploblepharus pictus* and for the whole dataset including the three aforementioned species as well as a more distantly related scyliorhinid species *Halaelurus natalensis*, with significant *P*-values indicated in bold.

Species	Source of variation	Variation (%)	F statistic	*P*-value
Haploblepharus edwardsii	Among populations	2.6	F_{ST} = 0.026	0.554
	Within populations	-14.0	F_{IS} = -0.144	1.000
	Within individuals	111.4	F_{IT} = -0.114	0.999
Haploblepharus fuscus	Among populations	0.5	F_{ST} = 0.005	0.981
	Within populations	-29.4	F_{IS} = -0.295	1.000
	Within individuals	128.9	F_{IT} = -0.289	1.000
Haploblepharus pictus	Among populations	6.9	F_{ST} = 0.069	**0.002**
	Within populations	-23.3	F_{IS} = -0.250	1.000
	Within individuals	116.4	F_{IT} = -0.164	1.000
All	Among species	15.1	F_{CT} =0.151	**0.000**
	Among populations within species	3.4	F_{SC} = 0.040	**0.000**
	Among individuals within populations	-15.6	F_{IS} = -0.192	1.000
	Within individuals	97.0	F_{IT} = 0.030	0.880

Table A3.5 Pairwise F_{ST} values (below the diagonal) and corresponding *P*-values (above the diagonal) between four sampling locations for the puffadder shyshark *Haploblepharus edwardsii* along the South African coastline.

	False Bay	Hermanus	Gansbaai	Agulhas Bank
False Bay		0.207	0.034	0.103
Hermanus	0.003		0.866	0.046
Gansbaai	0.020	0.000		0.079
Agulhas Bank	0.012	0.057	0.020	

Table A3.6 Pairwise F_{ST} values (below the diagonal) and corresponding *P*-values (above the diagonal) between three sampling locations for the brown shyshark *Haploblepharus fuscus* along the South African coastline.

	Riet River West	Kleinemonde West	Kleinemonde East
Riet River West		0.827	0.191
Kleinemonde West	0.000		0.113
Kleinemonde East	0.000	0.000	

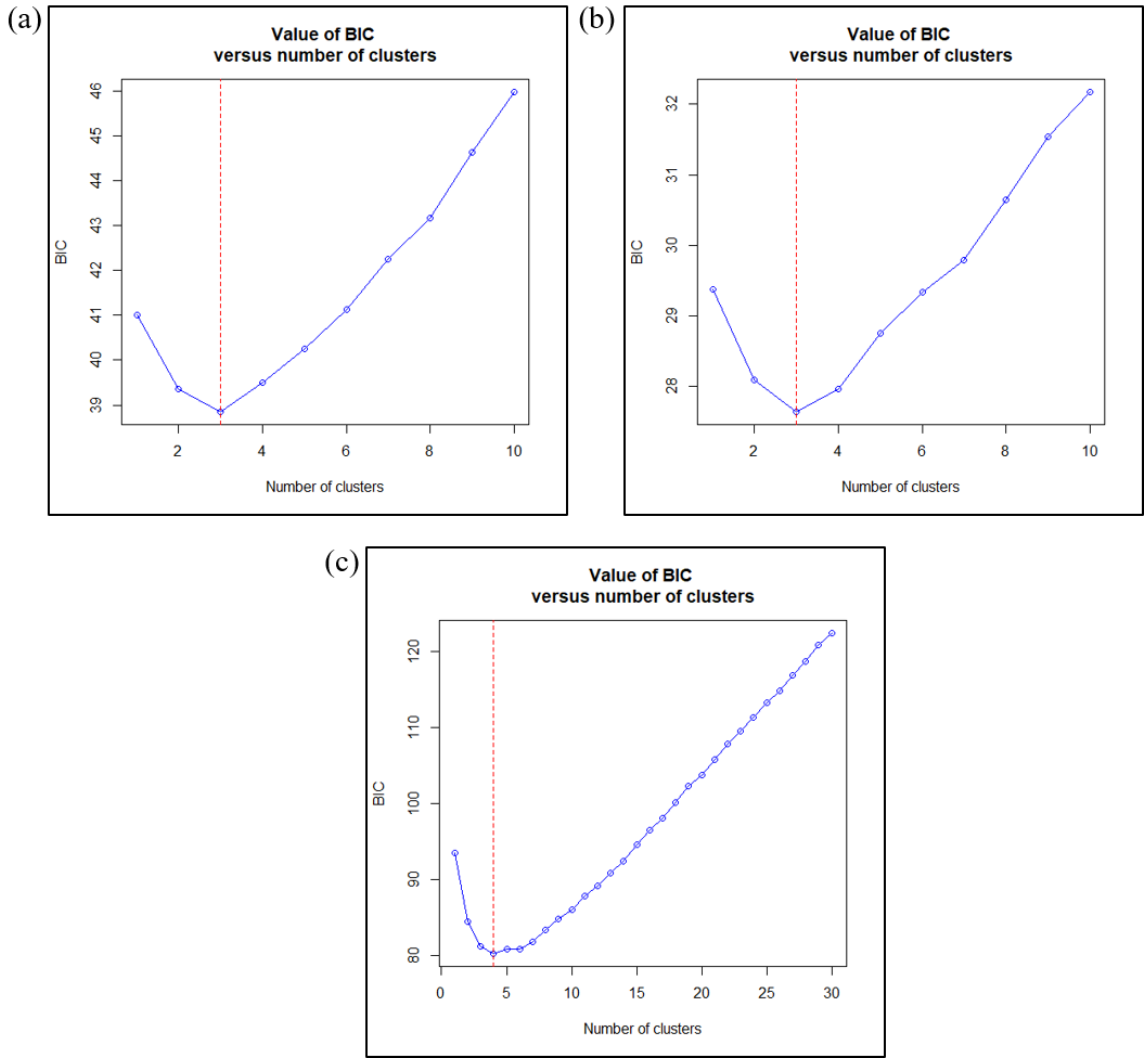


Figure A3.2 Bayesian Information Criterion (BIC) scores used to infer the number of genetic clusters present in each dataset when excluding location prior. (a) BIC score for the *Haploblepharus edwardsii* dataset with $K = 3$. (b) BIC score for the *Haploblepharus pictus* dataset with $K = 3$. (c) BIC score for the complete dataset including three *Haploblepharus* species (*H. edwardsii*, *H. fuscus* and *H. pictus*) with $K = 4$.

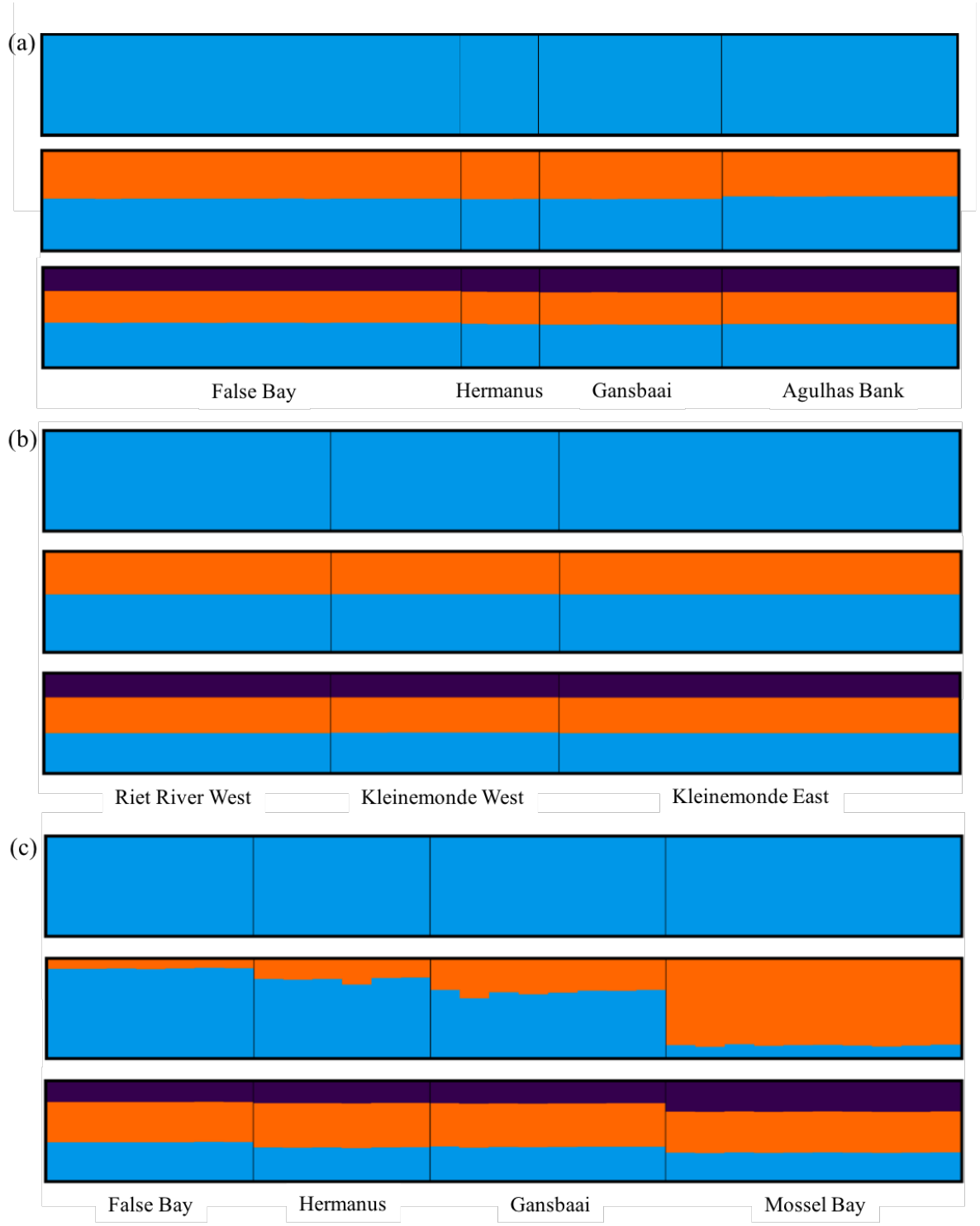


Figure A3.3 Individual cluster assignments based on STRUCTURE results for $K = 1$ to $K = 3$ for the three study species, where each vertical bar represents a single individual and each colour represents a genetic cluster. (a) *Haploblepharus edwardsii*. (b) *Haploblepharus fuscus*. (c) *Haploblepharus pictus*.

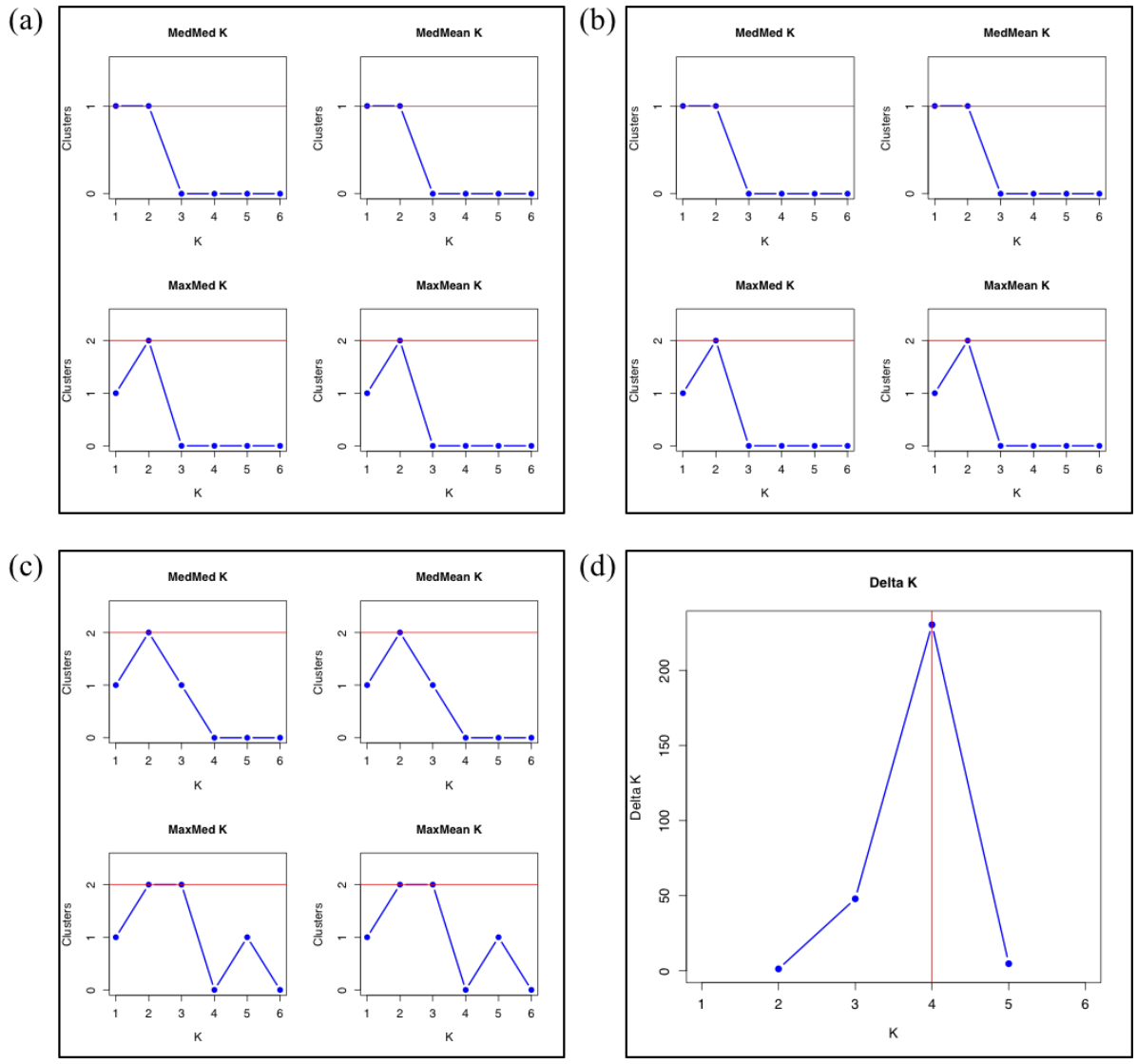


Figure A3.4 Number of clusters estimated from STRUCTURE results, with the most probable number of clusters indicated by the red line. (a) Four estimators of Puechmaille (2016) for the *Haploblepharus edwardsii* dataset. (b) Four estimators of Puechmaille (2016) for the *Haploblepharus fuscus* dataset. (c) Four estimators of Puechmaille (2016) for the *Haploblepharus pictus* dataset. (d) Delta *K* estimate for the whole dataset, including the three aforementioned *Haploblepharus* species and *Halaelurus natalensis*.

