Identification of *Macrourus* species (Teleostei: Gadiformes) found near Prince Edward Islands in the Southern Ocean using molecular and morphological analyses.

by

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in Biological Sciences.

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As the candidate's supervisor I have approved this dissertation for submission.

Signed: _____

Supervisor: Dr. T. Miya

Date: 05 October 2021

PREFACE

The research contained in this dissertation was completed by the candidate while based in the Discipline of Biological Sciences, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, South Africa. The research was financially supported by the National Research Foundation (NRF).

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

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Signed: Dr. T. Miya (Supervisor)

Date: 05 October 2021

DECLARATION 1: PLAGIARISM

I, Vineshree Shadamorgan, declare that:

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

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

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

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

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

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DECLARATION 2: RESEARCH OUTPUTS

Details of contributions to conferences that include research presented in this dissertation:

Conference 1: School of Life Sciences Research Day

Poster presentation (Population genetic structure between *Macrourus* species from the Prince Edward Islands in the Southern Ocean)

22 May 2019

University of KwaZulu-Natal (Westville Campus)

The candidate carried out experimental research, captured all the research data, complied the abstract and presented research outcomes during a poster presentation.

Conference 2: The College of Agriculture, Engineering and Science Postgraduate Research and Innovation Symposium (PRIS)

Poster presentation (Molecular and morphological relationship among *Macrourus* species from the Prince Edward Islands in the Southern Ocean)

17 October 2019

University of KwaZulu-Natal (Westville Campus)

The candidate carried out experimental research, captured all the research data, complied the abstract and presented research outcomes during a poster presentation.

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Signed: Vineshree Shadamorgan

Date: 05 October 2021

ABSTRACT

The genus *Macrourus* belongs to the family Macrouridae that consist mainly of deep-sea fish. *Macrourus* contains five species that have a polar distribution, with one species, *M. berglax*, found in the Atlantic Ocean, and the other four species, *M. carinatus*, *M. holotrachys*, *M. whitsoni* and *M. caml*, found in the Southern Ocean. The species found in the Southern Ocean are morphologically similar to each other and have overlapping distributional patterns, as a result there have been instances of misidentifications within this genus. Based on morphological characteristics, previous studies have grouped the Southern Ocean *Macrourus* species into two pairs, viz *M. carinatus* and *M. holotrachys*, and *M. whitsoni* and *M. caml*. Despite overlapping occurrence elsewhere, morphological studies conducted in the Prince Edward Islands (PEIs) located in the Southern Ocean, have identified only one *Macrourus* species, *M. carinatus*. Since there are known cases of species misidentification using morphological identification and distribution overlap within this genus, there is a need for a molecular study that will complement the morphological data. There are no published molecular studies that reviewed species identification in these islands. This study aimed to identify *Macrourus* specimens found near the PEIs using molecular and morphological analyses. This assessment was done by sequencing the cytochrome c oxidase subunit 1 gene, and by measuring and counting morphometric and meristic characters. The BLAST search which had a sequence similarity of 99 – 100%, showed that there are two species represented in this dataset which were identified as *M. carinatus* and *M. holotrachys*. This observation was supported by both the phylogenetic and haplotype network trees, which formed two distinct clades. On the other hand, the morphological data did not separate the two species, supporting previous studies that discovered high levels of morphological similarity between *M. carinatus* and *M. holotrachys*, which ultimately led to their grouping and/or misidentification. It can therefore be concluded that there are at least two *Macrourus* species inhabiting waters around PEIs, which are morphologically similar.

Keywords: Cytochrome c oxidase subunit 1, morphological similar species, meristics, misidentification, morphometrics.

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LIST OF ABBREVIATIONS

μL – Microlitre

μM - Micromolar

π – Nucleotide diversity

APF – Antarctic polar front

ACC – Antarctica circumpolar current

BLAST – Basic Local Alignment Search Tool

bp – Base pairs

CO1 – Cytochrome oxidase subunit 1

DNA – Deoxyribonucleic acid

DnaSP – DNA sequence polymorphism

H – Number of haplotypes

hd – Haplotype diversity

HRM – High Resolution Melt

Kb – Kilobases

MEGA – Molecular Evolutionary Genetic Analysis

mtDNA – Mitochondrial deoxyribonucleic acid

MS222 – Tricaine mesylate

F_{ST} – Fixation index

n – Sample size

NCBI – National Centre for Biotechnology Information

PC – Principal component

PCA – Principal component analysis

PCR – Polymerase chain reaction

PEIs – Prince Edward Islands

PFZ – Polar frontal zone

r – Raggedness index

S – Number of polymorphic sites

SAF – sub-Antarctic front

SAIAB – South African Institute of Aquatic Biodiveristy

SPSS – Statistical package for the social sciences

T92 – Tamura 3-parameter

Taq – *Thermus aquaticus*

CHAPTER 1: LITERATURE REVIEW AND STUDY RATIONALE

1.1 Introduction

Fishes comprise more than 50% of all living vertebrate species (Eschmeyer et al., 2010; Nelson et al., 2016). According to Nelson (2006), fishes are described as "aquatic vertebrates that have gills throughout life and limbs, if any, in the shape of fins". Fishes are very diverse in terms of their appearance, such as size, shape, or colour, and are regularly found in a variety of habitats ranging from extreme cold to temperate and tropical environments (Zemlak et al., 2009; Branch et al., 2010; Zhang and Hanner, 2011; Xu et al., 2019a). These organisms can be found in almost all aquatic environments (Magadan et al., 2015). Currently, there are approximately 35 934 fish species, of which 18 150 are found in freshwater ecosystems while 17 784 are found in marine ecosystems (Ivanova et al., 2007; Zemlak et al., 2009; Fricke et al., 2021). They play an essential role in the functioning of aquatic ecosystems by providing nutrients, regulating carbon flux, regulating food webs dynamics, *etc* (Homlund and Hammer, 1999). There are also several species that are commercially important and serve as an essential food source for humans (Rasmussen et al., 2009; Branch et al., 2010; Bingpeng et al., 2018).

Prior to the invention of molecular techniques, primarily fish identification was conducted using morphological characters (Strauss and Bond, 1990; Hebert et al., 2003; Zhang and Hanner, 2011). However, the usage of only morphological characters pose several issues, for example, the issue of cryptic species which is based on highly similar morphotypes and the diverse developmental stages of fish often resulted in misidentifications (Zhang and Hanner, 2011). Factors contributing to species misidentification using morphological analyses may include phenotypic plasticity specimens that are morphologically similar, rare species, unfamiliar life history stages of an organism, and diagnostic features of an organism that are damaged during collection (McCusker et al., 2013). An additional reason for the misidentification of fish species is that some taxa are poorly studied and/or identification has been based on a few specimens (Smith et al., 2012).

Information regarding the biology, morphology, and population structure of fish species is essential, as this information is required to develop management and conservation strategies (Praveena et al., 2017). Thus, the correct identification of specimens is imperative as the

information generated from these studies play an important role in ecological monitoring, environmental impact assessment, fishery compensation, resource management, smuggling prevention, and establishment of marine protected areas (Ko et al., 2013; Bingpeng et al., 2018; Rathipriya et al., 2019).

This study focuses on *Macrourus* species found near the Prince Edward Islands (PEIs) in the Southern Ocean. Four species have been identified in the Southern Ocean, and only one of these, *M. carinatus*, has been identified in the PEIs to date using morphological data (Cohen et al., 1990; Iwamoto, 1990; Duhamel et al., 2014). Studies that have reviewed *Macrourus* species using molecular data have discovered high levels of misidentification within this genus (Cohen et al., 1990; Smith et al., 2011; McMillan et al., 2012). This misidentification maybe due to the fact that these species have similar morphology and overlapping distributional patterns. *Macrourus carinatus* is known to have overlapping distribution with *M. holotrachys* and it is interesting that at the PEIs this species was not discovered. There is a lack of molecular studies that reviewed *Macrourus* species around these islands, therefore this study aimed to close this gap.

1.2 Taxonomy and distribution

1.2.1 Macrouridae

The family of deep-sea fish Macrouridae Bonaparte, 1831 belongs to the order Gadiformes of the class Actinopterygii (Cohen et al., 1990). Gadiformes consists of 17 families, namely Bergmacerotidae, Phycidae, Gaidropsaridae, Lotidae, Gadidae, Ranicipitidae, Merluccidae, Euclichthyidae, Muraenolepididae, Melanonidae, Trachyrincidae, Moridae, Macruronidae, Lyconidae, Bathygadidae, Steindachneriidae, and Macrouridae (Cohen et al., 1990; Fricke et al., 2021). There are approximately 615 species and 82 genera in this order, which are found throughout the world's oceans, as well as in the brackish and freshwater habitats around the temperate regions (Cohen et al., 1990; Kriwet and Hecht, 2008). Gadiformes are an economically significant group of teleosts as they form over one-quarter of the world's marine fish catch (Kriwet and Hecht, 2008). They include commercially important fish such as cods (Gadidae), grenadiers (Macrouridae), and hakes (Merluccidae) (Cohen et al., 1990; Swan and Gordon, 2001). The Macrouridae family is the most diverse group within this order in terms of

the number of species (Cohen et al., 1990; Smith et al., 2011; Münster et al., 2016). Due to the number of species and their biomass Macrouridae species are considered as a dominant part of the benthopelagic deep-sea fish fauna (Cohen et al., 1990; Smith et al., 2011; Sobrino et al., 2012; Münster et al., 2016; Lisney et al., 2018).

Macrouridae consists of four sub-families, including Bathygadinae, Macrourinae, Macrouridinae, and Trachyrinicinae (Cohen et al., 1990; Münster et al., 2016). There are 36 genera within this family; approximately there are 369 species are considered to be valid (Devine et al., 2012; Sobrino et al., 2012; Fricke et al., 2021, Froese and Pauly, 2019). In this family, the association that are shared between species and genera are not well known (Cohen et al., 1990; Swan and Gordon, 2001). The species in this family are characterised by the appearance of a large head and medium slender body that tapers uniformly to form a pointed tail that lacks a caudal fin (except for Trachyrinicinae that has a small caudal fin) (Cohen et al., 1990). Other features of this family include a chin barbel and eyes that appear to be moderate to large in size (Cohen et al., 1990). Additionally, these fish usually have a light organ located underneath their skin along the abdomen's midline (Gorny and Zapata-Hernández, 2018). Macrouridae is commonly referred to as macrourids, grenadiers, whiptails, and rattails, with the common names whiptails and rattails being related to their long tapering tails (Cohen et al., 1990; Devine et al., 2012).

Macrouridae species inhabit all oceans and can be found within a depth range of around 200 – 7000 m, with the majority of species (approximately 90%) found in the continental slopes ranging from 200 – 2000 m (Figure 1.1; Schneider et al., 2009; Devine et al., 2012; Sobrino et al., 2012; Münster et al., 2016). The remaining 10% can be found either in the abyssal plains, meso or bathypelagic zones of the oceans (Kriwet and Hecht, 2008; Münster et al., 2016). Macrouridae species are capable of living at great depths due to their specialised swim bladders (COSEWIC, 2007).

Macrouridae species are considered one of the main deep-sea fish groups and consist mainly of benthopelagic predators (Laptikhovsky, 2005; Smith et al., 2011; Sobrino et al., 2012; Münster et al., 2016). This group is known to have the most diverse diet as they feed on various animals, and are therefore referred to as generalist predators however, they also tend to feed on scavenged food (Laptikhovsky, 2005). The feeding habits of macrourids depend on various

factors such as species, size, depth, and the nature of the seabed (Münster et al., 2016). Majority of macrourid species feed on prey present in sediments or hunts benthic crustaceans, and only a few species prey on fish, cephalopods, and euphausiids in the water column (Münster et al., 2016).

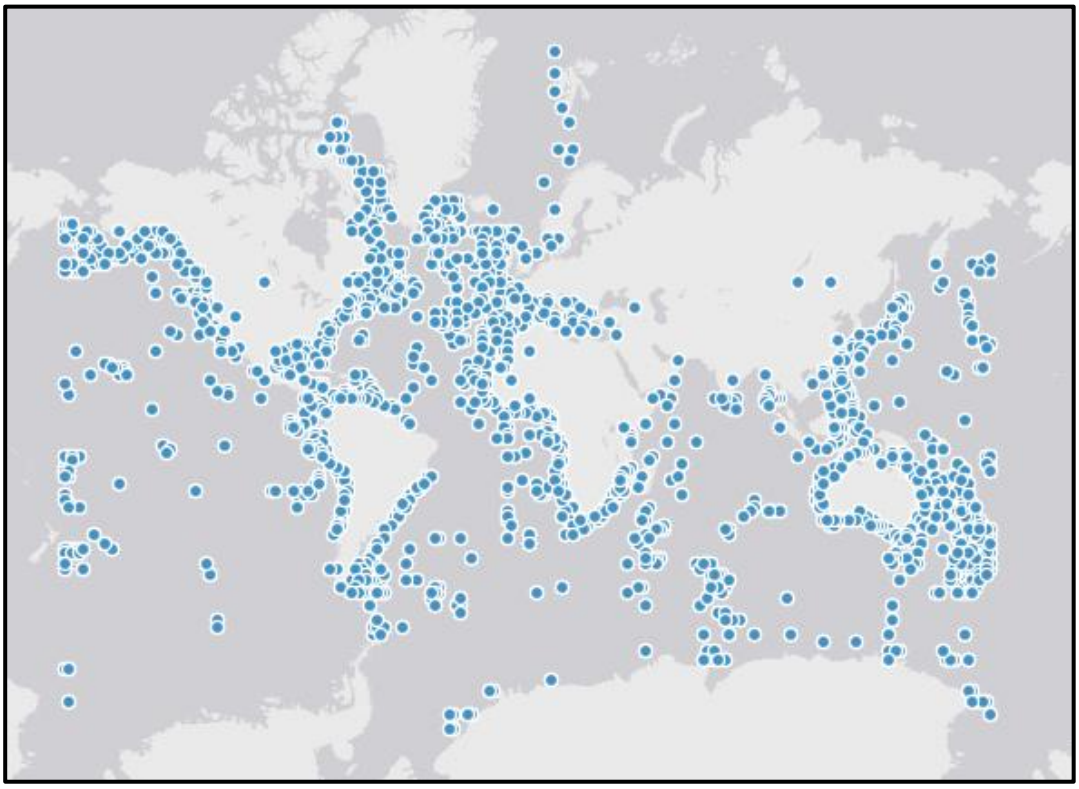

Figure 1.1: Distribution of Macrouridae fish throughout the world. Blue dots represent the occurrence of Macrouridae fish (accessed from the Global Biodiversity Information Facility (GBIF) website, 2020).

