A Genetic Approach to the Biodiversity of Shallow Water Alcyonacea in South Africa

by

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Biology, 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 SANBI's SeaKeys project and the National Research Foundation.

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

Signed: Dr. Angus H. H. Macdonald
Date: October 2018

DECLARATION 1: PLAGIARISM

I, Kerry-Lee Etsebeth, declare that:

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

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

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

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

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

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

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

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

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

Signed: Kerry-Lee Etsebeth

Date: October 2018

DECLARATION 2: RESEARCH OUTPUTS

The role of the dissertation author, K. Etsebeth in each paper, report and presentation is indicated. Several contributors have been critical to the development of this work and are/will be acknowledged in the form of co-authorship in the resultant publications from this work. In Chapters 2 to 5, the dissertation author was responsible for over 90% of the specimen collections, the design of each investigation (barring Chapter 2), specimen processing, laboratory work, data analysis and write up. The * indicates the corresponding author. The author's contribution to other aspects of the chapters such as taxonomic analyses, collection and laboratory work has varied because of the contribution of others. Their specific contributions are acknowledged below:

Chapter 2

1. Etsebeth K*, Alderslade PN, Macdonald AHH (2017) DNA barcoding supports morphological evidence for a new genus of Alcyoniidae (Cnidaria: Octocorallia). Paper presentation at the 16th Southern African Marine Science Symposium (SAMSS), 4th to 7th July 2017, Port Elizabeth, South Africa. Presented by K Etsebeth[1].
2. Etsebeth K*, Alderslade PN, Macdonald AHH (2017) A new genus of Alcyoniidae (Cnidaria: Octocorallia) as evidenced by genetic divergence. Paper and poster presentation to the 7th International Barcode of Life (iBOL) Conference, 20th to 24th November 2017, Kruger National Park, South Africa. Presented by K Etsebeth.

This chapter will be submitted as the molecular systematic component of a taxonomic paper co-authored with the principle author, Dr. PN Alderslade, to the Journal “Zootaxa.” The molecular contribution by K. Etsebeth as co-author included experimental design, collection, laboratory work, data analyses and the write up for this component. Dr. Macdonald, Dr Alderslade and Dr. Sink reviewed this work and R. Jackson contributed to the collection of specimens.

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[1] https://drive.google.com/file/d/0B9gcuGd4Fnz2ZnVjdElzSXBOY0U/view

Chapter 3

1. Etsebeth K*, Pooran S, Macdonald AHH (2017) DNA Barcoding of *Sinularia* morphotypes from the iSimangaliso Wetland Park. Poster presentation at the 16th Southern African Marine Science Symposium (SAMSS), 4th to 7th July 2017, Port Elizabeth, South Africa. Poster presented by K Etsebeth.[2]

This chapter will be submitted as a standalone publication to the journal "Marine Biodiversity." The study was designed solely by K. Etsebeth who exclusively carried out specimen collection, data analyses and write up. Microscopy and specimen identifications were done by Dr. Alderslade, K. Etsebeth and S. Pooran. Laboratory work was performed primarily by K. Etsebeth with the assistance of S. Pooran. Dr. Macdonald, Dr. Alderslade and Dr. Sink reviewed the work.

Chapter 4

1. Sink K, Bosman A, Etsebeth K, Chiazzari B, Sola E, Jones G, Olbers J, Harikishun A, Castelle-Subramoney A, Franken M (2015) Seakeys Project iSimangaliso Field report, May 2015. South African National Biodiversity Institute[3].
2. Etsebeth, K* (2017) Preservation of Alcyonacea (soft corals) for molecular analysis: protocols and considerations. South African National Biodiversity Institute.
3. Etsebeth K, Macdonald AHH (2014) Preliminary phylogenetic and systematic analyses of *Sarcophyton* in the south-western Indian Ocean. Poster presentation at the 15th Southern African Marine Science Symposium, 15th to 18th July 2014, Stellenbosch, South Africa. Poster presented by K Etsebeth[4].
4. Etsebeth K, Macdonald AHH (2013) Phylogeographic analysis of *Sarcophyton* in the south-western Indian Ocean using genetic markers cytochrome *c* oxidase I (*COI*), bacterial MutS homologue (*MutS*), and signal recognition particle 54kDa (*SRP54*) nuclear marker. Poster presentation at the 8th West Indian Ocean Marine

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[2] https://drive.google.com/file/d/0B9gcuGd4Fnz2N0poUHJKSE1tbVk/view

[3] DOI: 10.13140/RG.2.2.14035.58406

[4] DOI: 10.13140/RG.2.1.3849.1606

Science Association (WIOMSA) 28 October- 3rd November 2013, Maputo. Poster presented by K Etsebeth[5].

This chapter will be submitted as a standalone publication to the journal “Coral Reefs.” The study was designed solely by K. Etsebeth who exclusively carried out laboratory work, data analyses and write up. Microscopy and specimen identifications were done by Dr. Alderslade and K. Etsebeth. Specimen collections were carried out by K. Etsebeth and Dr. Sink. Dr. Macdonald, Dr Alderslade and Dr. Sink reviewed the work.