CHAPTER 4

Concluding remarks and future research

4.1 Introduction

Marine populations and ecosystems have been drastically altered over recent years, largely driven by the rapid expansion of fisheries and other anthropogenic effects (Cortés 2000; Stevens *et al*. 2000; Dudley and Simpfendorfer 2006; Ferretti *et al.* 2010; Dulvy *et al*. 2017). More so, compelling evidence for the threat that climate change poses to coastal marine ecosystems already exists (Richardson and Poloczanska 2008; Chin *et al*. 2010; Blamey *et al*. 2015). Human-induced climate change, resulting in vegetation shifts and the creation of new hybrid zones has led to a surge of research on hybridisation events as the consequences for the genetic integrity of the species are not well-documented (Mank *et al*. 2004; Brumfield 2010; DiBattista *et al*. 2016; Walter *et al*. 2017). The increasing presence of several direct and indirect threats to sharks have placed these species at a high risk of becoming endangered or extinct (Myers and Worm 2003), with studies reporting a widespread decline of chondrichthyan populations (Baum *et al*. 2003; Dulvy *et al.* 2003, 2008; Ferretti *et al*. 2008; Davidson *et al*. 2016). Accordingly, an estimated one quarter of chondrichthyan species are threatened with an elevated risk of extinction (Dulvy *et al*. 2014).

The need for conservation action focused on imperilled endemics in southern Africa has recently been highlighted (Davidson and Dulvy 2017; Stein *et al*. 2018). While Scyliorhinidae Gill 1862 was identified as one of the least threatened families globally (Dulvy *et al*. 2014), threat status ranges from Least Concern to Critically Endangered in southern African waters, especially within the genus *Haploblepharus* Garman 1913. Species identification within *Haploblepharus* has historically been problematic, stemming from the use of unreliable morphological characters in species identification keys (Human 2007*a*). Human (2007*a*) raised the possibility of interspecific hybridisation due to the difficulty in classifying some specimens which shared morphological features of different *Haploblepharus* taxa. In an attempt to increase species identification accuracy in this genus, a revised taxonomic dichotomous key was described for *Haploblepharus* (Human 2007*a*); however, misidentification between *Haploblepharus* species remains widespread (Gledhill *et al*. submitted). Previous studies using morphometric, meristic and genetic data to examine species group clustering have been unable to accurately delineate *Haploblepharus* species (Human 2007*b*; Gledhill *et al*. submitted).

With the first step towards biodiversity monitoring being fast and accurate species identification (Dayrat 2005), a frequent occurrence of species misidentification impedes species-specific conservation and management plans (Abercrombie *et al*. 2005; Bester-van der Merwe and Gledhill 2015; Smart *et al*. 2016). Therefore, the research presented in this thesis aimed to address crucial knowledge gaps on

species delineation in southern African endemic scyliorhinids by developing and applying molecular markers to assess species divergence in a morphologically conserved and threatened genus.

4.2 Overview and discussion of main research findings

Elasmobranchs are a poorly studied group in relation to other vertebrate classes (Naylor *et al*. 2012); possibly owing to their historically low economic value (Walker 1998). However, the pressing need to address critical conservation issues has led to a surge in molecular-based studies on elasmobranchs (Dudgeon *et al*. 2012), especially studies implementing molecular methods as tools for species identification. A previous study was unable to accurately differentiate between three *Haploblepharus* species (*H. edwardsii*, *H. fuscus* and *H. pictus*) based on three mitochondrial gene regions (*COI*, *cytb* and *ND2*) as well as the *ITS2* nuclear gene (Gledhill *et al*. submitted). Accordingly, Chapter 2 investigated the apparent lack of mtDNA sequence divergence evident among *Haploblepharus* species, and is the first study to report a complete mitogenome for a South African endemic catshark, *Poroderma pantherinum*, as well as partial mitogenomes for *H. edwardsii*, *H. pictus* and *Halaelurus natalensis*.

Mitogenome assemblies for *H. edwardsii* and *H. pictus* contained single nucleotide polymorphism sequence variants in various mitochondrial genes, including both synonymous and nonsynonymous variants. Nonsynonymous variants detected in this study were assessed through the comparison of elasmobranch amino acid sequences and all putative amino acids were identified in alternate species, illustrating the viability and functionality of the proteins. In contrast, only one of the two possible mitogenome sequences recovered for each of the *Haploblepharus* species is predicted to be functional, with Haplotype 2 exhibiting a premature stop codon due to a 14 bp frameshift deletion. Since there is no meiotic control in mtDNA replication, defected molecules can increase in frequency stochastically or due to faster replication attributed to a smaller size (Just *et al*. 2015); as could be the case for the truncated *ND4* gene. Following haplotype separation, interspecific sequence divergence was assessed for each protein-coding gene; with interspecific distances being congruent among all genes assessed. Interestingly, divergence estimates between the mitogenome haplotypes recovered from a single *Haploblepharus* specimen met previously proposed species discrimination thresholds (> 3.5% for *ND2*). Therefore, this is the first study to describe the presence of heteroplasmy in elasmobranchs, with evidence hinting at interspecific hybridisation and paternal leakage as possible factors responsible for the phenomenon. Heteroplasmy impedes traditional mtDNA species identification as the presence of multiple mitogenome templates produces ambiguities in the resulting Sanger sequences, thus only providing identification to the genus level.

Maisey (2012) suggested that the phylogeny of Scyliorhinidae be re-evaluated as morphological and molecular studies have indicated that the family, as presently recognised, may be paraphyletic (Chen *et al*. 2016; van Staden *et al*. 2018). Molecular data can provide a powerful tool for distinguishing between closely related species; however, different gene regions exhibit variable mutation rates which can result in discordance between gene trees and species trees (Maddison 1997). In comparison to single gene

approaches, the use of whole mitogenomes can potentially provide phylogenies with a higher resolution, as well as increased precision in divergence estimates (Arnason *et al*. 2008). The topology of the Bayesian tree generated in Chapter 2 was largely congruent with previous phylogenies reported for elasmobranchs using individual mtDNA and/or nuclear genes (Human *et al*. 2006; Naylor *et al*. 2012; Chen *et al*. 2016; van Staden *et al*. 2018). This study is the first to assess and confirm the monophyly of the subfamily Scyliorhininae using representative species from each of the respective genera, while the presence of the subfamily Pentanchinae was only supported by the monophyletic relationship observed among *Halaelurus* and *Haploblepharus*. The phylogeny illustrated the relatively low levels of interspecific genetic divergence between *Haploblepharus* Haplotype 1 and Haplotype 2, supporting the presence of recently diverged mitogenomes (Boomer *et al*. 2012; DiBattista *et al*. 2016). Furthermore, the clustering of co-distributed southern African endemic scyliorhinids with alternate scyliorhinid species displaying distributions endemic to the northern Pacific provides valuable insight into the origin of these species. The observed genetic divergence between *Poroderma pantherinum* and the rest of the study species (*H. natalensis*, *H. edwardsii* and *H. pictus*) suggests the occurrence of two separate colonisation events of the southern African coastline.

Despite conservation concerns, there is a lack of adequate scientific information on chondrichthyan population structure (Dudgeon *et al*. 2012) and few molecular markers are available for the group, especially for southern African endemics (Bester-van der Merwe and Gledhill 2015). The general lack of molecular markers for many elasmobranch species has previously delayed the study of population genetic structure, further impeding conservation action. More recently, high-throughput sequencing technologies have allowed for the efficient recovery of microsatellite loci from non-model organisms, as described in Chapter 3. This study is the first to develop species-specific microsatellite markers for a South African endemic catshark, *H. edwardsii*. Furthermore, two multiplex assays comprising 10 polymorphic microsatellites were successfully constructed and the cross-species utility of these markers assessed in *H. fuscus*, *H. pictus* and a more distantly related scyliorhinid *H. natalensis*. Similar to previous findings reported for shark species (Griffiths *et al*. 2011; Maduna *et al*. 2014; Pirog *et al*. 2015), a high cross-species amplification rate of success (100%) was observed for the three study species; suggesting that these markers may also be useful in future population genetic studies for catsharks.

The application of cross-species amplification enables the study and comparison of population genetic structure of co-distributed species (*e.g.*, Maduna *et al*. 2017); furthermore, these markers can be applied to identify different species in taxonomic disputes and to detect introgression (Khosravi *et al*. 2013; Abdul-Muneer 2014; Giresi *et al*. 2015; Ito *et al*. 2015). Through the application of the newly developed microsatellite markers, Chapter 3 assessed species differentiation; additionally, providing evidence for the suspected hybridisation among *Haploblepharus* species. As reported for other elasmobranchs (Veríssimo *et al*. 2010), the preliminary intraspecific population differentiation

estimates hinted at a positive correlation between population differentiation (F_{ST}) and geographic distance among populations. In this study the null hypothesis of panmixia was rejected for *H. pictus* based on all analyses, with populations being significantly differentiated once distance between sampling locations was in excess of 100km. Very little genetic differentiation was recorded for *H. edwardsii* between the offshore Agulhas Bank and coastal sampling sites; with the apparent lack of genetic differentiation over the ~280km region possibly being attributed to the Agulhas Bank forming a shallow plateau, providing a relatively continuous habitat for *H. edwardsii*. The difference in population genetic differentiation estimates between *H. edwardsii* and *H. pictus* hints at possible differences in dispersal ability and other life-history traits.

The correct species assignment obtained for the more distantly related species *H. natalensis*, based on both Bayesian (STRUCTURE) and multivariate (PCoA and DAPC) analyses, indicated the utility of these markers for species discrimination. In contrast, some of the *Haploblepharus* specimens presented a more complex assignment pattern. The index of admixture in Bayesian analysis has frequently been used to identify introgression (Barilani *et al*. 2006; Sanz *et al*. 2008; Scandura *et al*. 2009; Khosravi *et al*. 2013; Ito *et al*. 2015), and by implementing *qi* thresholds this study was able to accurately identify pure and admixed individuals. While the presence of admixture between *Haploblepharus* taxa was evident, distinct genetic clusters were also present. Approximately 59% of 88 specimens genotyped were unambiguously assigned ($qi > 0.95$) to a distinct genetic cluster that confirmed accurate taxonomic assignment. In closely related species such as *H. edwardsii*, *H. fuscus* and *H. pictus* it is difficult to discern whether the detected admixture is from contemporary hybridisation or a consequence of incomplete lineage sorting due to recent speciation. For recently diverged species, hybridisation appears to be the favoured explanation when interspecific mtDNA sequence divergence meets previously suggested divergence thresholds (van Herwerden *et al*. 2006; Yaakub *et al*. 2006; Marie *et al*. 2007; Montanari *et al*. 2014). Given that rates of mtDNA evolution are slower in elasmobranchs than in other taxa (Martin *et al*. 1992), the ~1.5% sequence divergence between the two *COI* haplotypes present for *H. edwardsii* and *H. pictus* in Chapter 2 suggests that hybridisation may be the more favourable explanation.

4.3 Project limitations and future perspectives

Although not exhaustive, the research presented in this thesis provided valuable insights into the evolutionary relationships, genetic diversity and population connectivity of southern African endemic scyliorhinids. The phylogeny reconstructed in this study supported the placement of scyliorhinids with relatively high posterior probability values, however, the *Halaelurus* and *Haploblepharus* clade was previously reported as unlikely due to contrasting results in the morphological phylogeny. Future research requires the inclusion of genetic data from a representative of *Holohalaelurus* to confidently ascertain interrelationships within the tribe Halaelurini. While this study described the presence of heteroplasmy in two whole mitogenome assemblies, heteroplasmy was also evident in numerous Sanger

sequences. Although heteroplasmy is often a transient state which may resolve to homoplasmy in a few generations (Santos *et al*. 2005, 2008; Irwin *et al*. 2009), future studies should investigate the presence of heteroplasmy in the alternate two *Haploblepharus* species (*H. fuscus* and *H. kistnasamyi*) and record the progression of this phenomenon. If heteroplasmy persists, species identification may be achieved through the accurate and widespread cataloguing of mitogenome haplotypes as some haplotypes, and thus ambiguous sites, may prove to be species-specific. Alternatively, mitogenome haplotypes may become differentially fixed in different populations, providing a completely new challenge to traditional mtDNA species identification.

Sample size and species representation was one of the main limitations in this study. Samples were selected to represent the widest possible geographic range, however, due to opportunistic sampling this did not encompass the entire distribution ranges of the respective species. Future studies should include additional samples, in terms of both an increased number of samples and sampling locations, to further explore the extent of population connectivity in each of the respective study species. Additionally, conventional or acoustic tracking data will aid in elucidating the movement patterns of these species, as the difference in population genetic differentiation estimates between *H. edwardsii* and *H. pictus* may be attributed to possible life-history differences (*e.g*. mobility).

The occurrence of species misidentification between closely related species is the other main limitation in this study, as misidentification is commonly cited as a possible explanation for the lack of genetic differentiation between species (Gledhill *et al*. submitted). While confusion exists between *Haploblepharus* taxa, species identification was performed by trained shark biologists according to taxonomic keys (Human 2007*a*) and genetic data was only generated for unambiguously identified specimens. However, comprehensive analyses of morphometric data are required to ensure accurate species identification. A comparative genomics study of *Haploblepharus* species will aid in elucidating whether the low sequence variation found among species in this study is consistent on a genomic scale. Future research requires an integrative approach, using morphological and meristic data in conjunction with additional diagnostic molecular markers to accurately tease apart the relative contribution of different factors influencing the signatures of admixture observed in this study.