1.2.2 Macrourus

The genus *Macrourus* Bloch, 1786 belongs to the sub-family Macrourinae (Cohen et al., 1990). Macrourinae is regarded as the biggest sub-family of the family Macrouridae consisting of more than 350 species (Cohen et al., 1990; Münster et al., 2016). Macrourinae is recognised from other sub-families by displaying a gap between the dorsal fins, and the long-based second dorsal fin rays are shorter in height compared to the rays on the anal fin (Figure 1.2; Cohen et al., 1990; Chiou et al., 2004). The genus *Macrourus* is a small group (in terms of the number of species) that consists of five benthopelagic species, including *M. berglax* Lacepède, 1801

commonly known as the rough-head or onion-eye grenadier, *M. holotrachys* Günther, 1878 the bigeye grenadier, *M. carinatus* (Günther, 1878) the ridge-scaled rattail, *M. whitsoni* (Regan, 1913) the circumpolar Whitson's grenadier and *M. caml* McMillan, Iwamoto, Stewart, and Smith, 2012 the caml grenadier. This fish group has no light organ and feeds on a variety of benthic and midwater fishes and invertebrates (Cohen et al., 1990; Morley et al., 2004). They are also slow-growing reaching a maximum growth size of ± 100 cm in total length and has a life span of at least 50 years (Cohen et al., 1990, O'Driscoll et al., 2012; Prut'ko, 2012). The maturity differs between sexes with males reaching maturity at approximately 10.6 years and females reaching maturity at approximately 13.6 years (O'Driscoll et al., 2012).

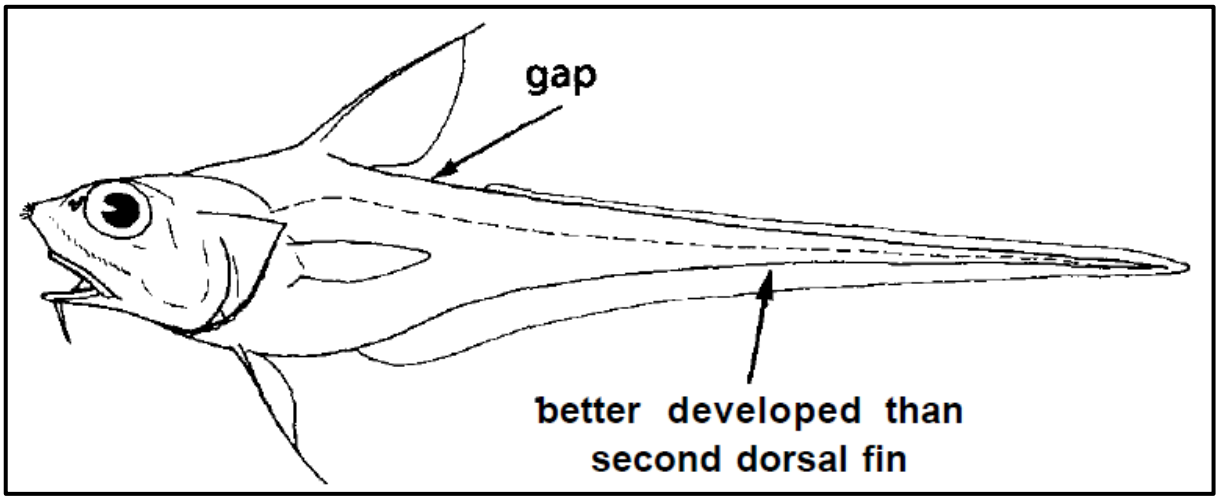

Figure 1.2: Distinguishable morphological features of the sub-family Macrourinae (Cohen et al., 1990).

Macrourus displays a polar distribution with species inhabiting the upper and middle continental slope in cold temperate and polar waters of the Atlantic and Southern Ocean (Cohen et al., 1990). Except for *M. berglax*, the other four *Macrourus* species are found in the Southern Ocean, with *M. whitsoni* and *M. caml* being endemic to this ocean (Figure 1.3; Marriott and Manning, 2006; Duhamel et al., 2014). *Macrourus* species found in the Southern Ocean are an integral component of the food chain (Gordeev, 2015). These species have been found to display overlapping distributional patterns along the Southern Ocean islands, and they also have a high similarity of morphological characteristics (Figure 1.4; Cohen et al., 1990; Iwamoto, 1990; Smith et al., 2011; McMillan et al., 2012). Morphological identification of these fish

species is difficult, with the identification of juveniles being the most challenging since features are not well developed (Cohen et al., 1990; Fitzcharles, 2012).

Macrourus holotrachys Günther, 1878 was originally described from an individual specimen caught off the East of Rio de la Plata mouth, Argentina, and was originally assigned to *Macrurus*. Subsequently it was assigned to *Coryphaenoides* by Nakamura in Nakamura et al. (1986) thereafter Trunov (1986) recognised *M. holotrachys* as a valid species. This decision was followed by the subsequent authors: Trunov and Konstantinov (1986), Iwamoto (1986), Arai in Amaoka et al. (1990), Iwamoto (1990), Iwamoto in Cohen et al. (1990), Trunov and Konstantinov (1990), Ruiz and Oyarzún (1993), Bianchi and Carpenter in Bianchi et al. (1993) Ruiz et al. (1995), López et al. (1996), Pequeño (1997), McMillan et al. (2012), McMillan and Iwamoto (2015), Nión et al. (2016), and Gon et al. (2021). *Macrourus holotrachys* is described as having large eyes, and the ventral surface of the head is naked (i.e., does not contain scales) (McMillan et al., 2012). Teeth in the lower and upper jaw are small and uniformed, with the lower jaw consisting of 2 – 5 rows of teeth while the upper jaw consists of 4 – 6 rows (McMillan et al., 2012). The pelvic fins consist of 8 – 9 rays and the pyloric caeca range from 9 to 16 (McMillan et al., 2012).

Macrourus carinatus (Günther, 1878) was first caught near Prince Edward Island and was originally assigned to *Coryphaenoides*. Iwamoto (1986) considered this species as a synonym of *M. holotrachys* but this decision was reversed by Trunov and Kinstantinoy (1986) who recognised *M. carinatus* as a valid species. This decision was followed by the subsequent authors: Pequeño (1989), Paulin et al. (1989), Iwamoto in Cohen et al. (1990) Trunov and Konstantinov (1990), Ruiz and Oyarzún (1993), Heemstra in Smith and Heemstra (1995), Olaso et al. (1997), Duhamel et al. (2005), Bray et al. (2006), Mabragaña et al. (2011), McMillan et al. (2012), McMillan and Iwamoto, (2015), Nión et al. (2016), and Gon et al. (2021). *Macrourus carinatus* is described as having a large head, short snout and the ventral surface of the head is mostly scale, with the snout being naked (McMillan et al., 2012). Teeth in the lower jaw are small and uniformed which consists of 2 – 5 rows while the teeth in the upper jaw have an enlarged outer row which consists of 3 – 6 rows (McMillan et al., 2012). The pelvic fins consist of usually eight rays and the pyloric caeca range from 13 to 20 (McMillan et al., 2012).

Macrourus whitsoni (Regan, 1913) was caught off Coats Land in the Weddall Sea between 1902 to 1904 during the Antarctic Fishes of the Scottish National Antarctic Expedition and was originally assigned to the genus *Chakinura*. Iwamoto (1986) questioned if this species was a synonym of *M. holotrachys* but Nakamura et al (1986) considered this species valid as *Coryphaenoides whitsoni*. Regan (1913) thereafter recognised *M. whitsoni* as a valid species. This decision was followed by the subsequent authors Trunov and Konstantinov (1989), Iwamoto (1990), Iwamoto in Cohen et al. (1990), Trunov and Konstantinov (1990), Miller (1993), Petrov (2011), McMillan et al. (2012), and Gon et al. (2021). *Macrourus whitsoni* is described as having a large head, a slightly pointed snout and the ventral surface of the head is mostly scale, with the snout being naked (McMillan et al., 2012). Teeth in the lower jaw are enlarged which consists of one row (two at the tip) while the teeth in the upper jaw have an enlarged outer row which consists of 2 – 5 rows (McMillan et al., 2012). The pelvic fins consist of usually nine rays and the pyloric caeca range from 15 to 26 (McMillan et al., 2012).

The last species to be identified was *Macrourus caml* McMillan, Iwamoto, Stewart, and Smith, 2012. Prior to 2012, this species was previously identified as *M. whitsoni* (Smith et al., 2011). McMillan et al. (2012) recognised *M. caml* as a valid species and this decision was followed by Gon et al. (2021). *Macrourus caml* was identified using molecular analyses as morphological analyses could not separate this species from *M. whitsoni* (Smith et al., 2011). Specimens were caught during the Ross Sea biodiversity survey of the International Polar Year - Census of Antarctic Marine Life (CAML) voyage in 2008, and this species was named after the voyage (McMillan et al., 2012). *Macrourus caml* is described as having a large head, a slightly rounded blunt snout and the ventral surface of the head is mostly scale, with the snout being naked (McMillan et al., 2012). Teeth in the lower and upper jaw are small and uniformed, with the lower jaw consisting of 2 – 3 rows of teeth while the upper jaw consists of 4 – 5 rows (McMillan et al., 2012). The pelvic fins consist of usually eight rays and the pyloric caeca range from 20 to 37 (McMillan et al., 2012).

As the search for *Macrourus* species continued, they were discovered in different islands around the Southern Ocean, surrounding both Antarctic and sub-Antarctic regions (Duhamel et al., 2014). *Macrourus holotrachys* was discovered around South America, Falkland Islands, and Shag Rocks with depths ranging between 150 and 1750 m (Laptikhovsky, 2005; McMillan et al., 2012). This species also inhabits the Southern Ocean's temperate waters, where they are

found at depths of 850 – 2000 m (Laptikhovsky, 2005; McMillan et al., 2012). *Macrourus carinatus* is distributed on the slopes of South America, South Africa, New Zealand, and in islands in the Southern Ocean, where it often inhabits temperate to sub-Antarctic waters of the Southern Ocean found at depths 300 – 1600 m (Laptikhovsky, 2005, 2010; McMillan et al., 2012). *Macrourus whitsoni* is distributed within the Antarctic convergence, close to the Falkland Islands, Cosmonaut Sea, D'Urville Sea, Ross Sea, Balleny and Scott Islands slope, Pacific Antarctic Ridge. This species inhabits depths ranging between 400 and 3185 m on the continental slope and found in abundance at depths ranging between 600 to 1500 m (Walter et al., 2002; Prut'ko, 2012; Gordeev, 2015). *Macrourus caml* is distributed in the Antarctic convergence, Ross Sea, Balleny and Scott Islands, Cosmonaut Sea, and south of South Georgia at depths of 350 – 2080 m (McMillan et al., 2012).

Characters that are used to separate the four *Macrourus* species found in the Southern Ocean include scales on the underside of the head, number of pelvic fin rays, size and shape of the teeth in the lower and upper jaw, and size and number of scales in a diagonal row from the anal fin origin forward to (but not including) the lateral line (McMillan et al., 2012). *Macrourus holotrachys* lacks scales on the underside side of the head, although if scales are present, it can consist of up to eight small patches (McMillan et al., 2012). Unlike *M. holotrachys* species, *M. whitsoni*, *M. carinatus* and *M. caml* all have scales present in rows on the underside of the head (McMillan et al., 2012). *Macrourus whitsoni* can be differentiated from *M. carinatus* and *M. caml* by characteristics such as nine pelvic fin rays and the lower jaw consisting of a single row (two rows at the tip) of slender, slightly elongated, and evenly spaced teeth (one tooth length spacing) (McMillan et al., 2012). In contrast, *M. carinatus* and *M. caml* have eight pelvic fin rays with the lower jaw consisting of 2 – 5 rows of small uniformed, closely spaced teeth (McMillan et al., 2012). *Macrourus carinatus* is differentiated from *M. caml* based on the enlarged outer row of teeth in the upper jaw and the large body scales, with 19 – 25 in a diagonal row from the anal fin origin forward to (but not including) the lateral line (McMillan et al., 2012). In comparison, *M. caml* is differentiated from *M. carinatus* by uniform sized teeth in the upper jaw and the small body scales, with 30 – 40 in a diagonal row from the anal fin origin forward to (but not including) the lateral line (McMillan et al., 2012).

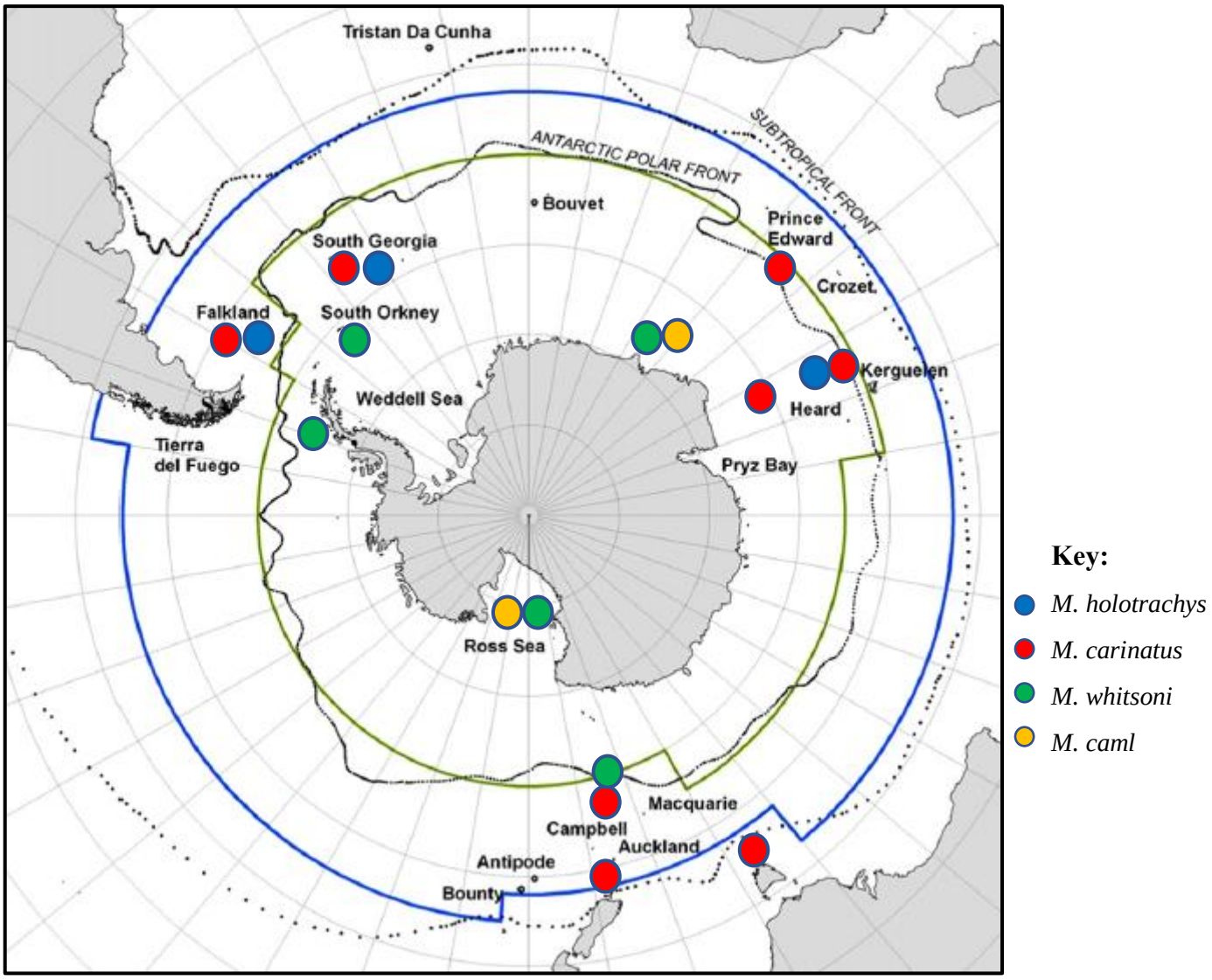

Figure 1.3: Distribution of *Macrourus* species in the Southern Ocean (De Broyer and Danis, 2011). The dots represent the distribution for the four *Macrourus* species blue – *M. holotrachys*, red – *M. carinatus*, green – *M. whitsoni* and yellow – *M. caml*.