Chapter 5

1. Etsebeth K* (2017) The national octocoral species list of South Africa. South African National Biodiversity Institute Report.

This chapter will be submitted as a standalone publication to the journal “African Journal of Marine Science”. The dissertation author designed, compiled and wrote the review.

Signed: Kerry-Lee Etsebeth

Date: October 2018

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[5] DOI: 10.13140/RG.2.1.1489.8647

ABSTRACT

The cnidarian order Alcyonacea (Octocorallia) represents a diverse group of marine invertebrates that are dominant taxa on shallow and deep reefs. These organisms constitute sensitive indicators of climate change and have bioprospecting potential. In South Africa, alcyonacean soft corals form the dominant faunal component of the high latitude marginal reefs on the east coast, an understudied region of interest that forms some of the southernmost distribution limits for key taxa in this order. Globally, soft corals are understudied whereby systematic, ecological, and biochemical studies are challenged by inconclusive taxonomy. This is in contrast to the growing understanding of the necessity for genetics in the systematics of Scleractinia, where large scale reclassification has been successful in reconstructing more accurate phylogenies of the order. The knowledge deficit in the systematics and evolution of Alcyonacea is paralleled in South African taxa. An integrative assessment using morphological classification in combination with phylogenetics of Alcyonacea is the most promising approach to assess their biodiversity in South Africa. Accordingly, the aims of these studies were to advance the taxonomic knowledge of soft corals in South Africa; assess species richness and provide a national checklist; and investigate evolutionary relationships between taxa. In so doing, this thesis also aimed to support the global taxonomy of Alcyonacea. Particular attention was given to Alcyonacea that dominate the reef biota in the UNESCO world heritage site, the iSimangaliso Wetland Park (iSWP). Three genetic markers, cytochrome *c* oxidase I (*COI*), bacterial *MutS* homolog (*MutS*) and nuclear 28S ribosomal RNA (*28S rRNA*) were used to determine genus and species boundaries in the closely related Alcyoniidae genera; *Cladiella*, *Aldersladum* and *Klyxum*. The use of these genes corroborated taxonomic analyses and demonstrated that morphologically aberrant members of the genus *Cladiella* should be reassigned to another as yet undescribed genus. The undescribed genus, Beta[6], is characterised by an absence of platelet sclerites in the polyp region, features that demonstrate major distinctions from the genera *Cladiella*, *Aldersladum* and *Klyxum*. The species richness of 67 *Sinularia* specimens in the iSWP was estimated

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[6] Beta was selected as a placeholder name until the full taxonomic descriptions of this new genus and any of its associated species are published.

using DNA barcoding (*MutS* and concatenated *MutS+28S* respectively). Phylogenetics and species delimitation methods were used to infer identifications for 63% of the specimens in the study, including a new record for *Sinularia grandilobata*. In addition, 16 putative species of *Sinularia* were discovered that are considered potentially undescribed or new records that require further taxonomic investigation by an octocoral expert. Furthermore, *S. brassica* exhibited two distinct morphotypes that were genetically differentiated and indicative of two distinct species. Taxonomic boundaries were investigated using integrative taxonomic approaches for 239 specimens of Alcyonacea from the iSWP. Specimens were assigned to ~61 putative species that were inferred from phylogenies, divergence estimates, colony photographs and sclerite characters for taxa in this study. Molecular evidence supported the reassignment of non-*S. brassica* congeners of *Sinularia* to an undescribed genus due to significant large scale polyphyly. Furthermore, this study reported new records for three genera; *Scleronephthya*, *Paraplexaura*, and *Chironephthya* and an undescribed species of *Lobophytum*. The results of this study, in conjunction with previously published records, contributed towards revised species estimates for the iSWP that increased from 37 to 81 species (with 12% endemism), 11 to 47 genera and 4 to 17 families. An updated checklist of the Alcyonacea across South Africa was compiled using records from chapters 2 to 4 in this thesis and published records. Two hundred and twenty-six different species from among 89 genera and within 25 families of Alcyonacea were reported. Seventy-five of these species are endemic to South Africa (33%). This represents a substantial increase from the last published estimates in South Africa of 130 species of Alcyonacea. This body of research represents a significant advancement in the knowledge of the biodiversity of an important group of marine invertebrates in South Africa. It provides resolution within some problematic identification assignations (*Cladiella*, *Sinularia brassica*). *Sinularia*, Beta and the *Lobophytum*-*Sarcophyton* complex require further taxonomic work for a better perspective on the diversity of this complex, marginal and neglected aspect of South Africa’s marine heritage.

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“The oceans deserve our respect and care, but you have to know something before you can care about it.”

Sylvia Earle

CHAPTER 1: INTRODUCTION

1.1 Alcyonacea (soft corals) introduction

Coral reefs and communities are among the most productive, diverse and oldest ecosystems on earth (McClanahan et al. 2000, Appeltans et al. 2012). These reefs are predominantly composed of hermatypic (reef building) corals and are mainly distributed throughout shallow, warm tropical waters confined to low latitudes (<25°) north and south of the equator (Veron & Stafford-Smith 2000).