4.4 Final remarks

In summary, the research presented in this thesis allowed the assembly of whole mitogenomes and the development of microsatellite markers; further demonstrating the utility of these genetic resources in generating knowledge on understudied scyliorhinids. This is the first study to describe the presence of heteroplasmy due to suspected hybridisation in elasmobranchs. This phenomenon requires careful consideration in future studies attempting species discrimination among *Haploblepharus* species, and possibly in additional species where heteroplasmy has remained undocumented. This study provides the first genetic insights into southern African endemic scyliorhinid population structure; providing evidence for population fragmentation in *Haploblepharus pictus* which should be accounted for in

future management and conservation efforts. While this study provides molecular evidence for admixture among *Haploblepharus* species, it also highlights the need for integrative research. To provide definitive conclusions, genetic results need to be placed in a broader context by acquiring biological and ecological data.

References

Abbott R, Albach D, Ansell S, Arntzen JW, Baird SJ, Bierne N, Boughman J, Brelsford A, Buerkle CA, Buggs R, Butlin RK. 2013. Hybridisation and speciation. *Journal of Evolutionary Biology* 26: 229-246.

Abdul-Muneer PM, Gopalakrishnan A, Musammilu KK, Mohindra V, Lal KK, Basheer VS, Lakra WS. 2009. Genetic variation and population structure of endemic yellow catfish, *Horabagrus brachysoma* (Bagridae) among three populations of Western Ghat region using RAPD and microsatellite markers. *Molecular Biology Reports* 36: 1779-1791.

Abdul-Muneer. 2014. Application of Microsatellite Markers in Conservation Genetics and Fisheries Management: Recent Advances in Population Structure Analysis and Conservation Strategies. *Genetics Research International* 691759.

Abercrombie DL, Clarke SC, Shivji MS. 2005. Global-scale genetic identification of hammerhead sharks: Application to assessment of the international fin trade and law enforcement. *Conservation Genetics* 6: 775-788.

Allendorf FW, Berry O, Ryman N. 2014. So long to genetic diversity, and thanks for all the fish. *Molecular Ecology* 23: 23-25.

Allendorf FW, England PR, Luikart G, Ritchie PA, Ryman N. 2008. Genetic effects of harvest on wild animal populations. *Trends in Ecology and Evolution* 23: 327-337.

Allendorf FW, Hohenlohe PA, Luikart G. 2010. Genomics and the future of conservation genetics. *Nature Reviews Genetics* 11: 697-709.

Allendorf FW, Leary RF, Spruell P, Wenburg JK. 2001. The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution* 16: 613-622.

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Brouin J, Eperron IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG. 1981. Sequence and organisation of the human mitochondrial genome. *Nature* 290: 457-465.

Anderson S. 1994. Area and endemism. *The Quarterly Review of Biology* 69: 451-471.

Andersson M. 1994. Sexual selection. New Jersey, USA: Princeton University Press.

Arnason E, Rand DM. 1992. Heteroplasmy of short tandem repeats in mitochondrial DNA of Atlantic cod, *Gadus morhua*. *Genetics* 132: 211-220.

Arnason U, Adegoke JA, Gullberg A, Harley EH, Janke A, Kullberg M. 2008. Mitogenomic relationships of placental mammals and molecular estimates of their divergences. *Gene* 421: 37-51.

Arnold ML, Bulger MR, Burke JM, Hempel AL, Williams JH. 1999. Natural hybridisation: How long can you go (and still be important)? *Ecology* 80: 371-381.

Arnold ML, Martin NH. 2010. Hybrid fitness across time and habitats. *Trends in Ecology and Evolution* 25: 530-536.

Arnold ML. 2006. Evolution through genetic exchange. Oxford, UK: Oxford University Press.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. 1987. Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. *Annual Review of Ecology and Systematics* 18: 489-522.

Avise JC. 2000. Phylogeography: The History and Formation of Species. Cambridge, MA: Harvard University Press.

Barbara T, Palma-Silva C, Paggi GM, Bered F, Fay MF, Lexer C. 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. *Molecular Ecology* 16: 3759-3767.

Barilani M, Sfougaris A, Giannakopoulos A, Mucci N, Tabarroni C, Randi E. 2006. Detecting introgressive hybridisation in rock partridge populations (*Alectoris graeca*) in Greece through Bayesian admixture analyses of multilocus genotypes. *Conservation Genetics* 8: 343-354.

Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A. 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439: 9.

Bass AJ, D'Aubrey JD, Kistnasamy N. 1975. Sharks of the East Coast of Southern Africa. II. The Families Scyliorhinidae and Pseudotriakidae. *Investigational Report of the Oceanographic Research Institute* 37: 1-64.

Baum JK, Myers RA, Kehler DG, Worm B, Harley SJ, Doherty PA. 2003. Collapse and Conservation of Shark Populations in the Northwest Atlantic. *Science* 299: 389-392.

Belkin IM. 2009. Rapid warming of Large Marine Ecosystems. *Progress in Oceanography* 81: 207-213.

Bellwood DR, Wainwright PC. 2002. The history and biogeography of fishes on coral reefs. In: Sale PF, (eds), *Coral Reef Fishes: Dynamics and Diversity on a Complex Ecosystem*. New York, NY: Academic Press. pp 5-32.

Benavides MT, Feldheim KA, Duffy CA, Wintner S, Braccini JM, Boomer J, Huveneers C, Rogers P, Mangel JC, Alfaro-Shigueto J, Cartamil DP, Chapman DD. 2011. Phylogeography of the copper shark (*Carcharhinus brachyurus*) in the southern hemisphere: implications for the conservation of a coastal apex predator. *Marine and Freshwater Research* 62: 861-869.

Benestan L, Ferchaud A-L, Honhenlohe P, Garner BA, Naylor GJP, Baums I, Schwartz M, Kelley JL, Luikart G. 2016. Conservation genomics of natural and managed populations: building a conceptual and practical framework. *Molecular Ecology* 25: 2967-2977.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Society: Series B (Statistical Methodology)* 1: 289-300.

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. *Nucleic Acids Research* 41: 36-42.

Bernardi G, Powers DA. 1992. Molecular phylogeny of the prickly shark, *Echinorhinus cookie*, based on a nuclear (18S rRNA) and a mitochondrial (cytochrome b) gene. *Molecular Phylogenetics and Evolution* 1: 161-167.

Bertonili A. 1993. Aspects of the biology of four Southern African catsharks. MSc thesis, University of Cape Town, South Africa.

Bester-van der Merwe AE, Bitalo D, Cuevas JM, Ovenden J, Hernández S, da Silva C, McCord M, Roodt-Wilding R. 2017. Population genetics of Southern Hemisphere tope shark (*Galeorhinus galeus*): Intercontinental divergence and constrained gene flow at different geographical scales. *PLoS ONE* 9: e0184481.

Bester-van der Merwe AE, Gledhill KS. 2015. Molecular species identification and population genetics of chondrichthyans in South Africa: current challenges, priorities and progress. *African Zoology* 50: 205-217.

Bird CE, Holland BS, Bowen BW, Toonen RJ. 2011. Diversification of endemic sympatric limpets (*Cellana* spp.) in the Hawaiian Archipelago. *Molecular Ecology* 20: 2128-2141.

Bitalo DN, Maduna SN, da Silva C, Roodt-Wilding R, Bester-van der Merwe AE. 2015. Differential gene flow patterns for two commercially exploited shark species, tope (*Galeorhinus galeus*) and common smoothhound (*Mustelus mustelus*) along the south-west coast of South Africa. *Fisheries Research* 172: 190-196.

Blamey LK, Shannon LJ, Bolton JJ, Crawford RJM, Dufois F, Evers-King H, Griffiths CL, Hutchings L, Jarre A, Rouault M, Watermeyer KE, Winker H. 2015. Ecosystem change in the southern Benguela and the underlying processes. *Journal of Marine Systems* 144: 9-29.

Blanco M, Perez-Martin RI, Sotelo CG. 2008. Identification of shark species in seafood products by forensically informative nucleotide sequencing (FINS). *Journal of Agricultural and Food Chemistry* 56: 9868-9874.

Blower DC, Corley S, Hereward JP, Riginos CR, Ovenden JR. 2015. Characterisation and cross-amplification of 21 novel microsatellite loci for the dusky shark, *Carcharhinus obscurus*. *Conservation Genetic Resources* 7: 909-912.

Bolton JJ, Anderson RJ, Smit AJ, Rothman MD. 2012. South African kelp moving eastwards?: the discovery of *Ecklonia maxima* (Osbeck) Papenfuss at De Hoop Nature Reserve. *African Journal of Marine Science* 34: 147-151.

Boomer JJ, Harcourt RG, Francis MP, Stow AJ. 2012. Genetic divergence, speciation and biogeography of *Mustelus* (sharks) in the central Indo-Pacific and Australasia. *Molecular Phylogenetics and Evolution* 64: 697-703.

Boomer JJ, Stow AJ. 2010. Rapid isolation of the first set of polymorphic microsatellite loci from the Australian gummy shark, *Mustelus antarticus* and their utility across divergent shark taxa. *Conservation Genetics Resources* 2: 393-395.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32: 314-331.

Bowen BW, Rocha LA, Toonen RJ, Karl SA, Craig MT, DiBattista JD, Eble JA, Gaither MR, Skillings D, Bird CE. 2013. Origins of tropical marine biodiversity. *Trends in Ecology and Evolution* 28: 359-366.

Branch GM, Griffiths CL, Branch MI, Beckley LE. 1994. Two Oceans – A Guide to the Marine Life of Southern Africa. Cape Town, RSA: David Philip.

Briggs JC, Bowen BW. 2012. A realignment of marine biogeographic provinces with particular reference to fish distributions. *Journal of Biogeography* 39: 12-30.

Broughton RE, Dowling TE. 1994. Length variation in mitochondrial DNA of the Minnow *Cyprinella spiloptera*. *Genetics* 138: 179-190.

Brumfield RT. 2010. Speciation genetics of biological invasions with hybridisation. *Molecular Ecology* 19: 5079-5083.

Buckley TR, Cordeiro M, Marshall DC, Simon C. 2006. Differentiating between Hypotheses of Lineage Sorting and Introgression in New Zealand Alpine Cicadas (*Maoricicada* Dugdale). *Systems Biology* 55: 411-425.

Cappetta H. 1987. Chondrichthyes II. Mesozoic and Cenozoic Elasmobranchii. In: Schultze HP, (eds), *Handbook of Paleoichthyology*. Stuttgart, NY: Gustav Fischer Verlag.

Carreras-Carbonell J, Macpherson E, Pascual M. 2007. Utility of pairwise mtDNA genetic distances for predicting cross-species microsatellite amplification and polymorphism success in fishes. *Conservation Genetics* 9: 181-190.

Carroll RL. 1988. Vertebrate Palaeontology and Evolution. New York, NY: WH Freeman and Company. pp 62-83.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540-552.

Castro ALF, Stewart BS, Wilson SG, Hueter RE, Meekan MG, Motta PJ, Bowen BW, Karl SA. 2007. Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). *Molecular Ecology* 16: 5183-5192.

Chabot CL, Nigenda S. 2011. Characterization of 13 microsatellite loci for the tope shark, *Galeorhinus galeus*, discovered with next-generation sequencing and their utility for eastern Pacific smooth-hound sharks (*Mustelus*). *Conservation Genetics Resources* 3: 553-555.

Chapman DD, Babcock EA, Gruber SH, DiBattista JD, Franks BR, Kessel SA, Guttridge T, Pikitch EK, Feldheim KA. 2005. Marine reserve design and evaluation using automated acoustic telemetry: a case-study involving coral reef-associated sharks in the Mesoamerican Caribbean. *Marine Technology Society* 39: 42-55.

Chapuis MP, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* 24: 621-631.

Chen H, Ding W, Shan L, Chen X, Ai W. 2016. Complete mitochondrial genome and the phylogenetic position of the blackspotted catshark *Halaelurus buergeri* (Carcharhiniformes: Scyliorhinidae). *Mitochondrial DNA Part B: Resources* 1: 369-370.

Chen H, Lin L, Chen X, Ai W, Chen S. 2015. Complete mitochondrial genome and phylogenetic position of the Blotchy swell shark *Cephaloscyllium umbratile*. *Mitochondrial DNA Part A* 27: 3045-3047.

Chin A, Kyne PM, Walker TI, McAuley RB. 2010. An integrated risk assessment for climate change: analysing the vulnerability of sharks and rays on Australia's Great Barrier Reef. *Global Change Biology* 16: 1936-1953.

Choat H. 2006. Phylogeography and reef fishes: bringing ecology back into the argument. *Journal of Biogeography* 33: 967-968.

Clarke SC, Magnussen JE, Abercrombie DL, McAllister MK, Shivji MS. 2006. Identification of Shark Species Composition and Proportion in the Hong Kong Shark Fin Market Based on Molecular Genetics and Trade Records. *Conservation Biology* 20: 201-211.

Clarke SC, Milner-Gulland EJ, Bjorndal T. 2007. Social, economic, and regulatory drivers of the shark fin trade. *Marine Resource Economics* 22: 305-327.

Cockcroft AC, van Zyl D, Hutchings L. 2008. Large-scale changes in the spatial distribution of South African West Coast rock lobsters: an overview. *African Journal of Marine Science* 30: 149-159.

Coleman RR, Gaither MR, Kimokeo B, Stanton F, Bowen BW, Toonen RJ. 2014. Large-scale introduction of the Indo-Pacific damselfish *Abudefduf vaigiensis* into Hawaii promotes genetic swamping of the endemic congener *A. abdominalis*. *Molecular Ecology* 23: 5552-5565.

Collins TM, Wimberger PH, Naylor GJP. 1994. Compositional bias, character-state bias, and character-state reconstruction using parsimony. *Systematic Biology* 43: 482-496.

Comeron JM, Kreitman M, Aguadé M. 1999. Natural selection on synonymous sites is correlated with gene length and recombination in *Drosophila*. *Genetics* 151: 239-249.

Compagno LJV, Dando M, Fowler S. 2005. Sharks of the World. Princeton, NJ: Princeton University Press.

Compagno LJV, Ebert DA, Smale MJ. 1989. Guide to the Sharks and Rays of Southern Africa. London, UK: New Holland Publishers.