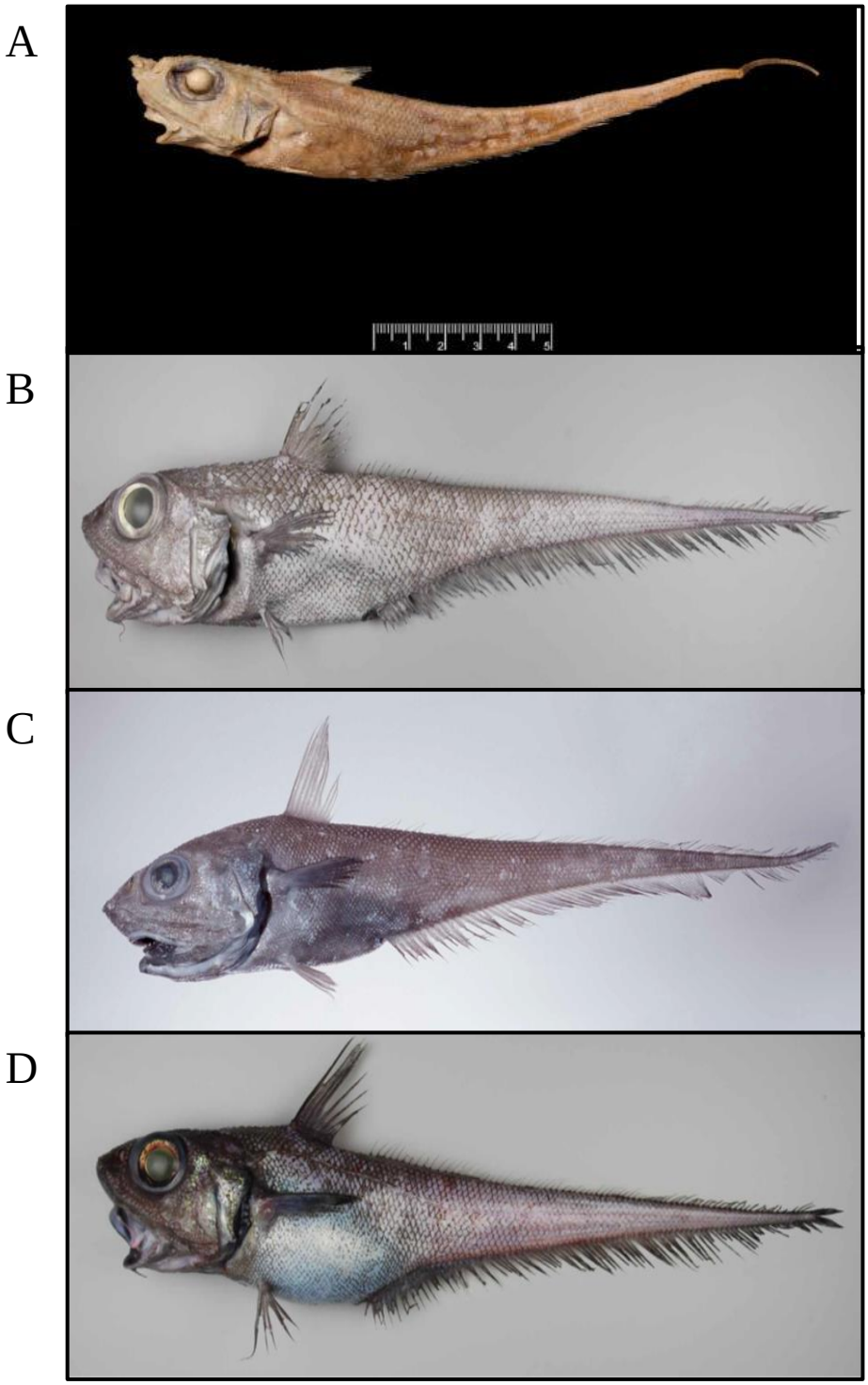

Figure 1.4: *Macrourus* species found in the Southern Ocean. A – *M. holotrachys*, B – *M. carinatus*, C – *M. whitsoni* and D – *M. caml* (McMillan et al., 2012; Fitzcharles, 2014).

1.3 Fish identification

1.3.1 Morphological techniques

The description of organisms was initially based on morphological characteristics, which has played an essential role in the development of the discipline of taxonomy (Anibaldi et al.,2016; Takács et al., 2016). Morphological methods consist of two groups, viz morphometrics, and meristics, which are used for the assessment/identification of organisms (Takács et al., 2016). Morphometrics involves measuring the length or distance between morphological features while meristics are defined as an organism's countable features (Muchlisin, 2013). In fish identification, some of the measurable features include head length, standard length, folk length, total length while, some of the countable features include the number of fin rays, scales, and gill rakers (González et al., 2016). Thus, morphometrics utilises continuous data since it is measurable features, whereas, meristics are non-continuous data since it is countable features (Turan, 1999; Kaouèche et al., 2017). Due to the different data types used, the datasets must be analysed separately (Turan, 1999). The information regarding other physical features such as shape, size, and colour can also be recorded during these procedures (Muchlisin, 2013). The fish's body weight is another variable that can be considered in this analysis for fish growth pattern, age determination, and fishery assessment (Fagbuaro et al., 2015, Fazazi et al., 2019).

Morphometrics is a technique that can be used to describe the body form of an organism, and it can also be used to assess the well-being of organisms and differentiate species from each other (Muchlisin, 2013; Anibaldi et al., 2016; González et al., 2016; Praveena et al., 2017). This technique allows for the quantitative description of an organism, which scientists frequently use from various science fields (Muchlisin, 2013). Furthermore, it is a widely used technique for the description of ichthyofauna in the study of ichthyological systematics and taxonomy (Muchlisin, 2013). Morphometrics can also be used for fish stock delineation and describing their spatial distribution (Ihssen et al., 1981; Turan, 1999; Palma and Andrade, 2002; Kaouèche et al., 2017; Jakubavičiūtė et al., 2018). The identification of fish stocks is based on changes in the specimen's size and shape (González et al., 2016; Turan, 1999). Despite several other methods for identifying fish stocks, morphological characteristics remain as a preferred choice (González et al., 2016; Takács et al., 2016).

Although fishes are very susceptible to factors such as temperature, food, and predation, over time they often adapt to these changes, and eventually, these changes are modelled into their morphology (Wimberger, 1992; Turan, 1999; Cabral et al., 2003; Hossain et al., 2010; González et al., 2016; Kaouèche et al., 2017). Subsequently, the way fishes react to these factors differ from species to species (González et al., 2016). Therefore, the morphology of fishes is considered a valuable tool for species discrimination (Begg and Waldman, 1999; Cadrin and Friedland, 1999; Jakubavičiūtė et al., 2018). Thus, the method of morphometric analysis can be used for differentiating closely related species (Manimegalai et al., 2010, Fazazi et al., 2019). The following includes some studies which have used morphological methods for their analyses: Turan at al. (2004), Yakubu and Okunsebor (2011), Takács (2012), González et al. (2016) and Gonzalez-Martinez et al. (2021).

1.3.2 Molecular techniques

The initial implementation of molecular tools was conducted on specimens from tropical and temperate regions, and after some time, these tools were eventually used on polar organisms (Kaiser et al., 2013). Samples that were initially collected from the Southern Ocean were stored in formaldehyde, and therefore could not be used for genetic studies (Bowen and Witzell, 1996; Díaz-Viloria et al., 2005; Kaiser et al., 2013). In the 1990s, the standard procedure for genetic based studies included using ethanol and freezing to preserve samples (Bowen and Witzell, 1996; Kaiser et al., 2013). Thus, these preservation methods enabled deoxyribonucleic acid (DNA) to be used for taxonomic and systematics related studies (Kaiser et al., 2013).

Genetic markers are commonly derived from two types of DNA, namely, mitochondrial (found in the cell's mitochondrion) and nuclear (found in the cell's nucleus) DNA (Sunnucks, 2000; Zhang and Hewitt, 2003). The nuclear DNA consists of coding and non-coding regions that evolve at different rates (Sunnucks, 2000). Since the non-coding region is more variable in comparison to the coding region, they are often used for population studies (Zhang and Hewitt, 2003). Nuclear DNA provides maternal and paternal inheritance information since it is bi-parentally inherited (Sunnucks, 2000). Some of the commonly used nuclear DNA for genetic-based studies include microsatellites, introns, and single-copy nuclear polymorphic DNA (Sunnucks, 2000; Zhang and Hewitt, 2003). Between the two types of DNA, mitochondrial DNA is the most popular marker used to assess molecular diversity in animals (Galtier et al.,

2009). Mitochondrial DNA is selected as a marker due to the following characteristics: lack of recombination, its inheritance as one locus (maternally inherited), a short coalescence compared to bi-parental loci, and evolves at a faster rate as compared to the nuclear DNA (Ballard and Whitlock, 2004; Liu et al., 2013; Adrian-Kalchhauser et al., 2017).

The mitochondrial genome is a circular molecule consisting of 37 genes that code for 13 proteins, 2 ribosomal RNAs, and 22 transfer RNAs and the control region (Yang et al., 2010; Liu et al., 2013; Mascolo et al., 2019). This DNA that is found in animals have a molecular size ranging from approximately 15 to 22 kilobases (Kb) in length (Liu et al., 2013). There are some concerns that are related to the genes on the mitochondrial genome and this includes the start/stop codons of the protein-coding genes and tRNA gene structure (Satoh et al., 2016). However, the data obtained from using mitochondrial DNA is beneficial for various studies such as taxonomy and phylogeny, genetic structure, biological identification, and conservation genetics (Yang et al., 2010; Liu et al., 2013). Some of the most used mitochondrial DNA markers for fish species include cytochrome b and cytochrome c oxidase subunit 1 (CO1). The CO1 gene is a type of mitochondrial DNA that is often used to determine molecular relationships. Cytochrome oxidise subunit 1 is a useful genetic marker for species identification (Hebert et al., 2003, 2004; Ward et al., 2005, Rathipriya et al., 2019). Hebert et al. (2004) stated that the CO1 gene enables closely related species to be discriminated across the animal phyla, and this has been successful for both marine and freshwater fish species (Rathipriya et al., 2019). Besides being used for identification purposes, CO1 data provides a very informative site for phylogenetic analysis (Ward et al., 2005; Fitzcharles, 2012).

Hebert et al. (2003) developed a concept known as DNA Barcoding, which involves using a short nucleotide sequence of a standardised gene region to identify and delineate organisms (Changizi et al., 2013). The short nucleotide sequence consists of 648 base pairs from the CO1 gene (Zhang and Hanner, 2011). The concept is based on the principle that interspecies variation is higher compared to intraspecies variation enabling one to differentiate species using nucleotide sequences (Khan et al., 2011). DNA barcoding has been used in various studies and proven to be a useful tool in the identification of samples regardless of the sample source (Changizi et al., 2013). Additionally, DNA barcoding can be utilised when identification based on morphology is problematic in cases involving cryptic species, damaged specimens, and larval life history stages (Ko et al., 2013; McCusker et al., 2013). Furthermore, it can also be used to cross-check identification that was initially based on the specimen's morphology and

reveal new species (Ko et al., 2013). DNA barcoding has played an essential role in correctly identifying marine fish and has proven to be fast, reliable, and easily accessible (Landi et al., 2014). The following includes some studies which have used molecular methods for their analyses: Ward et al. (2005), Zhang and Hanner (2011), Smith et al. (2012), Bingpeng et al. (2018) and Rathipriya et al. (2019).

1.4 Study rationale

Before 2012, it was assumed that the *Macrourus* genus consists of only three species in the Southern Ocean, which included *M. carinatus*, *M. holotrachys*, and *M. whitsoni*. A study conducted by Smith et al. (2011) raised questions regarding the taxonomic status of the *Macrourus* species when using the mitochondrial CO1 gene to review this group. This study revealed four well supported clades, with three clades representing known species, and the fourth clade consisted of specimens morphologically identified as the three *Macrourus* species dominated by *M. whitsoni* (Smith et al., 2011). McMillan et al. (2012) reviewed this group and concluded that Smith's et al. (2011) fourth clade consisted of the new species and named it *M. caml* (McMillan et al., 2012). Another study conducted by Fitzcharles (2012) used high-resolution melt (HRM) with species-specific primers for the mitochondrial CO1 gene to review the *Macrourus* species. This study was conducted simultaneously as Smith et al. (2011) and identified the presence of four *Macrourus* species in the Southern Ocean. Smith et al. (2011), and Fitzcharles (2012) are two studies that used genetic analyses and successfully identified the four *Macrourus* species in the Southern Ocean.