Compagno LJV. 1988. Sharks of the Order Carcharhiniformes. Caldwell, NJ: Blackburn Press.

Compagno LJV. 1990. Alternative life-history styles of cartilaginous fishes in time and space. *Environmental Biology of Fishes* 28: 33-75.

Compagno LJV. 1999. An overview of chondrichthyan systematics and biodiversity in southern Africa. *Transactions of the Royal Society of South Africa* 54: 75-120.

Corrigan S, Beheregaray LB. 2009. A recent shark radiation: Molecular phylogeny, biogeography and speciation of wobbegong sharks (family: Orectolobidae). *Molecular Phylogenetics and Evolution* 52: 205-216.

Corrigan S, Delser PM, Eddy C, Duffy C, Yang L, Li C, Bazinet AL, Mona S, Naylor GJP. 2017. Historical introgression drives pervasive mitochondrial admixture between two species of pelagic sharks. *Molecular Phylogenetics and Evolution* 110: 122-126.

Corrigan S, Huveneers C, Schwartz TS, Harcourt RG, Beheregaray LB. 2008. Genetic and reproductive evidence for two species of ornate wobbegong shark *Orectolobus* spp. on the Australian east coast. *Journal of Fish Biology* 73: 1662-1675.

Cortés E. 2000. Life History Patterns and Correlations in Sharks. *Reviews in Fisheries Science* 8: 299-344.

Cortés E. 2007. Chondrichthyan demographic modelling: an essay on its use, abuse and future. *Marine and Freshwater Research* 58: 4-6.

Costello C, Ovando D, Hilborn R, Gaines SD, Deschenes O, Lester SE. 2012. Status and solutions for the world's un-assessed fisheries. *Science* 338: 517-520.

Coyne JA, Orr HA. 2004. Speciation. Sunderland, MA: Sinauer Associates, Inc.

Cree LM, Samuels DC, de Sousa Lopes SC, Rajasimha HK, Wonnapinij P. 2008. A reduction of mitochondrial DNA molecules during embryogenesis explains the rapid segregation of genotypes. *Nature Genetics* 40: 249-254.

Cruz VP, Vera M, Mendonça FF, Pardo BG, Martinez P, Oliveira C, Foresti F. 2015. First identification of interspecies hybridisation in the freshwater stingrays *Potamotrygon motoro* and *P. falkneri* (Myliobatiformes, Potamotrygonidae). *Conservation Genetics* 16: 241-245.

Currat M, Ruedi M, Petit RJ, Excoffier L. 2008. The hidden side of invasions: Massive introgression by local genes. *Evolution* 62: 1908-1920.

da Silva C, Booth AJ, Dudley SFJ, Kerwath SE, Lamberth SJ, Leslie RW, McCord ME, Sauer WHH, Zweig T. 2015. The current status and management of South Africa's chondrichthyan fisheries. *African Journal of Marine Science* 37: 233-248.

da Silva C, Kerwath SE, Attwood CG, Thorstad EB, Cowley PD, Økland F, Wilke CG, Nsje TF. 2013. Quantifying the degree of protection afforded by a no-take marine reserve on an exploited shark. *African Journal of Marine Science* 35: 57-66.

da Silva C. 2007. The status and prognosis of the smoothhound shark (*Mustelus mustelus*) fishery in the southeastern and southwestern Cape Coasts. MSc thesis, Rhodes University, South Africa.

Dainty AM. 2002. Biology and ecology of four catshark species in the southwestern cape, South Africa. MSc thesis, University of Cape Town, South Africa.

Darwin CR. 1859. On the Origin of Species by Means of Natural Selection. London, UK: John Murray.

Davidson LNK, Dulvy NK. 2017. Global marine protected areas to prevent extinctions. *Nature Ecology and Evolution* 1: 0040.

Davidson LNK, Krawchuk MA, Dulvy NK. 2016. Why have global shark and ray landings declined: improved management or overfishing? *Fish and Fisheries* 17: 438-458.

Davies KF, Margules CR, Lawrence JF. 2004. A synergistic effect puts rare, specialized species at greater risk of extinction. *Ecology* 85: 265-271.

Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* 85: 407-415.

Degnan JH, Rosenberg NA. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology and Evolution* 24: 332-340.

Delarbre C, Spruyt N, Delmarre C, Gallut C, Barriel V, Janvier P, Laudet V, Gachelin G. 1998. The Complete Nucleotide Sequence of the Mitochondrial DNA of the Dogfish, *Scyliorhinus canicula*. *Genetics* 150: 331-344.

Dempster AP, Laird NM, Rubin DB. 1977. Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society Series B* 39: 1-38.

Díaz-Jaimes P, Bayona-Vásquez NJ, Adams DH, Uribe-Alcocer M. 2016. Complete mitochondrial DNA genome of bonnethead shark, *Sphyrna tiburo*, and phylogenetic relationships among main superorders of modern elasmobranchs. *Meta Gene* 7: 48-55.

DiBattista J, Whitney J, Craig MT, Hobbs J-PA, Rocha LA, Feldheim KA, Berumen ML, Bowen BW. 2016. Surgeons and suture zones: Hybridisation among four surgeonfish species in the Indo-Pacific with variable evolutionary outcomes. *Molecular Phylogenetics and Evolution* 101: 203-215.

Dobzhansky TH. 1937. Genetics and the Origin of Species. New York, NY: Columbia University Press.

Dobzhansky TH. 1970. Genetics of the Evolutionary Process. New York, NY: Columbia University Press.

Doiron S, Bernatchez L, Blier PU. 2002. A comparative mitogenomic analysis of the potential adaptative vlue of artic charr mtDNA introgression in brook charr populations (*Salvelinus fontinalis* Mitcnill). *Molecular Biology and Evolution* 19: 1902-1909.

Dosay-Akbulut M. 2008. The phylogenetic relationship within the genus *Carcharhinus*. *Comptes Rendus Biologies* 331: 500-509

Dudgeon CL, Blower DC, Broderick D, Giles JL, Holmes BJ, Kashiwagi T, Krück NC, Morgan JAT, Tillett BJ, Ovenden JR. 2012. A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *Journal of Fish Biology* 80: 1789-1843.

Dudgeon CL, Ovenden JR. 2015. The relationship between abundance and genetic effective population size in elasmobranchs: an example from the globally threatened zebra shark *Stegostoma fasciatum* within its protected range. *Conservation Genetics* 16: 1443-1454.

Dudley SFJ, Simpfendorfer CA. 2006. Population status of 14 shark species caught in the protective gillnets off KwaZulu-Natal beaches, South Africa, 1978-2003. *Marine and Freshwater Research* 57: 225-240.

Dudu A, Georgescu SE, Berrebi P, Costache M. 2012. Site heteroplasmy in the mitochondrial cytochrome b gene of the starlet sturgeon *Acipenser ruthenus*. *Genetics and Molecular Biology* 35: 886-891.

Dulvy NK, Baum JK, Clarke S, Compagno LJV, Cortés E, Domingo A, Fordham S, Fowler S, Francis MP, Gibson C, Martínez J, Musick JA, Soldo A, Stevens JD, Valenti S. 2008. You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation: Marine and Freshwater Ecosystems* 18: 459-482.

Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LNK, Fordham SV, Francis MP, Pollock CM, Simpendorfer CA, Burgess GH, Carpenter KE, Compagno LJV, Ebert DA, Gibson C, Heupel MR, Livingstone SR, Sanciangco JC, Stevens JD, Valenti S, White WT. 2014. Extinction risk and conservation of the world's sharks and rays. *eLife* 3: e00590.

Dulvy NK, Sadovy Y, Reynolds JD. 2003. Extinction vulnerability in marine populations. *Fish and Fisheries* 4: 25-64.

Dulvy NK, Simpfendorfer CA, Davidson LNK, Fordham SV, Bräutigam A, Sant G, Welch DJ. 2017. Challenges and Priorities in Shark and Ray Conservation. *Current Biology* 27: R565-R572.

Dulvy NK. 2013. Super-sized MPAs and the marginalization of species conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 23: 357-362.

Ebert DA, Compagno LJV, Cowley PD. 2006. Reproductive biology of catsharks (Chondrichthyes: Scyliorhinidae) off the west coast of southern Africa. *ICES Journal of Marine Science* 63: 1053-1065.

Ebert DA, Fowler SL, Compagno LJV. 2013. Sharks of the World: A Fully Illustrated Guide. Plymouth, UK: Wild Nature Press.

Ebert DA, van Hees KE. 2015. Beyond Jaws: rediscovering the 'lost sharks' of southern Africa. *African Journal of Marine Science* 37: 141-156.

Ebert DA. 2003. Sharks, rays, and chimaeras of California. Berkeley, CA: University of California Press.

Edwards AL, Civitello A, Hammond HA, Caskey CT. 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *American Journal of Human Genetics* 49: 746-756.

Eitner BJ. 1995. Systematics of the genus *Alopias* (Lamniformes: Alopiidae) with evidence for the existence of an unrecognized species. *Copeia* 3: 562-571.

Ellegren H. 2004. Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics* 5: 435-445.

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, Lischer HEL. 2010. ARLEQUIN suite version 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564-567.

Feder JL, Egan SP, Nosil P. 2012. The genomics of speciation with gene flow. *Trends in Genetics* 28: 342-350.

Feldheim KA, Clews A, Henningsen A, Todorov L, McDermott C, Meyers M, Bradley J, Pulver A, Anderson E, Marshall A. 2016. Multiple births by a captive swellshark *Cephaloscyllium ventriosum via* facultative parthenogenesis. *Journal of Fish Biology* 90: 1047-1053.

Feldheim KA, Gruber SH, Ashley MV. 2001. Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Molecular Ecology* 10: 295-303.

Ferguson MM, Danzmann RG. 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1553-1563.

Fernandes CRM, Martins CF, Ferreira KM, Del Lama MA. 2012. Gene variation, population differentiation, and sociogenetic structure of nests of *Partamona seridoensis* (Hymenoptera: Apidae, Meliponini). *Biochemical Genetics* 50: 325-335.

Ferretti F, Myers RA, Serena F, Lotze HK. 2008. Loss of Large Predatory Sharks from the Mediterranean Sea. *Conservation Biology* 22: 952-964.

Ferretti F, Worm B, Britten GL, Heithaus MR, Lotze HK. 2010. Patterns and ecosystem consequences of shark declines in the ocean. *Ecology Letters* 13: 1055-1071.

Feutry P, Kyne PM, Pillans RD, Chen X, Naylor GJP, Grewe PM. 2014. Mitogenomics of the Speartooth Shark challenges ten years of control region sequencing. *BMC Evolutionary Biology* 14: 232.

Fields PA, Graham JB, Rosenblatt RH, Somero GN. 1993. Effects of expected global climate change on marine faunas. *Trends in Ecology and Evolution* 8: 361-367.

Flammang BE, Ebert DA, Cailliet GM. 2008. Reproductive biology of deep-sea catsharks (Chondrichthyes: Scyliorhinidae) in the eastern North Pacific. *Environmental Biology of Fishes* 81: 35-49.

Forsman A, Ahnejö J, Caesar S, Karlsson M. 2008. A model of ecological and evolutionary consequences of colour polymorphism. *Ecology* 89: 34-40.

Frisk MG, Miller TJ, Fogarty MJ. 2001. Estimation and analysis of biological parameters in elasmobranch fishes: a comparative life history study. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 969-981.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* 34: 397-423.

Galtier N, Nabholz B, Glemin S, Hurst GDD. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* 18: 4541-4550.

Gardner JPA. 1997. Hybridisation in the sea. In: Baxter J, Southward A, (eds), *Advances in Marine Biology*. New York, NY: Academic Press. pp 1-78.

Giles JL, Riginos C, Naylor GJP, Dharmadi D, Ovenden JR. 2016. Genetic and phenotypic diversity in the wedgefish *Rhynchobatus australiae,* a threatened ray of high value in the shark fin trade. *Marine Ecology Progress Series* 548: 165-180.

Giresi MM, Grubbs RD, Portnoy DS, Driggers WB III, Jones L, Gold JR. 2015. Identification and Distribution of Morphologically Conserved Smoothhound Sharks in the Northern Gulf of Mexico. *Transactions of the American Fisheries Society* 144: 1301-1310.

Giresi MM, Grubbs RD, Portnoy DS, Gold JR. 2013. A Morphological Key to Distinguish Among Smoothhound Sharks (Genus *Mustelus*) in the Gulf of Mexico. *Gulf and Caribbean Fisheries Institute* 65: 143-146.

Gissi C, Jannelli F, Pesole G. 2008. Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. *Heredity* 101: 301-320.

Gledhill KS, van Staden M, Booth DJ, McCord ME, Bester-van der Merwe AE. Are molecular species identification markers able to differentiate among five South African endemic catsharks including the morphologically conserved genus *Haploblepharus*? *Marine Biodiversity*. Initial submission in July 2018.

Griffiths AM, Casane D, McHugh M, Wearmouth VJ, Sims DW, Genner MJ. 2011. Characterisation of polymorphic microsatellite loci in the small-spotted catshark (*Scyliorhinus canicula* L.). *Conservation Genetics Resources* 3: 705-709.

Griffiths CL, Robinson TB, Lange L, Mead A. 2010. Marine Biodiversity in South Africa: An Evaluation of Current Status of Knowledge. *PLoS ONE* 5: e12008.

Griffiths CL, van Stittert L, Best PB, Brown SC, Clark BM, Cook PA, Crawford RJM, David JHM, Davies BR, Griffiths MH, Hutchings K, Jerardino A, Kruger N, Lamberth S, Leslie RW, Melville-Smith R, Tarr R, van der Lingen CD. 2004. Impacts of human activities on marine life in the Benguela: a historical overview. *Oceanography and Marine Biology* 42: 303-392.

Gruber DF, Loew ER, Deheyn DD, Akkaynak D, Gaffney JP, Smith WL, Davis MP, Stern JH, Pieribone VA, Sparks JS. 2016. Biofluorescence in Catsharks (Scyliorhinidae): Fundamental Description and Relevance for Elasmobranch Visual Ecology. *Scientific Reports* 6: 24751.

Guastella LA. 1993. Marine linefish catch statistics: sharks. *Oceanographic Research Institute, Data Report* 93: 1-5.

Guo X, Liu S, Liu Y. 2006. Evidence for recombination of mitochondrial DNA in triploid crucian carp. *Genetics* 172: 1745-1749.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Science of the United States of America* 103: 968-971.

Harley CD, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJ, Thornber CS, Rodriguez LF, Tomanek L, Williams SL. 2006. The impacts of climate change in coastal marine ecosystems. *Ecology Letters* 9: 228-241.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B* 270: 313-321.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. 2004. Identification of Birds through DNA Barcodes. *PLoS Biology* 2: e312.

Heithaus MR, Wirsing AJ, Dill LM. 2012. The ecological importance of intact top-predator populations: a synthesis of 15 years of research in a seagrass ecosystem. *Marine and Freshwater Research* 63: 1039-1050.

Henderson AC, Reeve AJ, Jabado RW, Naylor GJP. 2016. Taxonomic assessment of sharks, rays and guitarfishes (Chondrichthyes: Elasmobranchii) from south-eastern Arabia, using the NADH dehydrogenase subunit 2 (NADH2) gene. *Zoological Journal of the Linnean Society* 176: 399-442.

Hendrix R, Susanne Hauswaldt J, Veith M, Steinfartz S. 2010. Strong correlation between cross-amplification success and genetic distance across all members of "True Salamanders" (Amphibia: Salamandridae) revealed by *Salamandra salamandra*-specific microsatellite loci. *Molecular Ecology Resources* 10: 1038-1047.

Henriques R, von der Heyden S, Matthee CA. 2016. When homoplasmy mimics hybridisation: a case study of Cape hakes (*Merluccius capensis* and *M. paradoxus*). *PeerJ* 4: e1827.

Heupel MR, Knip DM, Simpfendorfer CA, Dulvy NK. 2014. Sizing up the ecological role of sharks as predators. *Marine Ecology Progress Series* 495: 291-298.

Hinojosa-Alvarez S, Walter RP, Diaz-Jaimes P, Galván-Magaña F, Paig-Tran EM. 2016. A potential third Manta Ray species near the Yucatán Peninsula? Evidence for a recently diverged and novel genetic *Manta* group from the Gulf of Mexico. *PeerJ* 4: e2586.

Hobbs JPA, van Herwerden L, Pratchett MS, Allen GR. 2013. Hybridisation among butterflyfishes. In: Pratchett MS, Berumen ML, Kapoor B, (eds), *Biology of Butterflyfishes*. Boca Raton, FL: CRC Press.

Hoelzel A. 2001. Shark fishing in fin soup. *Conservation Genetics* 2: 69-72.

Hoffman JI, Nichols HJ. 2011. A novel approach for mining polymorphic microsatellite markers in silico. *PLoS ONE* 6: e23283.

Holland BR, Benthin S, Lockhart PJ, Moulton V, Huber KT. 2008. Using supernetworks to distinguish hybridisation from lineage-sorting. *BMC Evolutionary Biology* 8: 202.

Holmes BH, Steinke D, Ward RD. 2009. Identification of shark and ray fins using DNA barcoding. *Fisheries Research* 95: 280-288.

Hou WR, Chen Y, Wu X, Hu JC, Peng ZS, Yang J, Tang ZX, Zhou CQ, Li YM, Yang SK, Du YJ, Kong LL, Ren ZL, Zhang HY, Shuai SR. 2006. A complete mitochondrial genome sequence of Asian black bear Sichuan subspecies (*Ursus thibetanus mupinensis*). *International Journal of Biological Science* 3: 85-90.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

Human BA, Compagno LJV. 2006. Description of *Haploblepharus kistnasamyi*, a new catshark (Chondrichthyes: Scyliorhinidae) from South Africa. *Zootaxa* 1318: 41-58.

Human BA, Owen EP, Compagno LJV, Harley EH. 2006. Testing morphologically based phylogenetic theories within the cartilaginous fishes with molecular data, with special reference to the catshark family (Chondrichthyes; Scyliorhinidae) and the interrelationship within them. *Molecular Phylogenetics and Evolution* 39: 384-391.

Human BA. 2003. Taxonomy and Molecular Phylogeny of some southern African Catsharks (Scyliorhinidae; Chondrichthyes). PhD thesis, University of Cape Town, South Africa.

Human BA. 2006. Size-corrected shape variation analysis and quantitative species discrimination in a morphologically conservative catshark genus, *Poroderma* Smith, 1837 (Chondrichthyes: Carcharhinifomres: Scyliorhinidae). *African Natural History* 2: 1-15.

Human BA. 2007*a*. A taxonomic revision of the catshark genus *Haploblepharus* Garman 1913 (Chondrichthyes: Carcharhiniformes: Scyliorhinidae). *Zootaxa* 1451: 1-40.

Human BA. 2007*b*. Size-corrected shape variation analysis and quantitative species discrimination in a morphologically conservative catshark genus, *Haploblepharus* Garman, 1913 (Chondrichthyes: Carcharhiniformes: Scyliorhinidae). *African Natural History* 3: 59-73.

Human BA. 2009*a*. *Haploblepharus kistnasamyi*. The IUCN Red List of Threatened Species 2009: e.T161667A5476391. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161667A5476391.en [accessed on 5 August 2018].

Human BA. 2009*b*. *Haploblepharus edwardsii*. The IUCN Red List of Threatened Species 2009: e.T39345A10211065. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T39345A10211065.en [accessed on 5 August 2018].

Human BA. 2009*c*. *Haploblepharus fuscus*. The IUCN Red List of Threatened Species 2009: e.T39346A10211331. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T39346A10211331.en [accessed on 5 August 2018].

Human BA. 2009*d*. *Haploblepharus pictus*. The IUCN Red List of Threatened Species 2009: e.T161650A5472861. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161650A5472861.en [accessed on 5 August 2018].

Iglésias SP, Toulhoat L, Sellos DY. 2009. Taxonomic confusion and market mislabelling of threatened skates: important consequences for their conservation status. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 319-333.

Inoue JG, Miya M, Lam K, Tay BH, Danks JA, Bell J, Walker TI, Venkatesh B. 2010. Evolutionary origin and phylogeny of the modern holocephalans (Chondrichthyes: Chimaeriformes): a mitogenomic perspective. *Molecular Biology and Evolution* 27: 2576-2586.

Irwin JA, Saunier JL, Niederstätter H, Strouss KM, Sturk KA, Diegoli TM, Brandstätter A, Parson W, Parsons TJ. 2009. Investigation of heteroplasmy in the human mitochondrial DNA control region: A synthesis of observations from more than 5000 global population samples. *Journal of Molecular Evolution* 68: 516-527.

Ito H, Langenhorst T, Ogden R, Inoue-Murayama M. 2015. Population genetic diversity and hybrid detection in captive zebras. *Scientific Reports* 5: 13171.

Iwasaki W, Fukunaga T, Isagozawa R, Yamada K, Maeda Y, Satoh TP, Sado T, Mabuchi K, Takeshima H, Miya M, Nishida M. 2013. MitoFish and MitoAnnotator: a mitochondrial genome database of fish with an accurate and automatic annotation pipeline. *Molecular Biology and Evolution* 30: 2531-2540.

Jabado RW. 2018. The fate of the most threatened order of elasmobranchs: Shark-like batoids (Rhinopristiformes) in the Arabian Sea and adjacent waters. *Fisheries Research*. 204: 448-457.

Jombart T, Devillard S, Balloux F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* 11: 94.

Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403-1405.

Jukes TH, Cantor CR. 1969. Evolution of protein molecules. In: Munro HN, (eds), *Mammalian Protein Metabolism*. New York, NY: Academic Press. pp 21-132.

Just RS, Irwin JA, Parson W. 2015. Mitochondrial DNA heteroplasmy in the emerging field of massively parallel sequencing. *Forensic Science International: Genetics* 18: 131-139.

Karl SA, Castro ALF, Garla RC. 2012. Population genetics of the nurse shark (*Ginglymostoma cirratum*) in the western Atlantic. *Marine Biology* 159: 489-498.

Karl SA, Castro ALF, Lopez JA, Charvet P, Burgess GH. 2011. Phylogeography and conservation of the bull shark (*Carcharhinus leucas*) inferred from mitochondrial and microsatellite DNA. *Conservation Genetics* 12: 371-382.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647-1649.

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA. 2013. diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution* 4: 782-788.

Khosravi R, Rezaei HR, Kaboli M. 2013. Detecting hybridisation between Iranian wild wolf (Canis *lupus pallipes*) and free-ranging domestic dog (*Canis familiaris*) by analysis of microsatellite markers. *Zoological Science* 30: 27-34.

Khrapko K. 2008. Two ways to make an mtDNA bottleneck. *Nature Genetics* 40: 134-135.

Ki J-S, Hwang D-S, Park T-J, Han S-H, Lee J-S. 2010. A comparative analysis of the complete mitochondrial genome of the Eurasian otter *Lutra lutra* (Carnivora; Mustelidae). *Molecular Biology Reports* 37: 1943-1955.

Kimura M. 1980. A simple method of estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111-120.

Kmiec B, Woloszynska M, Janska H. 2006. Heteroplasmy as a common state of mitochondrial genetic information in plants and animals. *Current Genetics* 50: 149-159.

Knowlton N. 1993. Sibling species in the sea. *Annual Review of Ecology and Systematics* 24: 189-216.

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I. 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across *K*. *Molecular Ecology Resources* 15: 1179-1191.

Kousteni V, Kasapidis P, Kotoulas G, Megalofonou P. 2015. Strong population genetic structure and contrasting demographic histories for the small-spotted catshark (*Scyliorhinus canicula*) in the Mediterranean Sea. *Heredity* 114: 333-343.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870-1874.

Ladoukakis ED, Zouros E. 2017. Evolution and inheritance of animal mitochondrial DNA: rules and exceptions. *Journal of Biological Research-Thessaloniki* 24: 2.

Lanfear R, Calcott B, Ho SY, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695-1701.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772-773.

Larsson TA, Tay BH, Sundström G, Fredriksson R, Brenner S, Larhammar D, Venkatesh B. 2009. Neuropeptide Y-family peptides and receptors in the elephant shark, *Callorhinchus milii* confirm gene duplications before the gnathostome radiation. *Genomics* 93: 254-260.

Last P. 2007. The state of chondrichthyan taxonomy and systematics. *Marine and Freshwater Research* 58: 7-9.

Last PR, White WT, Gledhill DC, Hobday AJ, Brown R, Edgar GJ, Pecl G. 2011. Long-term shifts in abundance and distribution of a temperate fish fauna: a response to climate change and fishing practices. *Global Ecology and Biogeography* 20: 58-72.

Law R. 2007. Fisheries-induced evolution: present status and future directions. *Marine Ecology Progress Series* 335: 271-277.

Leduc G, Herbert CT, Blanz T, Martinez P, Schneider R. 2010. Contrasting evolution of sea surface temperature in the Benguela upwelling system under natural and anthropogenic climate forcings. *Geophysical Research Letters* 37: L20705.

Levsen N, Bergero R, Charlesworth D, Wolff K. 2016. Frequent, geographically structured heteroplasmy in the mitochondria of flowering plant, ribwort plantain (*Plantago lanceolata*). *Heredity* 117: 1-7.

Li C, Riethoven JJM, Ma L. 2010. Exon-primed intron-crossing (EPIC) markers for non-model teleost fishes. *BMC Evolutionary Biology* 10: 90.

Li YL, Liu JX. 2018. StructureSelector: A web based software to select and visualise the optimal number of clusters using multiple methods. *Molecular Ecology Resources* 18: 176-177.

Liu L, Yu L, Kubatko L, Pearl DK, Edwards SV. 2009. Coalescent methods for estimating multilocus phylogenetic trees. *Molecular Phylogenetics and Evolution* 53: 320-328.

Lombard AT. 2004. Marine component of the National Spatial Biodiversity Assessment for the development of South Africa's National Biodiversity Strategic Plan of Action. National Botanical Institute. pp 101.

López JA, Ryburn JA, Fedrigo O, Naylor GJP. 2006. Phylogeny of sharks of the family Triakidae (Carcharhiniformes) and its implications for the evolution of carcharhiniform placental viviparity. *Molecular Phylogenetics and Evolution* 64: 697-703.

Louis M, Viricel A, Lucas T, Peltier H, Alfonsi E, Berrow S, Brownlow A, Covelo P, Dabin W, Deaville R, de Stephanis R, Gally F, Gauffier P, Penrose R, Silva MA, Guinet C, Bouhet BS. 2014. Habitat-driven population structure of bottlenose dolphins, *Tursiops truncatus*, in the North-East Atlantic. *Molecular Ecology* 23: 857-874.

Lowry DB. 2012. Ecotypes and the controversy over stages in the formation of new species. *Biological Journal of the Linnean Society* 106: 241-257.

Lu X, Xu H, Li Z, Shang H, Adams RP, Mao K. 2014. Genetic diversity and conservation implications of four *Cupressus* species in China as revealed by microsatellite markers. *Biochemical Genetics* 52: 181-202.

Maddison W. 1997. Gene trees in species trees. *Systems Biology* 46: 523-536.

Maduna SN, da Silva C, Wintner SP, Roodt-Wilding R, Bester-van der Merwe AE. 2016. When two oceans meet: regional population genetics of an exploited coastal shark, *Mustelus mustelus*. *Marine Ecology Progress Series* 544: 183-196.

Maduna SN, Rossouw C, da Silva C, Soekoe M, Bester-van der Merwe AE. 2017. Species identification and comparative population genetics of four coastal houndsharks based on novel NGS-mined microsatellites. *Ecology and Evolution* 7: 1462-1486.

Maduna SN, Rossouw C, Roodt-Wilding R, Bester-van der Merwe AE. 2014. Microsatellite cross-species amplification and utility in southern African elasmobranchs: a valuable resource for fisheries management and conservation. *BMC Research Notes* 7: 352.

Maduna SN, Rossouw C, Slabbert R, Wintner SP, da Silva C, Bester-van der Merwe AE. 2018. New polymorphic microsatellite loci revealed for the dusky shark *Carcharhinus obscurus* through Ion Proton double-digest RAD sequencing. *Molecular Biology Reports* 1-5.