The available information on the geographical distribution of *Macrourus* specimens in PEIs have been based entirely on morphological characters and only one species has been recorded, *M. carinatus*, near these islands (Cohen et al., 1990; Iwamoto, 1990; Duhamel et al., 2014). The holotype of *M. carinatus* was based on a specimen collected near the PEIs in 1873 (Cohen et al., 1990). Although *M. carinatus* is the only *Macrourus* species that has been found near the PEIs, previous studies state that the pair of species, *M. carinatus* and *M. holotrachys* are found to have similar morphology and overlapping distributional patterns around the temperate to sub-Antarctic waters of the southern hemisphere (Cohen et al., 1990; Smith et al., 2011; McMillan et al., 2012). The specific locations where both these species have been identified includes the waters around South America, the South Pacific Ocean (Pacific-Antarctic Ridge), Heard and

McDonald Islands, South Georgia and Falkland Islands (Cohen et al., 1990; Laptikhovsky, 2005; Smith et al., 2011; McMillan et al., 2012; Fitzcharles, 2014). Thus, the identification of only one *Macrourus* species recorded near the PEIs remains to be interesting. Evidence provided by Cohen et al. (1990), Laptikhovsky (2005), Smith et al. (2011), McMillan et al. (2012), and Fitzcharles (2014) suggests that *M. carinatus* and *M. holotrachys* inhabit similar localities, hence, it could be possible that there are more than one *Macrourus* species found near PEIs.

A study conducted by Marriott et al. (2003) had mentioned that in 2002, observers had issues with identifying macrourid species from the Ross Sea region. A total of 348 specimens were collected at this sea and were identified as either *M. whitsoni*, *M. carinatus*, or *Coryphaenoides armatus*. When these specimens were reviewed it was found that the data consisted of two species, *M. whitsoni* and *M. holotrachys* (Marriott et al., 2003). This is an indication that *M. holotrachys* has previously been misidentified with other *Macrourus* species (also noted in Cohen et al. (1990)). Due to misidentifications that can occur when species are identified based on their morphological characteristics, molecular analyses are often used in conjunction with morphological analyses to verify the outcomes. Molecular methods have been successfully used to identify new species in the Southern Ocean which were previously misidentified morphologically. Studies by Smith et al. (2011) and Fitzcharles (2014) have proved this fact when reviewing *Macrourus* species. The current study aims to review *Macrourus* species associated with the PEIs using both molecular and morphological analyses. There are no available molecular studies that have reviewed this genus at these islands, and it has been proven by previous studies both molecular and morphological studies are needed to confirm identification in this group.

Objectives and research questions:

1. To identify *Macrourus* species found near PEIs using molecular analyses.
    - How many *Macrourus* species are found near PEIs?
    - If more than one species are found, how genetically different are they?
2. To compare morphological characteristics of species identified by molecular analyses (from objective 1).
    - What are the distinguishing characters for *Macrourus* species around PEIs?

1.5 Thesis Structure

Chapter one consists of background information elaborating on the importance of correct identification of fish species and a brief description of their role in the ecosystem, and the explanations of morphological and molecular analyses. Furthermore, it focuses on the description of the Macrouridae's family, the genus of *Macrourus*, and finally, the study taxa. It also includes the rationale for the study and the relevant aims and objectives. Chapter two explains the study site, PEIs, and the description of the material and methods used to achieve this study's objectives. Chapter three displays the results for both analyses. The first analysis involves the genetic data that determined which *Macrourus* species are found near the PEIs, and the genetic relationship among the *Macrourus* species. The second analysis involves the morphometric data that determined if species share similar/overlapping morphological features. Chapter four presents the discussion of this study, including recommendations for further and future research within this study field and the shortcomings that were experienced during the study.

CHAPTER 2: MATERIALS AND METHODS

2.1 Study site and sample collection

The Southern Ocean consists of two regions, the Antarctic and sub-Antarctic, separated by the Antarctic Polar Front (APF) (Duhamel et al., 2014). The sub-Antarctic region has much warmer temperatures reaching up to 12°C compared to the Antarctic region having the highest temperatures of 5°C in the low Antarctic zone (Duhamel et al., 2014). All the islands located in the sub-Antarctic region are referred to as hotspots for biological activity (Pakhomov and Chown, 2003; Oosthuizen et al., 2009; Gurney et al., 2014). The Prince Edward Islands (PEIs) are located in the sub-Antarctic region of the Indian Ocean sector, approximately 2000 km south-east of Africa's southern tip, Cape Town (Greve et al., 2020). These islands experience an annual mean temperature of 5.1°C with a rainfall of more than 2 500 mm (Schulze, 1971; Rheeder et al., 1990; Perissinotto et al., 2000; Oosthuizen et al., 2009; Lamont et al., 2019). Prince Edward Islands lie east of the Southwest Indian Ridge and Southeast of the Del Cano Rise and are found in the same path as the eastward flowing Antarctic Circumpolar Current (ACC) and within the Polar Frontal Zone (PFZ) between two fronts, the sub-Antarctic Front (SAF) to the north and the APF to the south (Figure 2.1; Perissinotto et al., 2000; Ansorge and Lutjeharms, 2003: Ryan and Bester, 2008; Ansorge et al., 2012; Carpenter-Kling et al., 2019).

The larger island of the two PEIs is Marion Island, which is approximately 270 km^2, and the smaller island is Prince Edward Island, which is approximately 45 km^2 (Figure 2.1; Oosthuizen et al., 2009; Ansorge et al., 2012; Carpenter-Kling et al., 2019; Lamont et al., 2019). These two islands are 19 km apart, and between them, there is a shallow inter-island shelf with a depth range of 40 to 400 m (Ansorge et al., 2012; Carpenter-Kling et al., 2019; Lamont et al., 2019). Since 1947, the PEIs were ruled by the South African government, and thereafter a meteorological station was proclaimed on Marion Island (Ansorge et al., 2012). Scientific research in these islands began in the 1960s, and these are South Africa's first offshore marine protected areas (Rastorgueff et al., 2016; Lamont et al., 2019). Biodiversity is negatively affected by climate change, biological invasions, and direct human impact (Rastorgueff et al., 2016). This habitat supports an extensive community of marine birds and mammals, and it serves as a breeding and molting location for a variety of animals such as flying seabirds, penguins, and seals (Perissinotto et al., 2000; Ryan and Bester, 2008; Oosthuizen et al., 2009;

Gurney et al., 2014; Carpenter-Kling et al., 2019). The shelf water surrounding the PEIs is rich in benthic fish fauna (Hunt and Pakhomov, 2003).

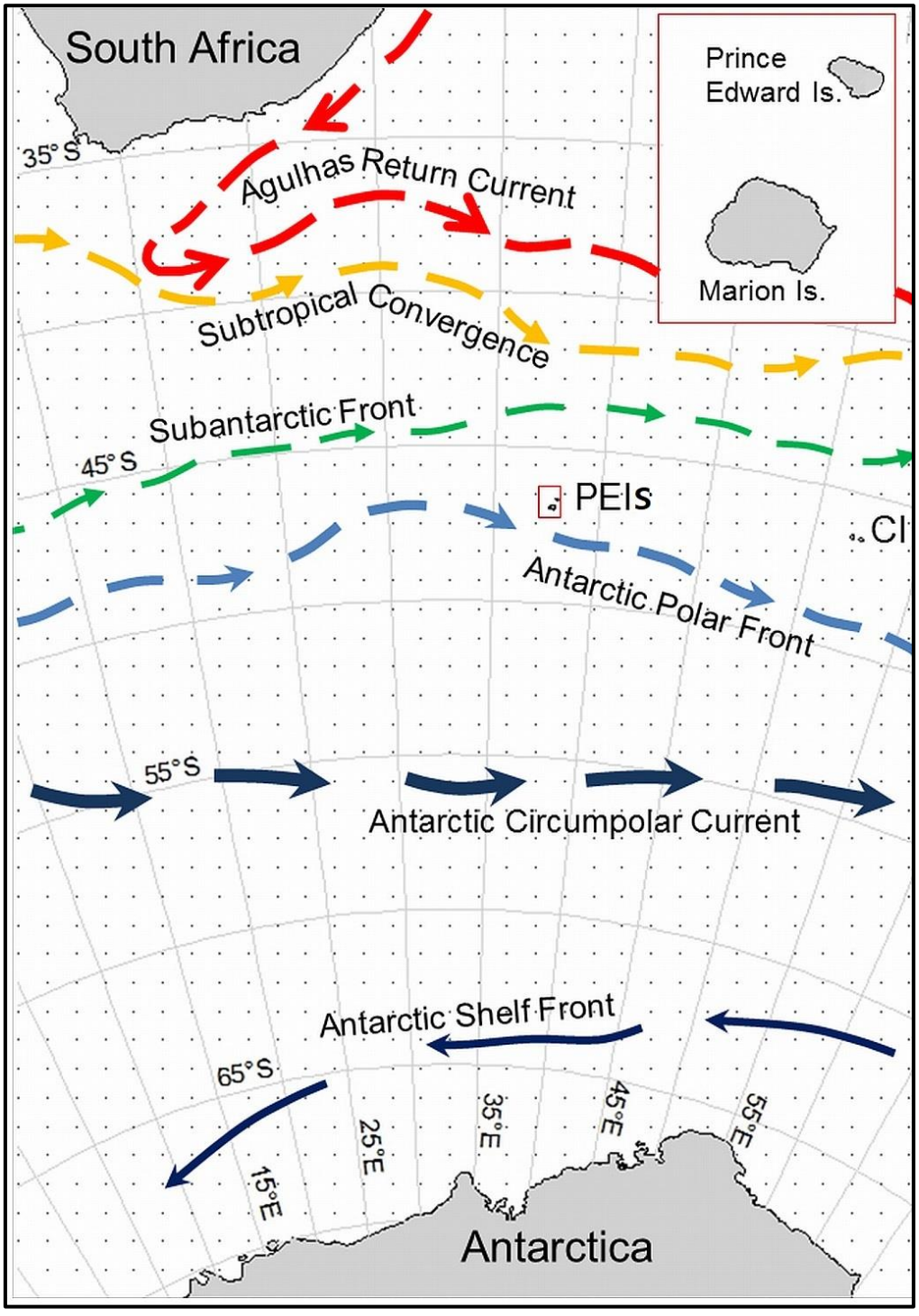

Figure 2.1: Location of the Prince Edward Islands (PEIs) (Kirkman et al., 2016).

Fish specimens for this study were collected north-west of the Prince Edward Islands (PEIs) in 2015 by Shinsei Maru No.3 vessel. The collection was done in depths ranging from 640 to 1800 m using long-line fishing techniques, and specimens were anaesthetised with clove oil, MS222, or quinaldine. Specimens were frozen at -80°C while in the field until they were transported to the South African Institute for Aquatic Biodiversity (SAIAB) for permanent storage in 96% ethanol. Tissue samples were harvested from the tail region of 69 specimens for the molecular analysis, and 22 of these specimens were used for the morphological analysis.

2.2 Molecular analyses

2.2.1 Extraction of genomic DNA

Total genomic DNA was obtained from the tissue samples using the ZYMO Miniprep kit (Zymo Research, USA) following the manufacturer's specifications. For each specimen, approximately 25 mg of tissue sample from the tail region was cut into small pieces and placed in a 1.5 mL microcentrifuge tube. The following reagents were added to each tube; 95 µL of molecular graded water, 95 µL digestion buffer, and 10 µL proteinase K. Samples were then vortexed to mix the contents, thereafter it was incubated at 55°C until the tissue was completely digested. A volume of 700 µL Genomic Lysis Buffer was added to the solution, which was then vortexed. The samples were then centrifuged for 1 minute at 10,000 *x g*. The supernatant was transferred to a Zymo-Spin column that was placed in a collection tube and thereafter it was centrifuged for 1 minute at 10,000 *x g*. A volume of 200 µL DNA Pre-Wash Buffer was added to the spin column and the collection tube was then replaced. Thereafter the sample was centrifuged for 1 minute at 10,000 *x g*. A volume of 400 µL g-DNA Wash Buffer was added to the spin column and the sample was centrifuged once again. The spin column was transferred to a microcentrifuge tube which, contained 60 µL DNA elution buffer that was incubated at room temperature. The samples were then stored at 4°C until used for further application. Thereafter, the DNA concentration of each sample was measured using ND1000 Nanodrop Spectrophotometer (Thermo Fisher Scientific, USA) and this information can be found in Appendix A.

2.2.2 PCR amplification, electrophoresis and sequencing

The partial cytochrome oxidase subunit 1 (CO1) gene region was amplified by polymerase chain reaction (PCR) from the extracted DNA using the universal forward primer dLCO1490 (5' – GGTCAACAAATCATAAAGATATTGG – 3') and the reverse fish primer Fish R1 (5' – ACTTCAGGGTGACCGAAGAATCAGAA – 3') (Folmer et al., 1994; Ward et al., 2005). The total reaction volume for PCR amplification was 25 µL, which constituted of 12.5 µL of one Taq 2X master mix with standard buffer (New England Bio Labs Inc., USA), 9.5 µL of nuclease-free water, 0.5 µL of each primer (10 µM) and 2 µL of genomic DNA (~ 38 – 1071 ng/µL). The PCR conditions used consisted of initial denaturation for 2 minutes at 95°C, followed by 35 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 45 – 54.5°C and extension for 50 seconds at 72°C, and a then final extension for 7 minutes at 72°C and these conditions were conducted using the Veriti 96 – well thermal cycler (Thermo Fisher Scientific, USA). Polymerase chain reaction products were run on a 1% agarose gel at 80 volts for 30 minutes. The agarose gel was stained with ethidium bromide to enable the visualisation of the amplicons. The amplicon's size was determined using Quick-Load 100 bp DNA ladder (New England Bio Labs Inc., USA). All amplicons were visualised using ChemiDoc XRS+ Imaging System (Bio-Rad Laboratories Inc., USA). The successful amplicons (approximately 600 – 700 bp) were sent to the Inqaba Biotech for Sanger sequencing using the forward primer.

2.2.3 Data analyses

Resulted sequences were edited manually by studying individual electropherograms using ChromasPro v2.6.5 (Technelysium, Australia) and saved as an ABI file. Sequences were loaded in MEGA v7.0 software (Kumar et al., 2016) and then aligned using Clustal W which is implemented in MEGA. Thereafter the sequences were trimmed, and the final aligned dataset was 545 base pairs (bp) long. These sequences were uploaded to a Basic Local Alignment Search Tool (BLAST) search engine implemented in National Center for Biotechnology Information (NCBI) website for species identification. Reference sequences for each *Macrourus* species found in the Southern Ocean were downloaded from GenBank (Appendix B – reference sequences from Smith et al. (2011)) and added to the subsequent analyses. Prior to constructing the phylogenetic tree, the best-fit nucleotide substitution model was determined for the current dataset in MEGA v7.0. The model selected for this dataset was the Hasegawa-

Kishino-Yano model plus gamma distribution (HKY+G) and it was used for the reconstruction of the maximum likelihood phylogenetic tree with 1000 bootstrap replications to assess the nodal support was constructed in MEGA v7.0. A minimum spanning tree of the haplotypes was generated using the median-joining method on Network software v5.0.1.1 (Bandelt et al., 1999). The sequence pairwise divergence within and among *Macrourus* species was analysed using MEGA v7.0. The genetic diversity indices were calculated as the number of haplotypes, haplotype diversity (H) and nucleotide diversity (π), and polymorphic sites using DnaSP v5.1 (Librado and Rozas, 2009). The DnaSP program was also used to generate the mismatch distribution curve and the associated raggedness index. The fixation index (F_{ST}) among *Macrourus* species was analysed using Arlequin v3.11 (Excoffier and Lischer, 2010).