Magoulas A, Zouros E. 1993. Restriction-site heteroplasmy in Anchovy (*Engraulis encrasicolus*) indicates incidental biparental inheritance of mitochondrial DNA. *Molecular Biology and Evolution* 10: 319-325.

Maisey JG. 2012. What is an 'elasmobranch'? The impact of palaeontology in understanding elasmobranch phylogeny and evolution. *Journal of Fish Biology* 80: 918-951.

Mank JE, Carlson JE, Brittingham MC. 2004. A century of hybridisation: decreasing genetic distance between American Black Ducks and Mallards. *Conservation Genetics* 5: 395-403.

Marie AD, van Herwerden L, Choat JH, Hobbs J-PA. 2007. Hybridisation of reef fishes at the Indo-Pacific biogeographic barrier: a case study. *Coral Reefs* 26: 841-850.

Marino IAM, Riginella E, Cariani A, Tinti F, Farrell ED, Mazzoldi C, Zane L. 2014. New Molecular Tools for the Identification of 2 Endangered Smooth-Hound Sharks, *Mustelus mustelus* and *Mustelus punctulatus*. *Journal of Heredity* 106: 123-130.

Marino IAM, Finotto L, Colloca F, Di Lorenzo M, Gristina M, Farrell ED, Zane L, Mazzoldi C. 2017. Resolving the ambiguities in the identification of two smooth-hound sharks (*Mustelus mustelus* and *Mustelus punctulatus*) using genetics and morphology. *Marine Biodiversity* 1-12.

Marino IAM, Riginella E, Gristina M, Rasotto MB, Zane L, Mazzoldi C. 2015. Multiple paternity and hybridisation in two smooth-hound sharks. *Scientific Reports* 5: 12919.

Martin AP, Naylor GJP, Palumbi SR. 1992. Rates of mitochondrial evolution in sharks are slow compared with mammals. *Nature* 357: 153-155.

Martin AP, Pardini AT, Noble LR, Jones CS. 2002. Conservation of a dinucleotide simple sequence repeat locus in sharks. *Molecular Phylogenetics and Evolution* 23: 205-213.

Mayr E. 1942. Systematics and the Origin of Species. New York, NY: Columbia University Press.

Mayr E. 1954. Geographic speciation in tropical echinoids. *Evolution* 8: 1-18.

Mayr E. 2001. Wu's genic view of speciation. *Journal of Evolutionary Biology* 14: 866-867.

McClenachan L, Cooper AB, Carpenter KE, Dulvy NK. 2012. Extinction risk and bottlenecks in the conservation of charismatic marine species. *Conservation Letters* 5: 73-80.

McMahon B, Teeling E, Höglund J. 2014. How and why should we implement genomics into conservation? *Evolutionary Applications* 7: 999-1007.

Montanari SR, Hobbs J-PA, Pratchett MS, Bay LK, van Herwerden L. 2014. Does genetic distance between parental species influence outcomes of hybridisation among coral reef butterflyfishes? *Molecular Ecology* 23: 2757-2770.

Moore ABM, White WT, Ward RD, Naylor GJP, Peirce R. 2011. Rediscovery and redescription of the smoothtooth blacktip shark, *Carcharhinus leiodon* (Carcharhinidae), from Kuwait, with notes on its possible conservation status. *Marine and Freshwater Research* 62: 528-539.

Moore WS. 1995. Inferring phylogenies from mtDNA variation: Mitochondrial gene trees versus nuclear-gene trees. *Evolution* 49: 718-126.

Morgan JAT, Harry AV, Welch DJ, Street R, White J, Geraghty PT, Macbeth WG, Tobin A, Simpfendorfer CA, Ovenden JR. 2012. Detection of interspecies hybridisation in Chondrichthyes: hybrids and hybrid offspring between Australian (*Carcharhinus tilstoni*) and common (*C. limbatus*) blacktip shark found in an Australian fishery. *Conservation Genetics* 13: 455-463.

Morin PA, Archer FI, Foote AD, Vilstrup J, Allen EE, Wade P, Durban J, Parsons K, Pitman R, Li L, Bouffard P, Abel Nielsen SC, Rasmussen M, Willerslev E, Gilbert MT, Harkins T. 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Research* 20: 908-916.

Moritz C, Cicero C. 2004. DNA barcoding: promise and pitfalls. *PLoS Biology* 2: 1529-1531.

Moritz C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology* 3: 401-411.

Munday PL, van Herwerden L, Dudgeon CL. 2004. Evidence for sympatric speciation by host shift in the sea. *Current Biology* 14: 1498-1504.

Musick JA, Harbin MM, Compagno LJV. 2004. Historical zoogeography of the Selachii. In: Carrier JC, Musick JA, Heithaus MR, (eds), *Biology of Sharks and Their Relatives*. Boca Raton, FL: CRC Press. pp 33-78.

Myers RA, Worm B. 2003. Rapid worldwide depletion of predatory fish communities. *Nature* 423: 280-283.

Myers RA, Worm B. 2005. Extinction, survival or recovery of large predatory fishes. *Philosophical Transactions of the Royal Society B* 360: 13-20.

Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA. 2013. Genotyping by sequencing in ecological and conservation genomics. *Molecular Ecology* 22: 2841-2847.

Naylor GJP, Martin AP, Mattison EG, Brown WM. 1997. Interrelationships of Lamniform sharks: testing phylogenetic hypotheses with sequence data. In: Kocher TD, Stepien CA, (eds), *Molecular Systematics of Fishes*. San Diego, CA: Academic Press.

Naylor GJP, Caira JN, Jensen K, Rosana KAM, White WT, Last PR. 2012. A DNA sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History* 367: 1-262.

Nosil P. 2012. Ecological Speciation. London, UK: Oxford University Press.

Nurk S, Bankevich A, Antipov D, Gurevich A, Korobeynikov A, Lapidus A, Prjibelsky A, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, McLean J, Lasken R, Clingenpeel SR, Woyke T, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling Genomes and Mini-metagenomes from Highly Chimeric Reads. *Research in Computational Molecular Biology* 7821: 158-170.

Ouborg NJ, Angeloni F, Vergeer P. 2010. An essay on the necessity and feasibility of conservation genomics. *Conservation Genetics* 11: 643-653.

Ovenden JR, Morgan J, Street R, Tobin A, Simpfendorfer CA, Macbeth W, Welch D. 2011. Negligible evidence for regional genetic population structure for two shark species (*Rhizoprionodon acutus*, Rüppell, 1837 and *Sphyrna lewini*, Griffith & Smith, 1834) with contrasting biology. *Marine Biology* 158: 1497-1509.

Ovenden JR. 2013. Crinkles in connectivity: combining genetics and other types of data to estimate movement and interbreeding between populations. *Marine and Freshwater Research* 64: 201-207.

Palkovacs EP. 2011. The overfishing debate: an eco-evolutionary perspective. *Trends in Ecology and Evolution* 26: 616-617.

Palumbi SR. 1989. Rates of molecular evolution and the proportion of nucleotide positions free to vary. *Journal of Molecular Evolution* 29: 180-187.

Palumbi SR. 1992. Marine speciation on a small planet. *Trends in Ecology and Evolution* 7: 114-118.

Palumbi SR. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annual Reviews Ecological Systems* 25: 547-572.

Pank M, Shivji MS, Stanhope M, Natanson L, Kohler N. 2001. Rapid and simultaneous identification of body parts from the morphologically similar sharks *Carcharhinus obscurus* and *Carcharhinus plumbeus* (Carcharhinidae) using multiplex PCR. *Marine Biotechnology* 3: 231-240.

Pardo-Diaz C, Salazar C, Baxter SW, Merot C, Figueiredo-Ready W, Joron M, McMillan WO, Jiggins CD. 2012. Adaptive introgression across species boundaries in *Heliconius* butterflies. *PLoS Genetics* 8: e002752.

Park S. 2001. The Excel microsatellite toolkit. Retrieved from http://animalgenomics.ucd.ie/sdepark/ms-toolkit (downloaded on 21 March 2018).

Patton JL, Smith MF. 1994. Paraphyly, polyphyly, and the nature of species boundaries in pocket gophers (genus *Thomomys*). *Systematic Biology* 43: 11-26.

Pavan-Kumar A, Gireesh-Babu P, Suresh Babu PP, Jaiswar AK, Hari Frishna V, Pani Prasasd K, Chaudhari A, Raje SG, Chakraborty SK, Krishna G, Lakra WS. 2014. Molecular phylogeny of elasmobranchs inferred from mitochondrial and nuclear markers. *Molecular Biology Reports* 41: 447-457.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 28: 2537-2539.

Pesole G, Gissi C, Chirrico AD, Saccone C. 1999. Nucleotide substitution rate of mammalian mitochondrial genomes. *Journal of Molecular Evolution* 48: 427-434.

Pirog A, Blaison A, Jaquemet S, Soria M, Magalon H. 2015. Isolation and characterization of 20 microsatellite markers from *Carcharhinus leucas* (bull shark) and cross-species amplification in *Galeocerdo cuvier* (tiger shark), *Carcharhinus obscurus* (dusky shark) and *Carcharhinus plumbeus* (sandbar shark). *Conservation Genetics Resources* 7: 121-124.

Pitcher GC, Calder DA. 2000. Harmful algal blooms of the southern Benguela Current: a review and appraisal of monitoring from 1989 to 1997. *South African Journal of Marine Science* 22: 255-271.

Pitcher GC, Probyn TA. 2011. Anoxia in southern Benguela during the autumn of 2009 and its linkage to a bloom of the dinoflagellate *Ceratium balechii*. *Harmful Algae* 11: 23-32.

Poliodoro BA, Livingstone SR, Carpenter KE, Hutchinson B, Mast RB, Pilcher NJ, Sadovy de Mitcheson Y, Valenti SV. 2008. Status of the world's marine species. In: Vié J-C, Hilton-Taylor C, Stuart SN, (eds), *Wildlife in a changing world: an analysis of the 2008 IUCN Red List of threatened species*. Gland, Switzerland: International Union for Conservation of Nature. pp 55-65.

Poorvliet M, Hoarau G. 2013. The complete mitochondrial genome of the spinetail devilray, *Mobula japonica*. *Mitochondrial DNA* 24: 28-30.

Pradervand P. 1999. National marine linefish system: recorded catches of cartilaginous fishes in the KwaZulu-Natal recreational line fishery (1995-1998). *Oceanographic Research Institute, Data Report* 99: 1-4.

Price KA, O'Bryhim JR, Jones KL, Lance SL. 2015. Development of polymorphic microsatellite markers for the bonnethead shark, *Sphyrna tiburo*. *Conservation Genetics Resources* 7: 69-71.

Primmer CR, Painter JN, Koskinen MT, Palo JU, Merilä J. 2005. Factors affecting avian cross-species microsatellite amplification. *Journal of Avian Biology* 36: 348-360.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Pritchard JK, Wen W, Falush D. 2010. Documentation for Structure Software: Version 2.3. University of Chicago, Chicago, Illinois, USA.

Puechmaille SJ, Ar Gouilh M, Piyapan P, Yokubol M, Mie Mie K, Bates PJ, Satasook C, New T, Si Hla Bu S, Mackie IJ, Petit EJ, Teeling EC. 2011. The evolution of sensory divergence in the context of limited gene flow in the bumblebee bat. *Nature Communications* 2: 573.

Puechmaille SJ. 2016. The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* 16: 608-627.

Qin J, Zhang Y, Zhou X, Kong X, Wei S, Ward RD, Zhang A. 2015. Mitochondrial phylogenomics and genetic relationships pf closely related pine moth (Lasiocampidae: Dendrolimus) species in China, using whole mitochondrial genomes. *BMC Genomics* 16: 428-439.

R Development Core Team. 2015. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/ (downloaded on 26 April 2017).

Rambaut A. 2016. FigTree (Version 1.4.3). Retrieved from http://tree.bio.ed.ac.uk/software/figtree (downloaded on 15 May 2018).

Randi E. 2008. Detecting hybridisation between wild species and their domesticated relatives. *Molecular Ecology* 17: 285-293.

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

Ravi V, Lam K, Tay BH, Tay A, Brenner S, Venkatesh B. 2009. Elephant shark (*Callorhinchus milii*) provides insights into the evolution of Hox gene clusters in gnathostomes. *Proceedings of the National Academy of Sciences of the United States of America* 106: 16327-16332.

Rheindt FE, Edwards SV. 2011. Genetic introgression: An integral but neglected component of speciation in birds. *The Auk* 128: 620-632.

Rhymer JM, Simberloff D. 1996. Extinction by hybridisation and introgression. *Annual Review of Ecology and Systematics* 27: 83-109.

Richards VP, Henning M, Witzell W, Shivji MS. 2009. Species delineation and evolutionary history of the globally distributed spotted eagle ray (*Aetobatus narinari*). *Journal of Heredity* 100: 273-283.

Richardson AJ, Poloczanska ES. 2008. Ocean science: Under-Resourced, Under Threat. *Science* 320: 1294-1295.

Richly E, Leister D. 2004. NUMTs in Sequenced Eukaryotic Genomes. *Molecular Biology and Evolution* 21: 1081-1084.

Rocha LA, Bowen BW. 2008. Speciation in coral reef fishes. *Journal of Fish Biology* 72: 1101-1121.

Rogers SI, Ellis JR. 2000. Changes in the demersal fish assemblages of British coastal waters during the 20th century. *ICES Journal of Marine Science* 57: 866-881.

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

Rubinoff D. 2006. Utility of mitochondrial DNA barcodes in species conservation. *Conservation Biology* 20: 1026-1033.

Ruck CL, Marra N, Shivji MS, Stanhope MJ. 2017. The complete mitochondrial genome of the endangered great hammerhead shark, *Sphyrna mokarran*. *Mitochondrial DNA Part B: Resources* 2: 246-248.

Ruxton GD, Sherratt TN, Speed M. 2004. Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry. Oxford, UK: Oxford University Press.

Saccone CC, DeCarla G, Gissi C, Pesole G, Reynes A. 1999. Evolutionary genomics in the Metazoa: the mitochondrial DNA as a model system. *Gene* 238: 195-210.

Saccone CC, Lanavé C, Pesole G, Preparata G. 1990. Influence of base composition on quantitative estimates of gene evolution. *Methods in Enzymology* 183: 570-583.

Saiki RK, Stephen S, Fallona F, Mullis KB, Horn GT, Henry AE, Arnheim N. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230: 1350-1354.

Sambrook J, Russell DW. 2001. Molecular cloning: a laboratory manual. New York, NY: Cold Spring Harbor Laboratory Press.

Sang T, Zhong Y. 2000. Testing Hybridisation Hypotheses Based on Incongruent Gene Trees. *Systems Biology* 49: 422-434.

Santos C, Montiel R, Sierra B, Bettencourt C, Fernandez E, Alvarez L, Lima M, Abade A, Aluja MP. 2005. Understanding differences between phylogenetic and pedigree-derived mtDNA mutation rate: A model using families from the Azores Islands (Portugal). *Molecular Biology and Evolution* 22: 1490-1505.

Santos C, Sierra B, Álvarez L, Ramos A, Fernández E, Nogués R, Aluja MP. 2008. Frequency and pattern of heteroplasmy in the control region of human mitochondrial DNA. *Journal of Molecular Evolution* 67: 191-200.

Sanz N, Araguas RM, Fernández R, Vera M, García-Marín JL. 2008. Efficiency of markers and methods for detecting hybrids and introgression in stocked populations. *Conservation Genetics* 10: 225-236.

Scandura M, Iacolina L, Apollonio M, Dessì-Fulgheri F, Baratti M. 2009. Current status of the Sardinian partridge (*Alectoris barbara*) assessed by molecular markers. *European Journal of Wildlife Research* 56: 33-42.

Schwartz MK, McKelvey KS. 2009. Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. *Conservation Genetics* 10: 441-452.

Sederoff RR. 1984. Structural variation in mitochondrial DNA. *Advances in Genetics* 22: 1-108.

Seehausen O, Terai Y, Magalhaes IS, Carleton KL, Mrosso HDJ, Miyagi R, van der Sluijs I, Schneider MV, Maan ME, Tachida H, Imai H, Okada N. 2008. Speciation through sensory drive in cichlid fish. *Nature* 455: 2.

Seehausen O, van Alphen JJM. 1999. Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria. *Ecology Letters* 2: 262-271.

Seehausen O. 2004. Hybridisation and adaptive radiation. *Trends in Ecology and Evolution* 19: 198-207.

Seehausen O. 2006. African cichlid fish: a model system in adaptive radiation research. *Proceeding of the Royal Society of London B: Biological Sciences* 273: 1987-1998.

Shepherd TD, Myers RA. 2005. Direct and indirect fishery effects on small coastal elasmobranchs in the northern Gulf of Mexico. *Ecology Letters* 8: 1095-1104.

Shigenbou Y, Saitoh K, Hayashizaki K, Hitoshi I. 2005. Nonsynonymous site heteroplasmy in fish mitochondrial DNA. *Genes and Genetic Systems* 80: 297-301.

Shivji M, Clarke SC, Pank M, Natanson L, Kohler N, Stanhope M. 2002. Genetic identification of pelagic shark body parts for conservation and trade monitoring. *Conservation Biology* 16: 1036-1047.

Shoemaker JS, Fitch WM. 1989. Evidence from nuclear sequences that invariable sites should be considered when sequence divergence is calculated. *Molecular Biology and Evolution* 6: 270-289.

Sidow A, Wilson AC. 1990. Compositional statistics: An improvement of evolutionary parsimony and its application to deep branches in the tree of life. *Journal of Molecular Evolution* 31: 51-68.

Simpfendorfer CA, Dulvy NK. 2017. Bright spots of sustainable shark fishing. *Current Biology* 27: R97-R98.

Smart JJ, Chin A, Baje L, Green ME, Appleyard SA, Tobin AJ, Simpfendorfer CA, White WT. 2016. Effects of Including Misidentified Sharks in Life History Analyses: A Case Study in the Grey Reef Shark *Carcharhinus amblyrhynchos* from Papua New Guinea. *PLoS ONE* 11: e0153116.

Smith PJ. 1986. Low genetic variation in sharks (Chondrichthyes). *Copeia* 1986: 202-207.

Soekoe M. 2016. Adaptations in allopatric populations of *Triakis megalopterus* isolated by the Benguela Current. Steps towards understanding evolutionary processes affecting regional biodiversity. PhD thesis, Rhodes University, South Africa.

Springer S. 1979. A revision of the catsharks, Family Scyliorhinidae. *NOAA Technical Report NMFS Circular* 422: 1-152.

Stapley J, Reger J, Feulner PGD, Smadja C, Galindo J, Ekblom R, Bennison C, Ball AD, Beckerman AP, Slate J. 2010. Adaptation genomics: the next generation. *Trends in Ecology and Evolution* 25: 705-712.

Steel M. 1994. Recovering a tree from the leaf colourations it generates under a Markov model. *Applied Mathematics Letters* 7: 19-23.

Stein RW, Mull CG, Kuhn TS, Aschliman NC, Davidson LNK, Joy JB, Smith GJ, Dulvy NK, Mooers AO. 2018. Global priorities for conserving the evolutionary history of sharks, rays and chimaeras. *Nature Ecology and Evolution* 2: 288-298.

Steinke D, Connell AD, Hebert PDN. 2016. Linking adults and immatures of South African marine fishes. *Genome* 59: 959-967.

Stelbrink B, von Rintelen T, Cliff G, Kriwet J. 2009. Molecular systematics and global phylogeography of angel sharks (genus *Squatina*). *Molecular Phylogenetics and Evolution* 54: 395-404.

Stephen VC, Hockey PAR. 2007. Evidence for an increasing incidence and severity of Harmful Algal Blooms in the southern Benguela region. *South African Journal of Science* 103: 223-231.

Stevens JD, Bonfil R, Dulvy NK, Walker PA. 2000. The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Sciences* 57: 476-494.

Storz JF. 2002. Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analysis of clinal variation. *Molecular Ecology* 11: 2537-2551.

Straube N, White WT, Ho H-C, Rochel E, Corrigan S, Li C, Naylor GJP. 2013. A DNA sequence-based identification checklist for Taiwanese chondrichthyans. *Zootaxa* 3752: 256-278.

Sullivan J, Holsinger KE, Simon C. 1995. Among site rate variation and phylogenetic analysis of 12S rRNA data in sigmodontine rodents. *Molecular Biology and Evolution* 12: 988-1001.

Sullivan J, Holsinger KE, Simon C. 1996. The effect of topology on estimates of among site rate variation. *Journal of Molecular Evolution* 42: 308-312.

Supungul P, Sootanan P, Klinbunga S, Kamonrat W, Jarayabhand P, Tassanakajon A. 2000. Microsatellite polymorphism and the population structure of the black tiger shrimp (*Penaeus monodon*) in ailand. *Marine Biotechnology* 2: 339-347.

Tautz D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* 17: 6463-6471.

Teletchea F. 2010. After 7 years and 1000 citations: Comparative assessment of the DNA barcoding and the DNA taxonomy proposals for taxonomists and non-taxonomists. *Mitochondrial DNA* 21: 206-226.

Teske PR, McQuaid CD, Froneman PW, Barker NP. 2006. Impacts of marine biogeographic boundaries on phylogeographic patterns of three South African estuarine crustaceans. *Marine Ecology Progress Series* 314: 283-293.

Teske PR, Papdopoulos I, Zardi GI, McQuaid CD, Edkins MT, Griffiths CL, Barker NP. 2007. Implication of life history for genetic structure and migration rates of five southern African coastal invertebrates: Planktonic, abbreviated and direct development. *Marine Biology* 152: 697-711.

Teske PR, von der Heyden S, McQuaid C, Barker NP. 2011. A review of marine phylogeography in southern Africa. *South African Journal of Science* 107: 1-11.

van der Lingen CD, Coetzee JC, Hutchings L. 2002. Temporal shifts in the spatial distribution of anchovy spawners and their eggs in the Southern Benguela: implications for recruitments. *Global Ocean Ecosystems Dynamics* 16: 46-48.

van Herwerden L, Choat JH, Dudgeon CL, Carlos G, Newman SJ, Frisch A, van Oppen M. 2006. Contrasting patterns of genetic structure in two species of the coral trout *Plectropomous* (Serranidae) from east and west Australia: Introgressive hybridisation or ancestral polymorphism. *Molecular Phylogenetics and Evolution* 41: 420-435.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in the microsatellite data. *Molecular Ecology Notes* 4: 535-538.

van Staden M, Gledhill KS, Rhode C, Bester-van der Merwe AE. 2018. The complete mitochondrial genome and phylogenetic position of the leopard catshark, *Poroderma pantherinum*. *Mitochondrial DNA Part B: Resources* 3: 750-752.

Velez-Zuazo X, Argarsson I. 2011. Shark tales: a molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). *Molecular Phylogenetics and Evolution* 58: 207-217.

Veríssimo A, McDowell JR, Graves JE. 2010. Global population structure of the spiny dogfish *Squalus acanthias*, a temperate shark with an antitropical distribution. *Molecular Ecology* 19: 1651-1662.

Veríssimo A, Sampaio Í, McDowell, JR, Alexandrino P, Mucientes G, Queiroz, N, da Silva C, Jones CS, Noble LR. 2017. World without borders - genetic population structure of a highly migratory marine predator, the blue shark (*Prionace glauca*). *Ecology and Evolution* 7: 4768-4781.

Via S. 2001. Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology and Evolution* 16: 381-390.

Vignaud T, Clua E, Mourier J, Maynard J, Planes S. 2013. Microsatellite analyses of blacktip reef sharks (*Carcharhinus melanopterus*) in a fragmented environment show structured clusters. *PLoS ONE* 8: e61067.

Volmer SV, Palumbi SR. 2002. Hybridisation and evolution of reef coral diversity. *Science* 296: 2023-2025.

Von Bonde C. 1945. The External Development of the Banded Dogfish or Pofadderhaai *Haploblepharus edwardsii* (M. & H.). *The Biological Bulletin* 88: 1-11.

Walker TI. 1998. Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. *Marine and Freshwater Research* 49: 553-572.

Walsh MR, Munch SB, Chiba S, Conover DO. 2006. Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. *Ecology Letters* 9: 142-148.

Walter RP, Roy D, Hussey NE, Stelbrink B, Kovacs KM, Lydersen C, McMeans BC, Svavarsson J, Kessel ST, Porsmoguer SB, Wildes S, Tribuzio CA, Campana SE, Peterson SD, Grubbs RD, Heath DD, Hedges KJ, Fisk AT. 2017. Origins of the Greenland shark (*Sominosus microcephalus*): Impacts of ice-olation and introgression. *Ecology and Evolution* 7: 8113-8125.

Ward RD, Hanner R, Hebert PD. 2009. The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology* 74: 329-356.

Ward RD, Holmes BH, White WT, Last PR. 2008. DNA barcoding Australasian chondrichthyans: results and potential uses in conservation. *Marine and Freshwater Research* 59: 57-71.

Ward RD, Holmes BH. 2007. An analysis of nucleotide and amino acid variability in the barcode region of cytochrome c oxidase I (coxI) in fishes. *Molecular Ecology Notes* 7: 899-907.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B* 360: 1847-1857.

Weigmann S. 2016. Annotated checklist of the living sharks, batoids, and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. *Journal of Fish Biology* 88: 837-1037.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.

White DJ, Wolff JN, Pierson M, Gemmell NJ. 2008. Revealing the hidden complexities of mtDNA inheritance. *Molecular Ecology* 17: 4925-4942.

White WT, Last PR. 2012. A review of the taxonomy of chondrichthyan fishes: a modern perspective. *Journal of Fish Biology* 80: 901-917.

Whitfield AK, James NC, Lamberth SJ, Adams JB, Perissinotto R, Rajkaran A, Bornman TG. 2016. The role of pioneers as indicators of biogeographic range expansion caused by global change in southern African coastal waters. *Estuarine, Coastal and Shelf Science* 172: 138-153.

Whitney NM, Robbins WD, Schultz JK, Bowen BW, Holland KN. 2012. Oceanic dispersal in a sedentary reef shark (*Triaenodon obesus*): genetic evidence for extensive connectivity without a pelagic larval stage. *Journal of Biogeography* 39: 1144-1156.

Worm B, Davis B, Kettemer L, Ward-Paige CA, Chapman D, Heithaus MR, Kessel ST, Gruber SH. 2013. Global catches, exploitation rates, and rebuilding options for sharks. *Marine Policy* 40: 194-204.

Wright S. 1943. Isolation by distance. *Genetics* 28: 139-156.

Yaakub SM, Bellwood DR, van Herwerden L, Walsh FM. 2006. Hybridisation in coral reef fishes: introgression and bi-directional gene exchange in *Thalassoma* (family Labridae). *Molecular Phylogenetics and Evolution* 40: 84-100.

Yang L, Matthes-Rosana KA, Naylor GJP. 2014. Complete mitochondrial genome of the blacknose shark *Carcharhinus acronotus* (Elasmobranchii: Carcharhinidae). *Mitochondrial DNA* 27: 169-170.

Yang Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution* 39: 306-314.

Yang Z. 1996. Among site rate variation and its impact on phylogenetic analyses. *Trends in Ecology and Evolution* 11: 367-372.

You FM, Huo N, Gu YQ, Luo M, Ma Y, Hane D, Lazo GR, Dvorak J, Anderson OD. 2008. BatchPrimer3: a high throughput web application for PCR and sequencing primer design. *BMC Bioinformatics* 9: 253.

Zardoya R, Vollmer DM, Craddock C, Streelman JT, Karl S, Meyer A. 1996. Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). *Proceedings of the Royal Society B: Biological Sciences* 263: 1589-1598.

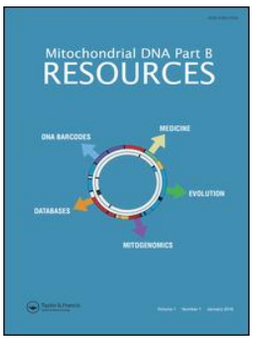
Zemlak TS, Ward RD, Connell AD, Holmes BH, and Hebert PDN. 2009. DNA barcoding reveals overlooked marine fishes. *Molecular Ecology Resources* 9: 237-242.