2.3 Morphological analyses

2.3.1 Identification

Prior to morphological analyses, study specimens were separated into *M. holotrachys* and *M. carinatus* following the molecular identification. The morphological relationship between these species was compared by analysing both morphometric and meristic characters.

2.3.2 Measurements of specimens

The weight (g) of each specimen was recorded using an electrical scale. Measurements were recorded on each specimen's same side, with 22 morphometric and five meristic characters (Table 2.1). The method of morphometrics employed in this study is regarded as the "traditional" distance-based method, which includes the use of standard linear measurements (Maderbacher et al., 2008). The morphometric measurements were done using a Vernier caliper with an accuracy increment of 0.1 mm. The standard and total lengths of the fish specimens could not be measured due to the tapering tail being bent or damaged. The meristic characters were counted manually for the different fin rays, and scales were counted with the aid of a dissecting microscope.

2.3.3 Data analyses

All statistical analyses were performed using R Software v4.1.0 (R Development Core Team 2013). Univariate analyses, such as an independent sample *t*-test, and multivariate analyses including the permutational multivariate analysis of variance (PERMANOVA) and a principal component analysis (PCA) were used to analyses the study data. The independent sample *t*-test was used to determine if there was a significant difference or not for each variable between the two species. Assumptions such as homogeneity of variance and normality of data were tested. When the assumption of normality was not normally distributed, a non-parametric Mann Whitney *U* test was conducted. The PERMANOVA was used to determine if there was an overall significant difference or not between the two species. The data used for the PERMANOVA was transformed prior to analysis. The PCA was used to determine if the species could be separated from each other based on the variables measured. The data for PCA was transformed by scaling prior to analysis. Assumptions such as linearity and variables that are correlated with each other were tested. These analyses were performed for morphometric and meristic characters.

Table 2.1: Morphological and meristic characters measured for each fish specimen

Morphological character	Meristic character
Head length	Dorsal fin rays
Snout length	Pectoral fin rays
Orbital length	Pelvic fin rays
Postorbital length	Scales from anal fin to lateral line (diagonal)
Orbit to preopercle length	Scales from anal fin to lateral line (vertical)
Interspace between first and second dorsal fin	
First dorsal fin height	
Pelvic fin length	
Pectoral fin length	
Snout to dorsal fin length	
Dorsal fin to pectoral fin	
Dorsal fin to operculum tip	
Pectoral fin to pelvic fin	
Dorsal fin length	
Dorsal fin to anal fin	
Pelvic fin to anal fin	
Dorsal fin back to anal fin	
Body depth	
Snout to pectoral fin	
Snout to pelvic fin	
Pectoral fin to anal fin	
Dorsal fin to pelvic fin	

CHAPTER 3: RESULTS

3.1 Molecular analyses

The BLAST search revealed that of the 69 *Macrourus* specimens analysed, 53 were *M. holotrachys*, and 16 were *M. carinatus* (Table 3.1), with similarity percentages ranging from 99 – 100%. The maximum likelihood tree formed two distinctive clades with bootstrap support values of 72% and 93%, respectively (Figure 3.1 (a)). Similarly, the haplotype network tree also showed two main clades separated by nine mutational steps (Figure 3.1 (b)). The results of these trees support that of the BLAST search indicating the presence of two *Macrourus* species in this dataset. Furthermore, the sequence divergence between these two species was 2.1%, with F_{ST} value of 0.98 (Table 3.2). These values are high enough to indicate the presence of high level of genetic differentiation between *M. holotrachys* and *M. carinatus*, thus confirming that there are different species.

The overall genetic variation test for the analysed samples showed a low haplotype diversity and a high nucleotide diversity (Table 3.1). When comparing the variations within the two species, *M. carinatus* showed the highest haplotype diversity (0.505), nucleotide diversity (0.00154), the number of polymorphic sites (5), and the number of haplotypes (5) (Table 3.1). Conversely, the nucleotide diversity was low, and therefore, the genetic variation results of this species indicate a population that has expanded. Consistently, the mismatch distribution for this species had a unimodal curve and a low raggedness index (*r*) value of 0.113 (Figure 3.2 and Table 3.1), which are also indicators of a population undergoing an expansion. The results of *M. holotrachys*, on the other hand, showed low haplotype (0.069) and nucleotide (0.00013) diversities, which is an indication of a population that underwent a population bottleneck or founder event. In contrast, the mismatch distribution curve results and the high *r* value of 0.748 (Table 3.1 and Figure 3.3) indicated characteristics of a stable population for *M. holotrachys*.

Table 3.2: Genetic variation for *Macrourus carinatus* and *Macrourus holotrachys* from Prince Edward Islands.

Species	n	S	H	hd	π	*r**
M. carinatus	20	5	5	0.505	0.00154	0.113
M. holotrachys	57	1	2	0.069	0.00013	0.748
Total	77	15	7	0.460	0.00826	

n – sample size, S – number of polymorphic sites, H – number of haplotypes, hd – haplotype diversity, π – nucleotide diversity, *r** – significant raggedness index.

Table 3.3: Pairwise distance percentages are given in ranges within species and between species (below diagonal), and pairwise F_{ST} values in above diagonal for *Macrourus* species.

Species	Ranges within species	*M. caml*	*M. whitsoni*	*M. carinatus*	*M. holotrachys*	Outgroup
M. caml	0.00 – 0.20		0.90	**0.92**	**0.98**	**0.64**
M. whitsoni	0.00	0.60		**0.92**	**0.98**	**0.64**
M. carinatus	0.00 – 0.60	2.00	1.70		**0.98**	**0.89**
M. holotrachys	0.00 – 0.20	1.00	0.70	2.10		**0.96**
Outgroup	0.00 – 15.2	16.4	16.2	16.3	16.1	

*Pairwise F_{ST} values in **BOLD** are significant.

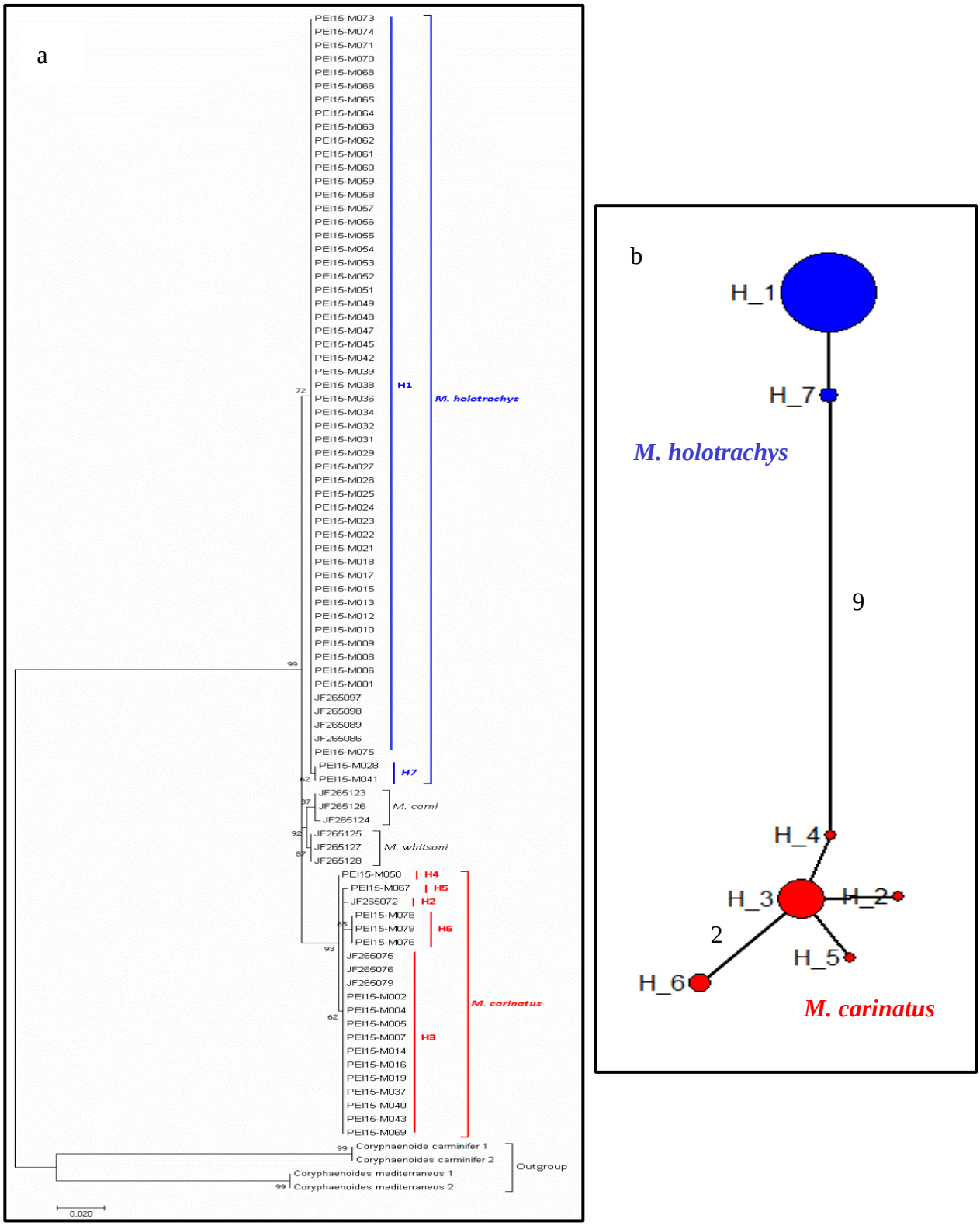

Figure 3.1: Maximum likelihood tree (a) and median-joining network (b) of *Macrourus* specimens reconstructed with mtDNA CO1 dataset. The size of haplotypes in (b) is proportional to the frequency of a haplotype, and where there is no number between haplotypes, it indicates one mutational step.

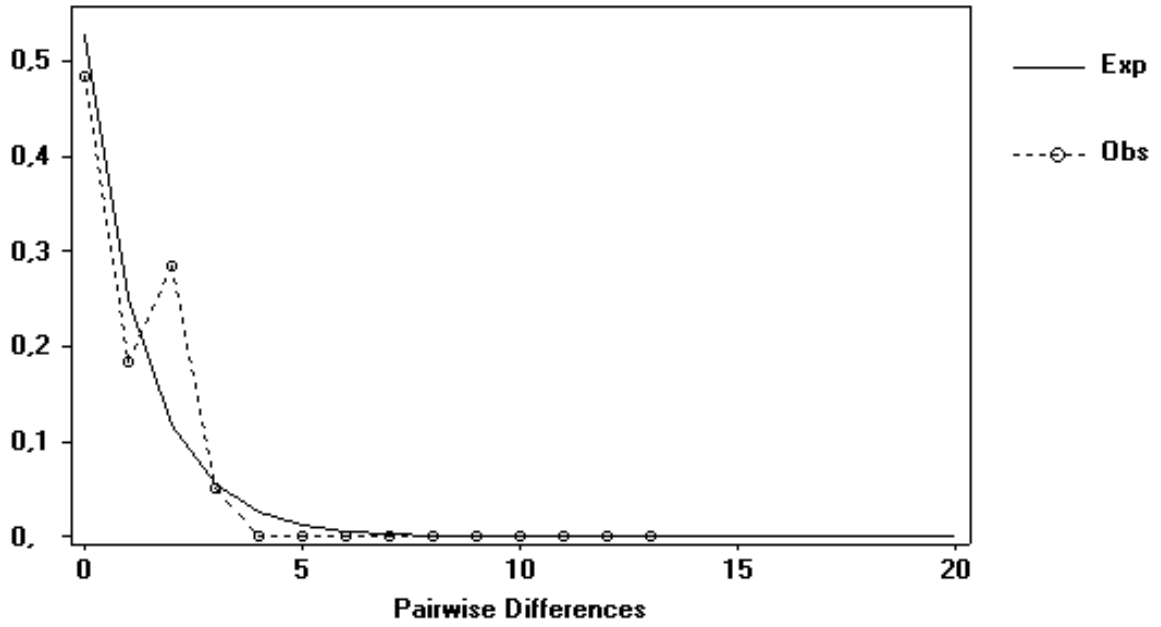

Figure 3.2: Mismatch distribution graph of *Macrourus carinatus* under constant population growth model. Obs is observed pairwise difference, and Exp is expected mismatch distributions under this model.

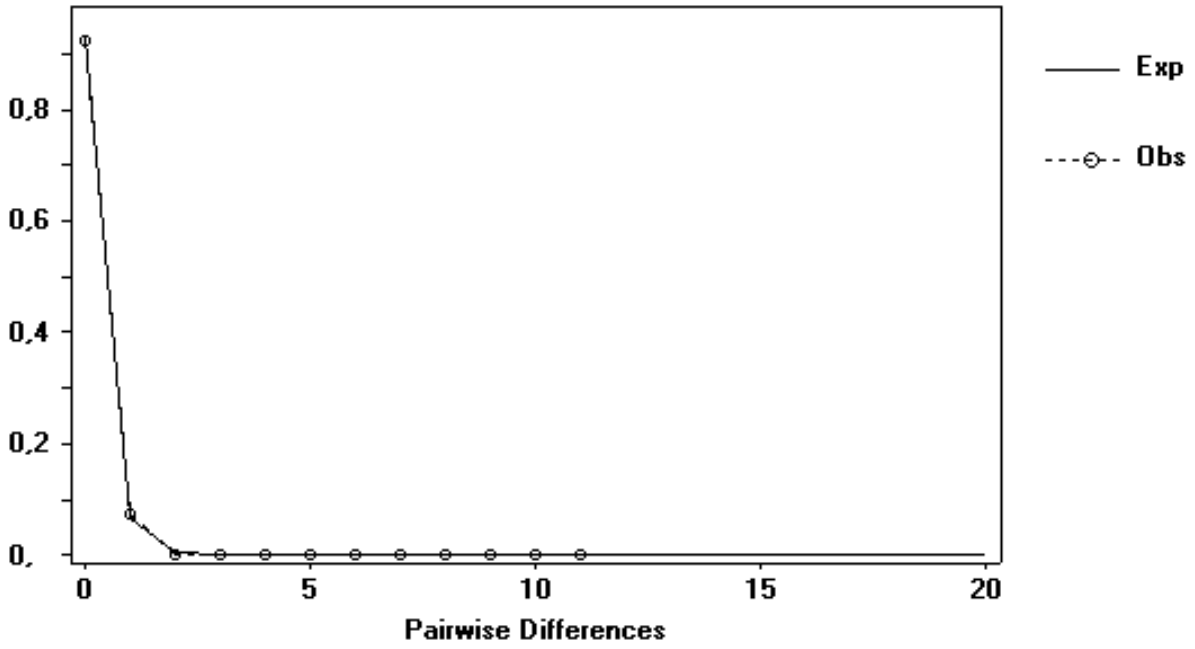

Figure 3.3: Mismatch distribution graph of *Macrourus holotrachys* under constant population growth model. Obs is observed pairwise difference, and Exp is expected mismatch distributions under this growth model.