Zhang DX, Hewitt GM. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution* 11: 247-251.

Appendix A

Published Papers

van Staden M, Gledhill KS, Rhode C, Bester-van der Merwe AE. 2018. The complete mitochondrial genome and phylogenetic position of the leopard catshark, *Poroderma pantherinum*. *Mitochondrial DNA Part B: Resources* 3: 750-752.



Taylor & Francis
Taylor & Francis Group

Mitochondrial DNA Part B

Resources

ISSN: (Print) 2380-2359 (Online) Journal homepage: http://www.tandfonline.com/loi/tmdn20

The complete mitochondrial genome and phylogenetic position of the leopard catshark, *Poroderma pantherinum*

Michaela van Staden, Katie S. Gledhill, Clint Rhode & Aletta E. Bester-van der Merwe

To cite this article: Michaela van Staden, Katie S. Gledhill, Clint Rhode & Aletta E. Bester-van der Merwe (2018) The complete mitochondrial genome and phylogenetic position of the leopard catshark, *Poroderma pantherinum*, Mitochondrial DNA Part B, 3:2, 750-752, DOI: 10.1080/23802359.2018.1483772

To link to this article: https://doi.org/10.1080/23802359.2018.1483772

Published online: 10 Jul 2018.

MITOCHONDRIAL DNA PART B
2018, VOL. 3, NO. 2, 750–752
https://doi.org/10.1080/23802359.2018.1483772

Taylor & Francis
Taylor & Francis Group

MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome and phylogenetic position of the leopard catshark, *Poroderma pantherinum*

Michaela van Staden[a], Katie S. Gledhill[a,b], Clint Rhode[a] and Aletta E. Bester-van der Merwe[a]

[a]Department of Genetics, Stellenbosch University, Stellenbosch, South Africa; [b]South African Shark Conservancy, Old Harbour Museum, Hermanus, South Africa

ABSTRACT

We present the first mitochondrial genome of a South African endemic catshark, *Poroderma pantherinum*. The complete mitogenome is 16,686 bp in length, comprising 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, and one non-coding control region. Similar to other shark mitogenomes, it is AT rich (61.1%), with a GC content of 38.9%. Protein-coding genes used one of two start codons (ATG and GTG) and one stop codon (TAA/TA-/T-). Phylogenetic analysis of the leopard catshark and 34 carcharhinid species showed that it clusters with two other scyliorhinid species (*Cephaloscyllium umbratile* and *Scyliorhinus canicula*) with 100% support.

ARTICLE HISTORY
Received 17 May 2018
Accepted 29 May 2018

KEYWORDS
mitochondrial genome; catshark; *Poroderma pantherinum*; Scyliorhinidae

The leopard catshark *Poroderma pantherinum* (Carcharhiniformes: Scyliorhinidae) is a bottom-dwelling South African endemic shark, predominantly distributed in inshore waters along the south and south-east coasts (Human 2006). There is a lack of data on the population trends of *P. pantherinum* and it is currently assessed as Data Deficient using the International Union for the Conservation of Nature (IUCN) Red List Criteria (Human 2009). Southern Africa is a biodiversity hotspot with many endemic catsharks (Ebert and van Hees 2015); however, the lack of genetic resources for South African sharks delays the understanding of species delineation, population genetics, and reproductive behaviour (Bester-van der Merwe and Gledhill 2015). Here, we present the first complete mitogenome sequence and phylogenetic position of a South African endemic catshark.

A tissue sample (fin clip) was taken from one female *P. pantherinum* individual in Walker Bay, Hermanus, South Africa (geospatial coordinates: –34.421111, 19.244010) in 2016. The fin clip sample (FWB387) is stored in 100% ethanol at Stellenbosch University, Department of Genetics. Total genomic DNA was isolated using a standard cetyltrimethylammonium bromide (CTAB) extraction protocol (Sambrook and Russell 2001). Low coverage whole genome sequencing was performed on an Ion Torrent S5™ platform (Thermo Fisher Scientific, Waltham, MA, USA). Libraries with a mean insert size of 600 bp were prepared for sequencing using the Ion Plus Fragment Library Kit (Thermo Fisher Scentific), following the manufacturer's protocol. The generated sequence reads were quality filtered using Torrent Suite™ Software (Thermo Fisher Scientific) and mapped to a reference mitogenome from *Scyliorhinus canicula* (NC_001950.1) in Geneious® v.10.2.3 (Kearse et al. 2012). The final assembly was annotated using MitoAnnotator (Iwasaki et al. 2013). A MUSCLE alignment, excluding *ND6* and the control region, was performed in Geneious® with 36 publicly available elasmobranch mitogenomes. A Bayesian tree (Figure 1) was generated in MrBayes v.3.2.6 (Huelsenbeck and Ronquist 2001) using the best substitution model (GTR + I + G) determined by the Bayesian Information Criterion in jModelTest2 v.0.1.10 (Darriba et al. 2012). The Bayesian analysis was run for 1,000,000 generations, sampled every 1000 generations and the first 25% of trees were omitted as burn-in with the remaining trees used to calculate the posterior probabilities.

The complete mitogenome of the leopard catshark (accession MH321446) is 16,686 bp in length, containing 13 protein-coding, 22 tRNA, 2 rRNA genes, and one non-coding control region. The nucleotide base composition is rich in A (31.0%) + T (30.1%) and low in C (25.0%) + G (13.9%), which is common for elasmobranch mitogenomes (Ruck et al. 2017). All genes started with the standard ATG codon, except *COI*, which started with the alternate GTG codon. Majority of the genes ended with the TAA stop codon, with *ND2*, *COII*, *ND3*, *ND4,* and *CYTB* ending with an incomplete stop codon (TA–/T–). The Bayesian tree (Figure 1) shows that *P. pantherinum* clusters with two other catshark species (*Cephaloscyllium umbratile* and *Scyliorhinus canicula*) with 100% support, whereas *Halaelurus buergeri* clusters with the remaining five families supporting earlier work by Chen et al. (2016) that the family Scyliorhinidae is paraphyletic.

CONTACT Michaela van Staden 17665299@sun.ac.za Department of Genetics, Stellenbosch University, Private Bag X1 Matieland 7602, Stellenbosch, South Africa

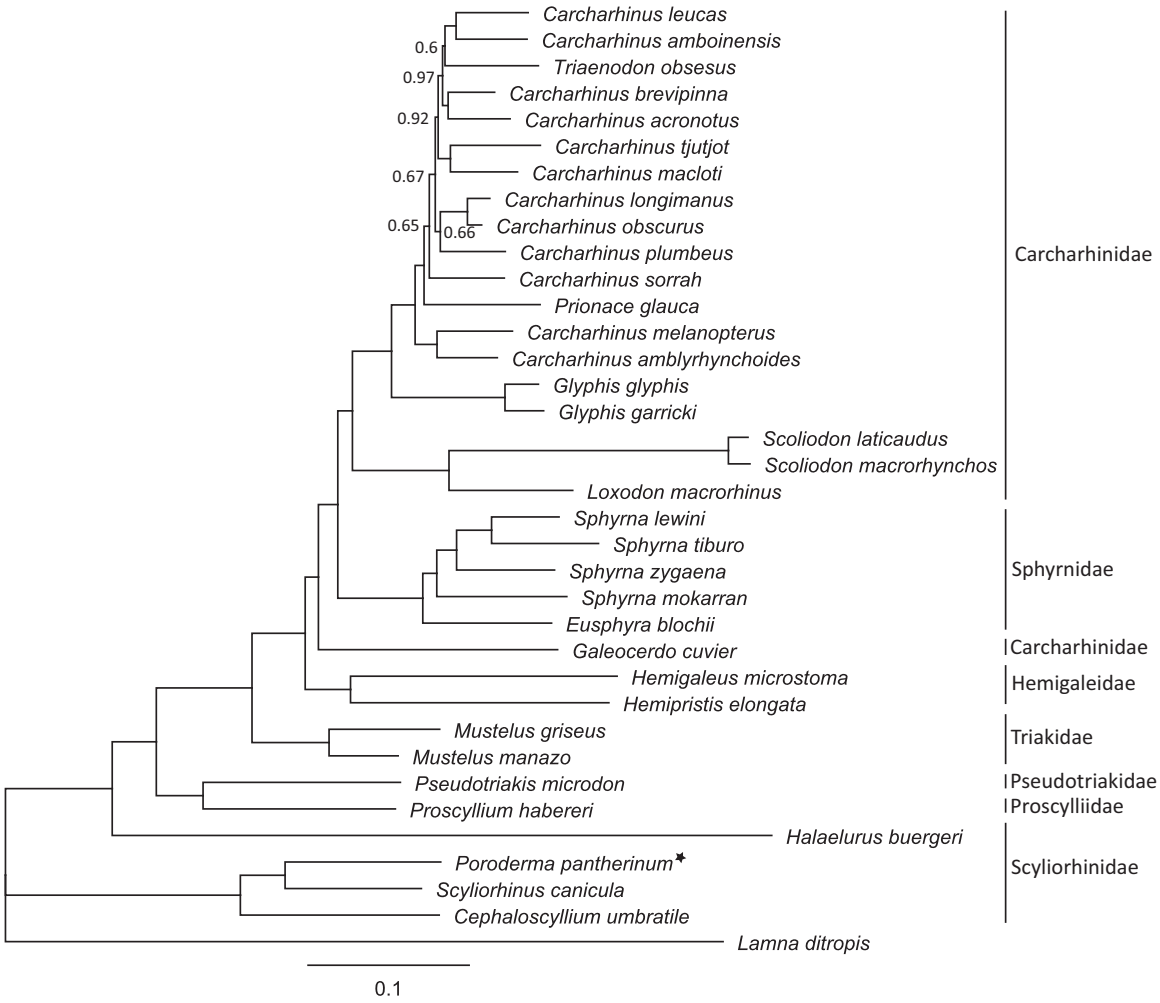


Figure 1. Bayesian tree depicting the phylogenetic position of *Poroderma pantherinum* (posterior probability values only shown if below 1.0). Based on 35 mitochondrial genomes (excluding *ND6* and the control region) of sharks from the order Carcharhiniformes, using *Lamna ditropis* (KF962053.1) as an outgroup. (*Carcharhinus acronotus*: NC_024055.1, *Carcharhinus amblyrhynchoides*: NC_023948.1, *Carcharhinus amboinensis*: NC_026696.1, *Carcharhinus brevipinna*: KM244770.1, *Carcharhinus leucas*: KF646785.1, *Carcharhinus longimanus*: NC_025520.1, *Carcharhinus macloti*: NC_024862.1, *Carcharhinus melanopterus*: NC_024284.1, *Carcharhinus obscurus*: NC_020611.1, *Carcharhinus plumbeus*: NC_024596.1, *Carcharhinus sorrah*: NC_023521.1, *Carcharhinus tjutjot*: KP091436.1, *Cephaloscyllium umbratile*: NC_029399.1, *Eusphyra blochii*: NC_031812.1, *Galeocerdo cuvier*: NC_022193.1, *Glyphis garricki*: KF646786.1, *Glyphis glyphis*: NC_021768.2, *Halaelurus buergeri*: NC_0311811.1, *Hemigaleus microstoma*: KT003687.1, *Hemipristis elongata*: KU508621.1, *Loxodon macrorhinus*: KT347599.1, *Mustelus griseus*: NC_023527.1, *Mustelus manazo*: NC_000890.1, *Prionace glauca*: NC_022819.1, *Proscyllium habereri*: KU721838.1, *Pseudotriakis microdon*: NC_022735.1, *Scoliodon laticaudus*: KP336547.1, *Scoliodon macrorhynchos*: NC_018052.1, *Scyliorhinus canicula*: NC_001950.1, *Sphyrna lewini*: NC_022679.1, *Sphyrna mokarran*: KY464952.1, *Sphyrna tiburo*: KM453976.1, *Sphyrna zygaena*: NC_025778.1 and *Triaenodon obsesus*: KJ748376.1).

Acknowledgements

The authors thank the South African Shark Conservancy (SASC) for providing the tissue sample. Sequence data were generated at the Central Analytical Facility, Stellenbosch University, the authors thank all staff who assisted in the sequencing of this sample.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Michaela van Staden http://orcid.org/0000-0003-2798-7821
Katie S. Gledhill https://orcid.org/0000-0002-4614-8671
Clint Rhode https://orcid.org/0000-0001-7969-7282
Aletta E. Bester-van der Merwe https://orcid.org/0000-0002-0332-7864

References

Bester-van der Merwe AE, Gledhill KS. 2015. Molecular species identification and population genetics of chondrichthyans in South Africa: current challenges, priorities and progress. Afr Zool. 50:205–217.

Chen H, Ding W, Shan L, Chen X, Ai W. 2016. Complete mitochondrial genome and the phylogenetic position of the blackspotted catshark *Halaelurus buergeri* (Charcarhiniformes: Scyliorhinidae). Mitochondrial DNA B: Resour. 1:369–370.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 9:772

Ebert DA, van Hees KE. 2015. Beyond Jaws: rediscovering the 'lost sharks' of southern Africa. Afr J Mar Sci. 37:141–156.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17:754–755.

Human BA. 2006. A taxonomic revision of the catshark genus *Poroderma* Smith, 1837 (Chondricthyes: Carcharhiniformes: Scyliorhinidae). Zootaxa. 1229:1–32.

Human BA. 2009. *Poroderma pantherinum*. The IUCN Red List of Threatened Species. 2009:e.T161515A5440910.

Iwasaki W, Fukunaga T, Isagozawa R, Yamada K, Maeda Y, Satoh TP, Sado T, Mabuchi K, Takeshima H, Miya M, Nishida M. 2013. MitoFish and MitoAnnotator: a mitochondrial genome database of fish with an accurate and automatic annotation pipeline. Mol Biol Evol. 30:2531–2540.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28:1647–1649.

Ruck CL, Marra N, Shivji MS, Stanhope MJ. 2017. The complete mitochondrial genome of the endangered hammerhead shark, *Sphyrna mokarran*. Mitochondrial DNA B: Resour. 2:246–248.

Sambrook J, Russell DW. 2001. Molecular cloning: a laboratory manual. New York (NY): Cold Spring Harbor Laboratory Press.