3.2 Morphological analyses

The separation of the species/specimens were based on the molecular analyses outcomes. The visual and microscopic examination of species showed that, *M. holotrachys* had few to no scales present on the underside of the head while *M. carinatus* had much more scales present on the underside of the head. Another character that differed between the two species was the spinules present on their scales. *Macrourus carinatus* displayed many spinules compared *M. holotrachys*, which was clearly seen using the dissecting microscope.

The parallel plot for the morphometric and meristic characters showed high mean values for *M. holotrachys* and low for *M. carinatus*, except for two morphometric measurements – pectoral fin to pelvic fin and postorbital length (Figure 3.4). However, the coefficient of variance plot indicated there was a high level of variations in *M. carinatus*, while *M. holotrachys* showed low variation, except for three meristic characters – scales from anal fin to lateral line (vertically), pelvic and pectoral fin rays (Figure 3.5). Based on the independent *t*-test, the species weights were not significantly different ($p > 0.05$). Out of the 22 morphometric characters analysed, the mean of five characters (snout length, orbit to preopercle length, dorsal fin length, pelvic fin to anal fin length, snout to pectoral length) was significantly different between *M. carinatus* and *M. holotrachys* (Table 3.4). Approximately 77% of the morphological characters measured between the two species were not significantly different. The results from the PERMANOVA analyses indicated that there is no significant difference for the morphological characters between the two species (Table 3.5). The independent *t*-test and Mann Whitney *U* test analyses of the meristic characters was only significantly different for one character (dorsal fin rays) while the rest of the characters were not significantly different between *M. carinatus* and *M. holotrachys* (Table 3.6). This was further supported by the PERMANOVA results which also found no significant difference between the two species (Table 3.7).

The first two axes of principle component analyses (PCA) explained approximately 83% and 63% of species variation, respectively, for morphological and meristic characters (Figure 3.6; Figure 3.7). However, both these results did not clearly separate the two *Macrourus* species from each other instead they had overlapped (Figure 3.6; Figure 3.7).

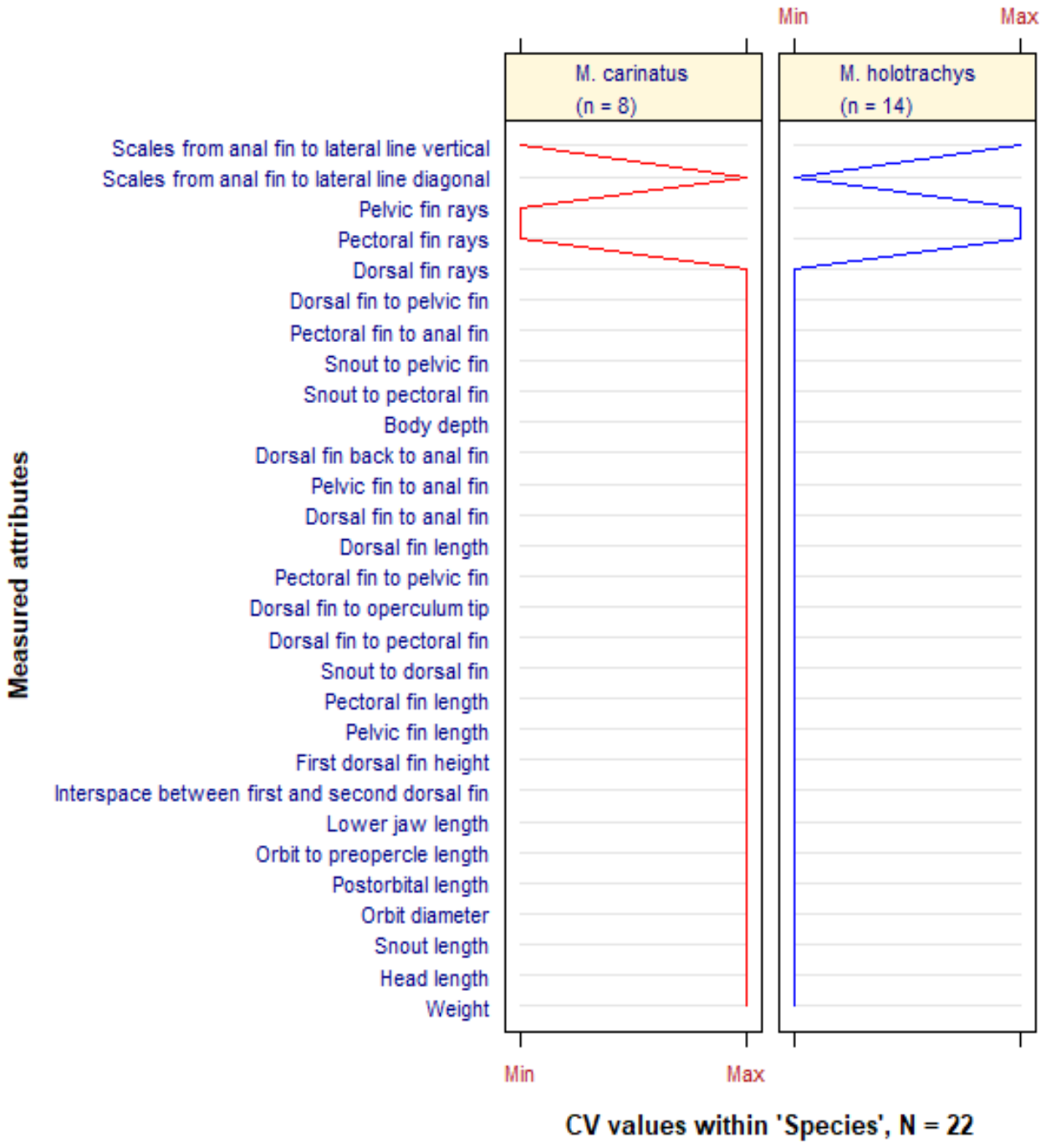

Figure 3.4: Parallel plot showing the minimum and maximum mean values for morphometric and meristic characters for *Macrourus carinatus* and *Macrourus holotrachys*.

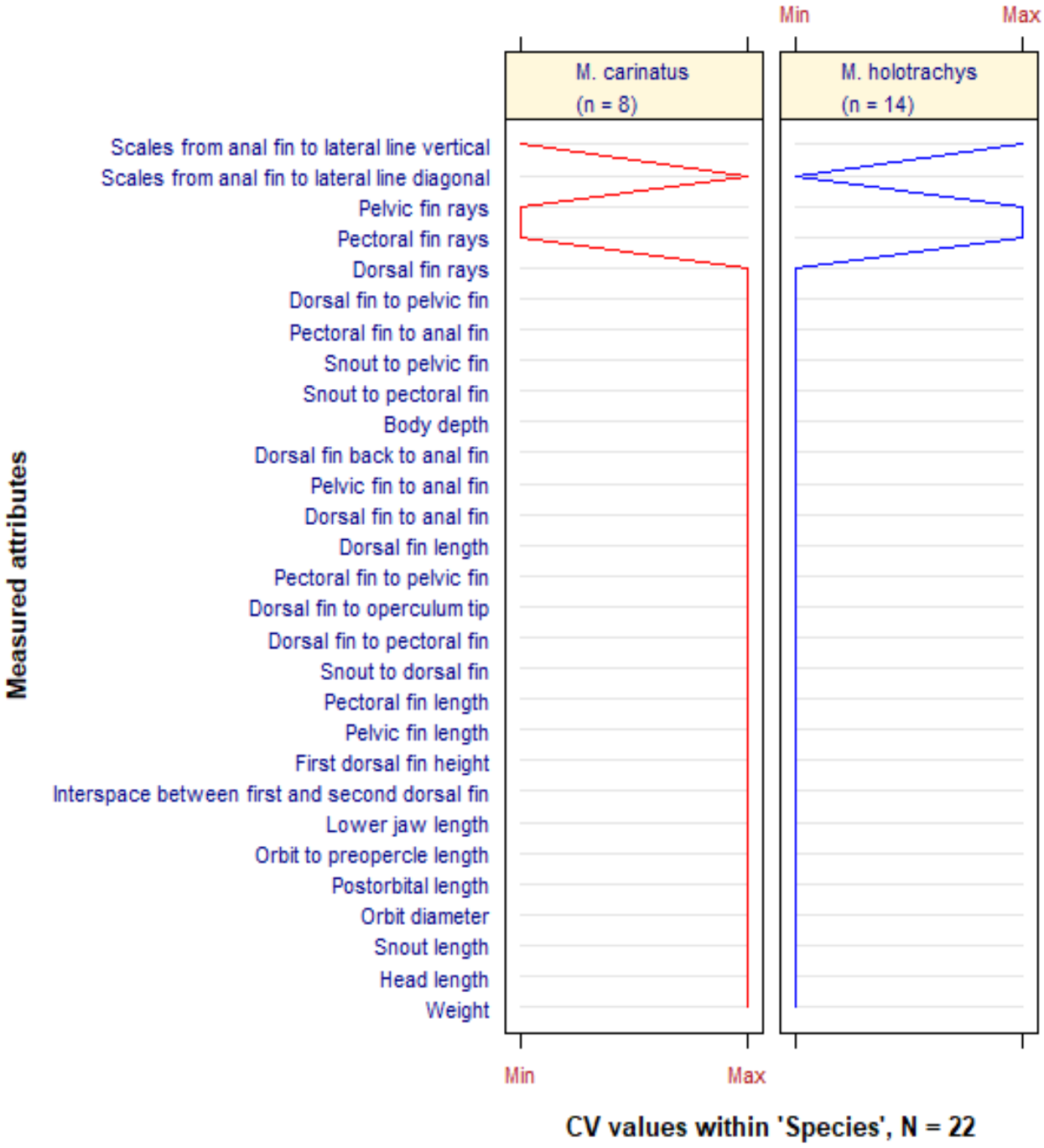

Figure 3.5: Parallel plot showing the minimum and maximum coefficient of variance values for morphometric and meristic characters for *Macrourus carinatus* and *Macrourus holotrachys*.

Table 3.4: Weight in grams (g) and morphometric characters used to analyse *Macrourus* specimens, including averages for each character in millimetres (mm) and their test statistic data determined by an independent *t*-test.

Morphological character	Mean±SD(CV%) *M. carinatus* (n=8)	Mean±SD(CV%) *M. holotrachys* (n=14)	*p* value
Weight (g)	634±376(59.4)	803±279(34.7)	0.241
Head length	123±18.3(14.8)	137±14.3(10.4)	0.075
Snout length	35.8±4.64(13.0)	41.1±3.67(8.9)	**0.008**
Orbit diameter	44.0±4.85(11.0)	48.0±4.47(9.3)	0.063
Postorbital length	57.6±17.1(29.7)	55.1±9.68(17.6)	0.653
Orbit to preopercle length	88.1±15.5(17.6)	104±15.1(14.5)	**0.029**
Lower jaw length	53.3±10.3(19.3)	55.7±8.03(14.4)	0.547
Interspace between first and second dorsal fin	22.3±5.64(25.3)	23.7±4.68(19.7)	0.534
First dorsal fin height	67.1±12.0(17.9)	73.1±8.00(11.0)	0.179
Pelvic fin length	45.5±10.0(22.0)	46.5±8.22(17.7)	0.800
Pectoral fin length	65.2±13.6(20.8)	70.0±6.40(9.1)	0.275
Snout to dorsal fin	139±21.6(15.6)	155±15.2(9.8)	0.052
Dorsal fin to pectoral fin	44.4±8.75(19.7)	50.0±7.51(15.0)	0.127
Dorsal fin to operculum tip	42.0±9.82(23.4)	47.4±5.63(11.9)	0.112
Pectoral fin to pelvic fin	48.6±11.4(23.5)	46.8±6.55(14.0)	0.628
Dorsal fin length	28.7±5.64(19.6)	33.4±4.29(12.8)	**0.040**
Dorsal fin to anal fin	96.1±19.9(20.7)	110±16.9(15.3)	0.086
Pelvic fin to anal fin	70.0±11.3(16.2)	86.9±13.6(15.7)	**0.008**
Dorsal back to anal fin	74.8±17.2(22.9)	84.7±14.8(17.5)	0.169
Body depth	86.0±18.0(21.0)	92.2±14.3(15.5)	0.387
Snout to pectoral fin	120±19.3(16.1)	137±14.2(10.4)	**0.025**
Snout to pelvic fin	126±21.8(17.3)	143±18.1(12.6)	0.060
Pectoral fin to anal fin	86.7±17.1(19.8)	101±16.7(16.6)	0.073
Dorsal fin to pelvic fin	82.1±16.0(19.4)	86.8±11.3(13.0)	0.432

n – sample size, values in **BOLD** are significantly different.

Table 3.5: Permutational multivariate analysis of variance results of the morphometric characters for *Macrourus carinatus* and *Macrourus holotrachys*.

	Degrees of freedom	Sum of squares	R2	F	*p* value
Species	1	0.01317	0.07715	1.6721	0.08709
Residual	20	0.15752	0.92285		
Total	21	0.17070	1.00		

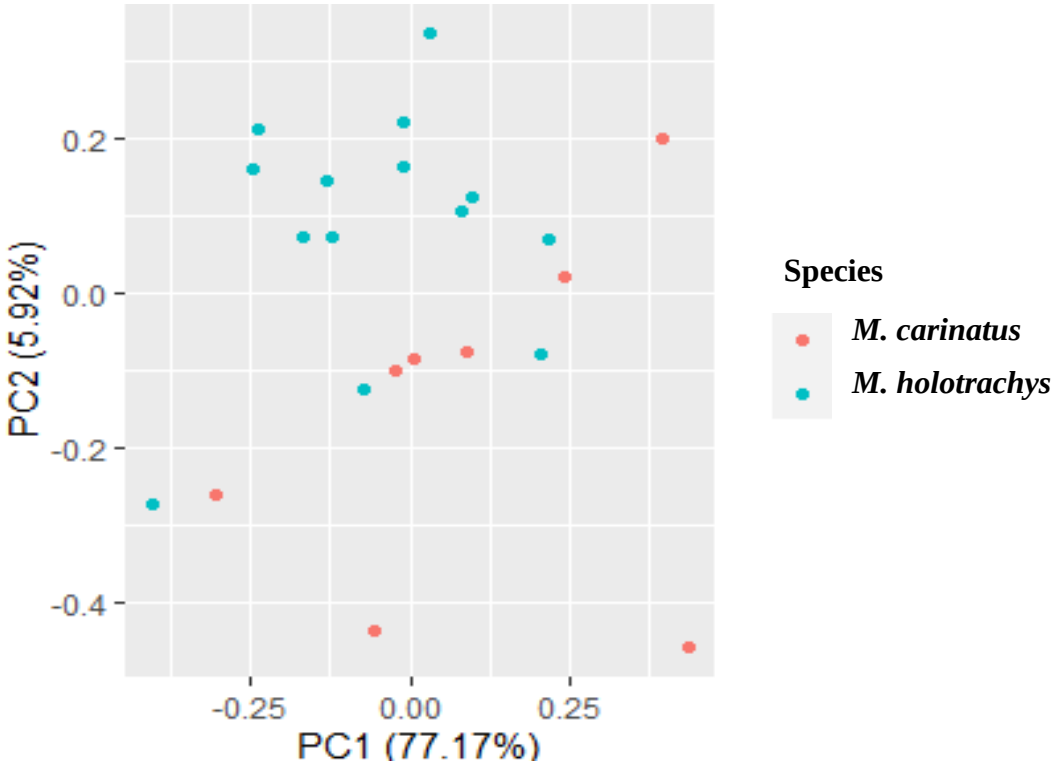

Figure 3.6: Principal component analysis (PCA) based on morphometric characters for *Macrourus* species.

Table 3.6: Meristic characters used to analyse *Macrourus* specimens, including counts for each character and their test statistic data determined by an independent *t*-test.

Meristic character	*M. carinatus* (n=8) Counts	*M. holotrachys* (n=14) Counts	*p* value
Dorsal fin rays	10 – 11	10 – 11	**0.044***
Pectoral fin rays	16 – 19	16 – 20	0.173
Pelvic fin rays	8 – 9	8 – 9	0.646*
Scales from anal fin to lateral line (diagonal)	18 – 26	18 – 25	0.231
Scales from anal fin to lateral line (vertical)	9 – 14	8 – 16	0.652

n – sample size, *Mann Whitney *U* test conducted, values in **BOLD** are significantly different.

Table 3.7: Permutational multivariate analysis of variance results of the meristic characters for *Macrourus carinatus* and *Macrourus holotrachys*.

	Degrees of freedom	Sum of squares	R2	F	*p* value
Species	1	0.006400	0.06157	1.3122	0.1976
Residual	20	0.097545	0.93843		
Total	21	0.103945	1.00		

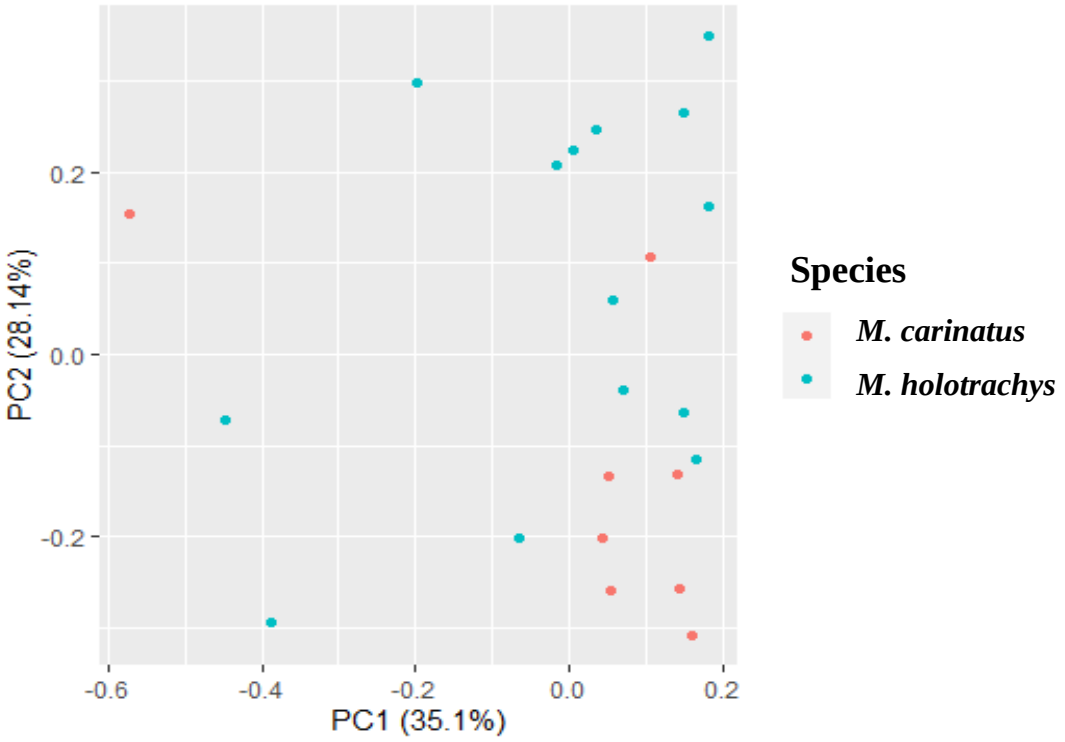

Figure 3.7: Principal component analysis (PCA) based on meristic characters for *Macrourus* species.

CHAPTER 4: DISCUSSION

4.1 Summary

The aim of this study was to review *Macrourus* species found near the Prince Edward Islands (PEIs), which are islands found in the sub-Antarctic region of the Southern Ocean. The review was done by analysing data collected around these islands using both molecular and morphological methods. Molecular analyses revealed that the dataset consists of two species, *M. carinatus* and *M. holotrachys*, with majority of the dataset identified as *M. holotrachys*. Morphological analyses, on the other hand, could not separate these two species indicating that they are morphologically similar.

Previous morphological studies did not indicate the presence of *M. holotrachys* near PEIs, only *M. carinatus* was recorded (Cohen et al., 1990; Iwamoto, 1990; Duhamel et al., 2014). Given that, in the current study, approximately 77% of the specimens analysed by the CO1 molecular marker were identified as *M. holotrachys*, there might be two possible explanations for these observations. The first explanation might be a possible misidentification, as the two species are known to exhibit similar morphological appearance and characters, which was evident in the current study's morphological analyses. The second explanation might be that *M. holotrachys* is a recent migrate to these islands, hence it was not collected by previous studies. However, it is not unusual to have identified *M. holotrachys* in the same area (near PEIs) as *M. carinatus* since they are referred to as sibling species and are noted to occur in similar habitats (Laptikhovsky, 2005; Smith et al., 2011). When these species are in the same habitat, they feed on different food sources, i.e., *M. carinatus* feeds on pelagic prey such as salps, amphipods, euphausid, while *M. holotrachys* feed on more benthic prey such as amphipods, decapods, isopods, and polychaetes. As such, competition for food sources is reduced between the two species, and therefore, they can thrive in the same environment (Laptikhovsky, 2005).

4.2 Molecular analyses

The current study analysed the CO1 gene to identify species as this method was successfully used by Smith et al. (2011) to identify *Macrourus* species and highlighted a cryptic species. The final aligned sequences used in the present study were 545 bp long, which according to Hajibabaei et al. (2006), is considered a sufficient length for species identification. The BLAST

search platform was used for species identification, and the sequence matches that were considered reliable were 99% and 100%, as they fall within the acceptable sequence match percentages (97 to 100%) for species identification (Wong and Hanner, 2008; Panprommin and Panprommin, 2017). During the BLAST search, sequences with a higher similarity percentage were those published by Smith et al. (2011), and resulted in the successful identification of the two *Macrourus* species, *M. carinatus* and *M. holotrachys*. Like Smith et al. (2011), the sequence distance between these species was low, a common feature for marine species (Ward et al., 2005). This was also evident when comparing sequence distances among all *Macrourus* species (Table 3.2), which could imply a recent evolutionary divergence and/or a low rate of nucleotide substitution within this genus (Smith et al., 2011).

According to Grant and Bowen (1998), mitochondrial DNA enables marine fish to be divided into four categories based on haplotype and nucleotide diversity values. The first category contains species that have low values for both haplotype (hd < 0.5) and nucleotide ($\pi < 0.005$) diversities; the second category contains species that have high haplotype and low nucleotide diversity values; the third category contains species that have low haplotype and high nucleotide diversity values; and lastly, the fourth category contains species that have high values for both haplotype and nucleotide diversities. The overall results for the *Macrourus* species analysed in the present study had characteristics of the third category, indicating divergence between subdivided populations, which is the presence of separate/different populations that are reproductively isolated (Grant and Bowen, 1998). The presence of two genetically different populations was further supported by a high significant F_{ST} value.

Macrourus carinatus displayed characteristics of the second category indicating a recent population expansion, which is usually associated with population growth and an increase in mutations following the decline in the population size (Grant and Bowen, 1998). Thus, this may imply that the species had previously experienced a population bottleneck associated with a rapid decline of the population size due to environmental events or human activities (Parmaksiz and Eksi, 2017; Xu et al., 2019b). The PEIs are declared as marine protected areas due to high human activities that led to the decline of some marine communities, including fish communities (Rastorgueff et al., 2016). *Macrourus* species were caught as by-catch in many toothfish fisheries around the Southern Ocean (Devine et al., 2012; Pinkerton et al., 2015). These, as a result, might have contributed to the past decline of *M. carinatus* population. Since the PEIs are marine protected areas, this would have reduced the fishing intensity resulting in

population growth. This characteristic is further supported by the observed star topology haplotype network tree and unimodal mismatch distribution curve (Figure 3.1 (b); Figure 3.2).

Macrourus holotrachys, on the other hand, had characteristics of the first category, with low genetic diversities that are usually associated with a recent population bottleneck or a founder event by a single or few mtDNA lineages (Grant and Bowen, 1998; Keskin et al., 2012; Parmaksiz and Eksi, 2017). A founder event is when a few specimens start a new population from the original population (Parmaksiz and Eksi, 2017; Sun et al., 2017). It could be a possibility that *M. holotrachys* had recently migrated near the PEIs, hence not identified in this area before. This could be the reason for the mismatch distribution and the associated significant raggedness index indicating that *M. holotrachys* is a stable population.

4.3 Morphological analyses

Unlike molecular analyses, morphological analyses did not separate the study specimens/species into two distinctive groups. The difference between the two analyses is that molecular analyses use nucleotide sequences while morphology relay on phenotypes. The species phenotype is the primary way in which they interact with the environment. Thus, changes or adaptations to the environment result in changes to the organism's physiology and behaviour and, overall, it causes changes to their phenotype/morphology (Turan, 1999; Rawat et al., 2017). Due to *M. holotrachys* and *M. carinatus* occupying the same habitat, they appear to respond similarly in terms of adapting to their environmental factors such as food availability or prolonged swimming and fishing intensity (Turan, 2004; González et al., 2016; Kaouèche et al., 2017; Chen et al., 2018). As a result, these two species have overlapping morphological characters**.** The findings of this study are in agreement with Cohen et al. (1990) and Smith et al. (2011) which mentioned that species within the *Macrourus* group appear to be morphologically similar. Even though these species are phenotypically similar, there is no interspecific hybridisation between them as evident from the genetic analysis (Figure 3.1; Takács, 2012).

Based on previous studies, separating species using morphology can be difficult when species are morphologically similar (Teletchea, 2009). This was also noted within the *Macrourus* genus, where *M. holotrachys* have been morphologically confused with other species. Cohen et al. (1990) reported that *M. carinatus* was previously considered a synonym of *M. holotrachys*,

but these species were later identified as separate species. The characters used to separate these species were stated to overlap but was more efficient when used in combination (Cohen et al., 1990). An important feature that was noted between these species was related to the amount of scales found on the underside of the head, with *M. holotrachys* having a file or a narrow patch of scales towards the jaw while *M. carinatus* being densely covered with scales on the underside of the head (Cohen et al., 1990). However, using this character becomes an issue when the scales are sloughed off during the collection process (Cohen et al., 1990). Another distinguishing character between these species is the arrangement of the central row of enlarged spinules, *M. carinatus* have lateral ridges that has small spines while *M. holotrachys* having lateral ridges that lack spinules McMillan et al. (2012). Notes on these differences were recorded in the current study, and other significantly different characters included snout length, orbit to preopercle length, dorsal fin length, pelvic fin to anal fin length, snout to pectoral length (Table 3.3).

4.4 Conclusion

The molecular results from this study indicated the presence of two genetically different *Macrourus* species near the PEIs. The species formed two distinctive clades on the maximum likelihood and haplotype network trees, and they had an evolutionary distance of 2.1%. The morphological results could not separate the two species, although the main distinguishing characters were observed. These findings indicate the presence of two *Macrourus* species near the PEIs, which are morphologically similar.

Correct species identification is essential for all fish species, including *Macrourus*, as this information plays an important role when implementing conservation management strategies. Identification of *Macrourus* species based on morphological characters is challenging as these species appear similar. Using both molecular and morphological approaches are recommended when reviewing this genus, as it gives an insight into the genetic and phenotype structures of the genus.

4.5 Recommendations for future studies

Information provided by this study is based on data collected from a single location and, therefore, samples from multiple locations around the PEIs should be added for more information. *Macrourus* species previously identified using morphological analyses should also be reviewed genetically, especially since species in this group appear to be morphologically similar. For the molecular analysis, multiple primers had to be tested using several different combinations of forward and reverse primers before the correct combination was obtained, thus there is a need for species-specific primers to be designed. Using species-specific primers will be less time-consuming in comparison to using the trial-and-error method. To accurately determine if this species did experience a population bottleneck, nuclear microsatellites should be considered (Chuluunbat et al., 2014; Wang et al., 2019). Other types of morphometric analyses that should be considered include, 1) geometric morphometrics, which is based on landmarks and is more sensitive to changes (Fazazi et al., 2019); 2) otoliths, which have phylogenetic signals, high level of morphological variability and are species-specific (Teimori et al., 2019); and 3) scales, which have shapes that are highly variable at different classification levels and they are species-specific (Ibáñez and Jawad, 2018). Using some of these methods are more cost effective and less time consuming than molecular analyses (Ibáñez and Jawad, 2018).

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APPENDIX A: DNA CONCENTRATIONS AND TYPE OF ANALYSES CONDUCTED ON EACH *MACROURUS* FISH SPECIMENS.

	Species name	**DNA concentrations (ng/µL)**	**Molecular analysis**	**Morphological analysis**
PEI15-M001	*M. holotrachys*	255.67	✓	
PEI15-M002	*M. carinatus*	259.36	✓	✓
PEI15-M003	*M. carinatus*			✓
PEI15-M004	*M. carinatus*	196.32	✓	✓
PEI15-M005	*M. carinatus*	101.57	✓	✓
PEI15-M006	*M. holotrachys*	265.32	✓	✓
PEI15-M007	*M. carinatus*	116.21	✓	✓
PEI15-M008	*M. holotrachys*	391.64	✓	✓
PEI15-M009	*M. holotrachys*	144.9	✓	✓
PEI15-M010	*M. holotrachys*	72.8	✓	
PEI15-M011	*M. holotrachys*			✓
PEI15-M012	*M. holotrachys*	204.12	✓	
PEI15-M013	*M. holotrachys*	174.94	✓	✓
PEI15-M014	*M. carinatus*	109.45	✓	✓
PEI15-M015	*M. holotrachys*	78.31	✓	
PEI15-M016	*M. carinatus*	138.22	✓	✓
PEI15-M017	*M. holotrachys*	200.65	✓	✓
PEI15-M018	*M. holotrachys*	92.72	✓	✓
PEI15-M019	*M. carinatus*	252.18	✓	✓
PEI15-M021	*M. holotrachys*	151.74	✓	
PEI15-M022	*M. holotrachys*	199.78	✓	✓
PEI15-M023	*M. holotrachys*	223.12	✓	
PEI15-M024	*M. holotrachys*	98.63	✓	✓
PEI15-M025	*M. holotrachys*	121.07	✓	✓
PEI15-M026	*M. holotrachys*	143.70	✓	
PEI15-M027	*M. holotrachys*	67.99	✓	✓
PEI15-M028	*M. holotrachys*	78.83	✓	✓

PEI15-M029	*M. holotrachys*	35.91	✓	✓
PEI15-M031	*M. holotrachys*	149.87	✓	
PEI15-M032	*M. holotrachys*	118.03	✓	
PEI15-M034	*M. holotrachys*	84.97	✓	
PEI15-M036	*M. holotrachys*	149.23	✓	
PEI15-M037	*M. carinatus*	77.37	✓	
PEI15-M038	*M. holotrachys*	160.02	✓	
PEI15-M039	*M. holotrachys*	93.88	✓	
PEI15-M040	*M. carinatus*	116.85	✓	
PEI15-M041	*M. holotrachys*	411.69	✓	
PEI15-M042	*M. holotrachys*	368.20	✓	
PEI15-M043	*M. carinatus*	410.46	✓	
PEI15-M045	*M. holotrachys*	176.41	✓	
PEI15-M047	*M. holotrachys*	138.43	✓	
PEI15-M048	*M. holotrachys*	108	✓	
PEI15-M049	*M. holotrachys*	568.76	✓	
PEI15-M050	*M. carinatus*	91.50	✓	
PEI15-M051	*M. holotrachys*	63.24	✓	
PEI15-M052	*M. holotrachys*	166	✓	
PEI15-M053	*M. holotrachys*	85.83	✓	
PEI15-M054	*M. holotrachys*	108.06	✓	
PEI15-M055	*M. holotrachys*	135.31	✓	
PEI15-M056	*M. holotrachys*	38.5	✓	
PEI15-M057	*M. holotrachys*	155.5	✓	
PEI15-M058	*M. holotrachys*	129.43	✓	
PEI15-M059	*M. holotrachys*	174.7	✓	
PEI15-M060	*M. holotrachys*	184.9	✓	
PEI15-M061	*M. holotrachys*	128.97	✓	
PEI15-M062	*M. holotrachys*	76.76	✓	
PEI15-M063	*M. holotrachys*	185.42	✓	
PEI15-M064	*M. holotrachys*	124.52	✓	
PEI15-M065	*M. holotrachys*	195.15	✓	

PEI15-M066	*M. holotrachys*	77.82	✓	
PEI15-M067	*M. carinatus*	93.73	✓	
PEI15-M068	*M. holotrachys*	91.96	✓	
PEI15-M069	*M. carinatus*	61	✓	
PEI15-M070	*M. holotrachys*	515.44	✓	
PEI15-M071	*M. holotrachys*	176.1	✓	
PEI15-M073	*M. holotrachys*	27.17	✓	
PEI15-M074	*M. holotrachys*	108.97	✓	
PEI15-M075	*M. holotrachys*	114.02	✓	
PEI15-M076	*M. carinatus*	244.9	✓	
PEI15-M078	*M. carinatus*	1071	✓	
PEI15-M079	*M. carinatus*	533.8	✓	

APPENDIX B: REFERENCE SEQUENCE ACCESSION NUMBERS OF SPECIES USED FROM GENBANK DATABASE.

Accession number	Species	Voucher ID	Locality
JF265125	*M. whitsoni*	WGR1	Ross Sea
JF265127	*M. whitsoni*	P42316	Ross Sea
JF265128	*M. whitsoni*	P42295 TS1600	Durmont D'Urville Sea
JF265123	*M. caml*	WGR4	Ross Sea
JF265124	*M. caml*	WGR3	Ross Sea
JF265126	*M. caml*	P42587	South Georgia
JF265089	*M. holotrachys*	BW-A4421	Heard and McDonald Islands
JF265086	*M. holotrachys*	BW-A4424	Heard and McDonald Islands
JF265098	*M. holotrachys*	P42574	South Georgia
JF265097	*M. holotrachys*	P42575	South Georgia
JF265072	*M. carinatus*	BW-A4418	Heard and McDonald Islands
JF265075	*M. carinatus*	MCA1	Chatham Rise, New Zealand
JF265076	*M. carinatus*	MCA2	Chatham Rise, New Zealand
JF265079	*M. carinatus*	MCA7	Southern Plateau, New Zealand
MF956605	*Coryphaenoides carminifer*		
MF956604	*Coryphaenoides carminifer*		
KY345269	*Coryphaenoides mediterraneus*		
KY345268	*Coryphaenoides mediterraneus*		

APPENDIX C: DESCRIPTIVE STATISTICS FOR MORPHOLOGICAL CHARACTERS.

Morphological character	***M. carinatus* (n=8)**	***M. holotrachys* (n=14)**
Weight (g)		
Median(Q1-Q3)	635(354-751)	740(639-959)
Min-Max	218-1410	420-1450
Head length		
Median(Q1-Q3)	125(109-134)	133(128-145)
Min-Max	101-154	113-167
Snout length		
Median(Q1-Q3)	37.1(32.3-38.2)	41.0(38.5-44.2)
Min-Max	28.3-42.9	35.5-47.5
Orbit diameter		
Median(Q1-Q3)	45.3(42.4-46.8)	48.2(45.2-50.0)
Min-Max	35.9-50.1	39.3-55.8
Postorbital length		
Median(Q1-Q3)	52.8(46.3-70.7)	53.0(48.4-58.4)
Min-Max	37.1-82.7	43.2-81.0
Orbit to preopercle length		
Median(Q1-Q3)	89.5(79.7-97.0)	103(92.6-112)
Min-Max	66.4-110	82.1-136
Lower jaw length		
Median(Q1-Q3)	55.4(48.6-58.4)	56.5(50.8-59.0)
Min-Max	35.7-68.5	41.3-76.2
Interspace between first and second dorsal fin		
Median(Q1-Q3)	20.8(18.1-27.8)	22.8(21.3-26.5)
Min-Max	15.2-29.6	17.0-33.1
First dorsal fin height		
Median(Q1-Q3)	68.4(59.8-74.8)	72.2(67.0-77.2)
Min-Max	47.3-85.1	62.7-88.7
Pelvic fin length		

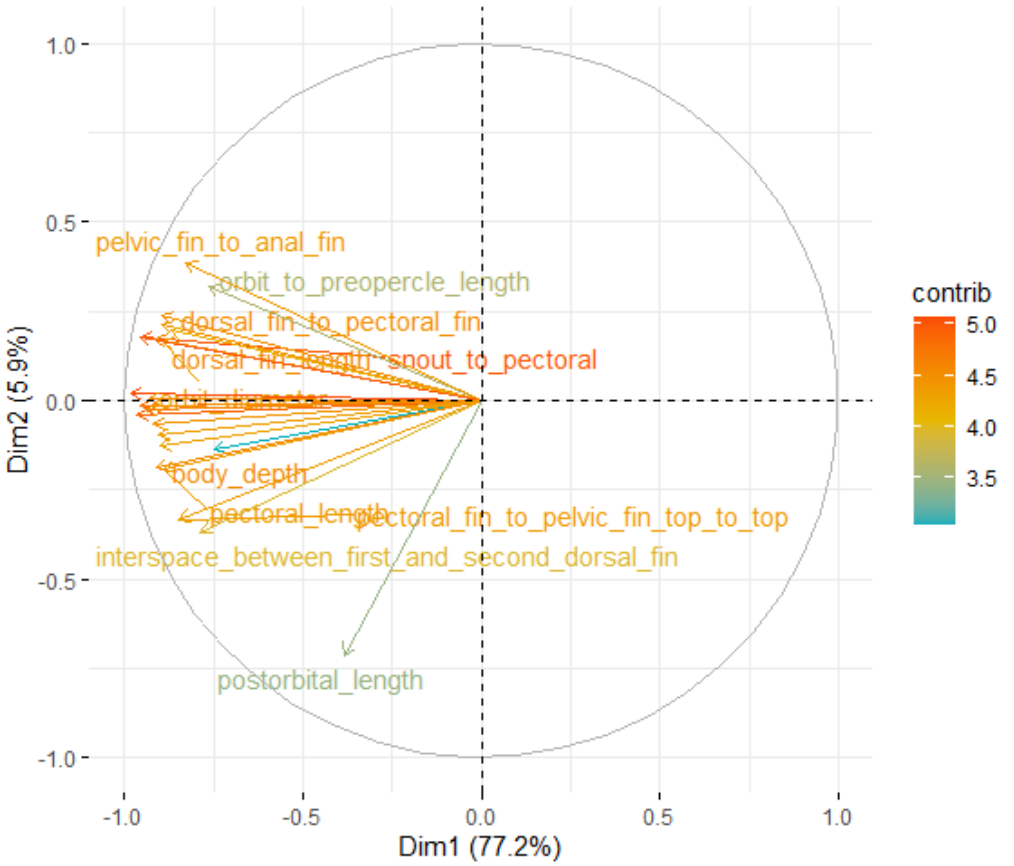
Median(Q1-Q3)	48.3(42.3-50.8)	46.7(40.8-49.9)
Min-Max	26.9-56.5	36.2-67.9
Pectoral fin length		
Median(Q1-Q3)	65.4(57.9-73.1)	70.8(64.7-74.9)
Min-Max	43.5-84.3	58.2-81.6
Snout to dorsal		
Median(Q1-Q3)	140(126-149)	153(143-167)
Min-Max	111-177	134-187
Dorsal fin to pectoral fin		
Median(Q1-Q3)	42.8(40.6-48.2)	51.7(46.5-53.3)
Min-Max	30.8-60.7	37.8-65.1
Dorsal fin to operculum tip		
Median(Q1-Q3)	39.8(37.6-43.9)	47.6(44.3-48.6)
Min-Max	29.1-62.8	35.6-56.5
Pectoral fin to pelvic fin		
Median(Q1-Q3)	49.1(41.2-52.9)	47.2(42.2-49.7)
Min-Max	34.2-68.2	37.1-59.6
Dorsal fin length		
Median(Q1-Q3)	29.3(24.8-31.3)	33.7(31.2-35.9)
Min-Max	21.2-37.8	26.1-41.5
Dorsal fin to anal fin		
Median(Q1-Q3)	99.6(76.5-112)	107(98.8-126)
Min-Max	71.1-123	84.7-137
Pelvic fin to anal fin		
Median(Q1-Q3)	69.5(66.1-73.7)	85.2(79.6-98.6)
Min-Max	50.9-91.4	60.0-104
Dorsal back to anal fin		
Median(Q1-Q3)	78.4(57.1-87.8)	82.8(73.6-94.2)
Min-Max	53.4-98.4	63.1-107
Body depth		
Median(Q1-Q3)	91.2(71.6-98.3)	90.3(79.9-104)
Min-Max	57.0-109	71.5-117
Snout to pectoral fin		

Median(Q1-Q3)	124(108-128)	137(125-145)
Min-Max	87.5-149	114-163
Snout to pelvic fin		
Median(Q1-Q3)	129(109-136)	138(132-152)
Min-Max	98.5-163	118-178
Pectoral fin to anal fin		
Median(Q1-Q3)	88.9(72.3-95.1)	99.4(89.6-114)
Min-Max	63.2-114	68.7-127
Dorsal fin to pelvic fin		
Median(Q1-Q3)	82.5(73.5-95.4)	84.6(78.8-96.3)
Min-Max	60.2-103	69.2-106

APPENDIX D: LOADING PLOT SHOWING CONTRIBUTION OF EACH MORPHOLOGICAL CHARACTER ON PRINCIPLE COMPONENT'S 1 AND 2.

Morphological character	PC1	PC2
Head length	0.80	0.55
Snout length	0.78	0.49
Orbit diameter	0.66	0.59
Orbit to preopercle length	0.59	0.48
Lower jaw length	0.72	0.52
Interspace between first and second dorsal fin	0.48	0.65
First dorsal fin height	0.81	0.51
Pectoral fin length	0.62	0.62
Snout to dorsal	0.81	0.57
Dorsal fin to pectoral fin	0.75	0.50
Dorsal fin to operculum tip	0.81	0.40
Pectoral fin to pelvic fin	0.50	0.67
Dorsal fin length	0.68	0.60
Dorsal fin to anal fin	0.51	0.85
Pelvic fin to anal fin	0.70	0.46
Dorsal back to anal fin	0.46	0.88
Body depth	0.46	0.85
Snout to pectoral fin	0.82	0.53
Snout to pelvic fin	0.77	0.54
Pectoral fin to anal fin	0.66	0.66
Dorsal fin to pelvic fin	0.58	0.69

APPENDIX E: THE CONTRIBUTION OF EACH MORPHOLOGICAL CHARACTER IN THE PRINCIPAL COMPONENT ANALYSIS.



APPENDIX F: LOADING PLOT SHOWING CONTRIBUTION OF EACH MERISTIC CHARACTER ON PRINCIPLE COMPONENT'S 1 AND 2.

Meristic character	PC1	PC2
Dorsal fin rays		0.65
Pectoral fin rays	-0.96	
Pelvic fin rays	0.74	0.33
Scales from anal fin to lateral line (diagonal)		0.49

APPENDIX G: THE CONTRIBUTION OF EACH MERISTIC CHARACTER IN THE PRINCIPAL COMPONENT ANALYSIS.