Soft corals (Order Alcyonacea) form essential components of this reef ecology. Alcyonacea Lamouroux, 1812 date back to the Ordovician Period (480-475 Mya) and are a diverse group of anthozoans with over 3300 extant species (Cope 2005, Horton et al. 2017). These colonial benthic organisms are ubiquitous and exhibit a wide bathymetric range from intertidal to deep sea waters (Fabricius & Alderslade 2001, Daly et al. 2007, Benayahu et al. 2017). They differ from hard corals (Order Scleractinia) in that they produce calcareous elements (sclerites) that support tissue structure (Konishi 1981, Jeng et al. 2011), and their polyp bodies are partitioned by eight mesenteries that give rise to eight tentacles adorned with rows of pinnules (Figure 1.1) (Bayer et al. 1983, Menzel et al. 2015).

1.1.1 General biology

Alcyonacea have primary polyps (autozooids) that play key roles in feeding and reproduction (Fabricius & Alderslade 2001). Taxa that exclusively contain autozooid polyps are termed 'monomorphic' (Bayer et al. 1983). A secondary, reduced polyp (siphonozooid) that is responsible for siphoning water into the colony and potentially involved in reproduction, may also be present in certain taxa which are deemed 'dimorphic' (Bayer et al. 1983). Colony size and shape vary considerably amongst taxa for which growth forms are categorized into encrusting, massive, membranous, lobate, digitate, bushy, arborescent, fan-shaped and whip-like (Bayer et al. 1983, Williams 1992a, Fabricius & Alderslade 2001).

Soft corals are largely suspension feeders whereby autozooids filter suspended microparticles from the water column (Fabricius & Alderslade 2001). They may possess stinging nematocyst cells for active capture of larger prey (Coma et al. 1994). Furthermore, shallow water soft corals and some gorgonians are host to photosynthetic algal symbionts (*Symbiodinium* Freudenthal, 1962) on which they rely for additional nutrition (van Oppen et al. 2005, Berkelmans & van Oppen 2006).

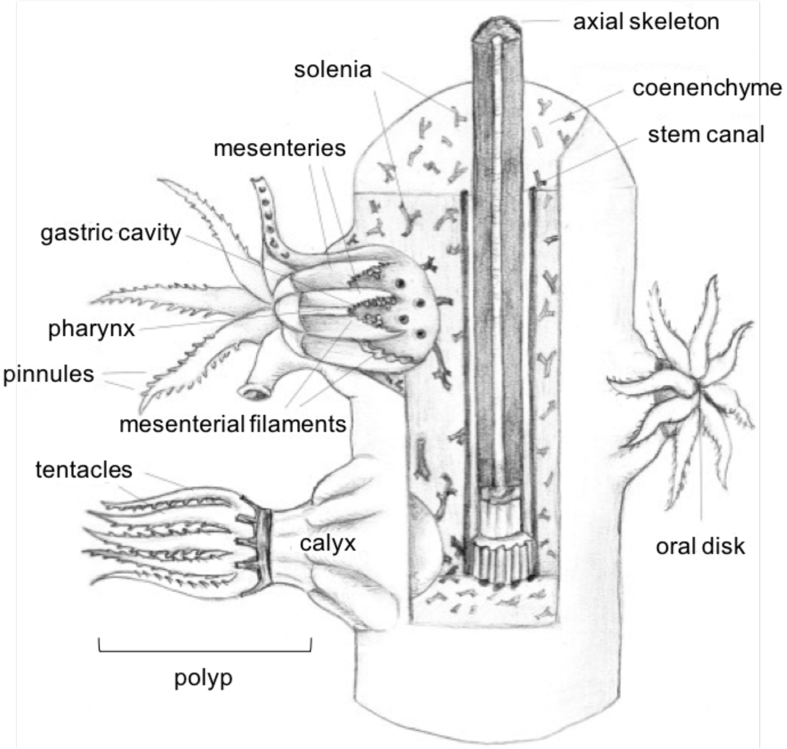

Figure 1.1 Diagram of an octocoral indicating polyp structure. Image adapted from Menzel et al. (2014) based on Bayer et al. (1983).

Habitat preference and suitability differs significantly among Alcyonacea taxa though they are often found in tropical and subtropical coastal waters at shallow depths (<30 m) (Fabricius & De'ath 2001). Their distribution is dependent on factors such as exposure to wave action, sedimentation and light availability; the quality of the water in terms of salinity, temperature and pH; water motion, turbidity and nutrient levels (Fabricius & Alderslade 2001, Fabricius & De'ath 2008, Baum et al. 2016).

1.1.2 Reproduction

Soft corals are mostly gonochoric, with a few reports of hermaphroditism, and reproduce both sexually and asexually (Schleyer et al. 1997, 2004). Sexual reproduction takes place seasonally in one of two forms: broadcast spawning and brooding (Benayahu 1997, Benayahu & Chou 2010). Broadcast spawning occurs when gametes are mass spawned into the water column and brooding is further subdivided into internal or external, wherein planulae are fertilized and brooded either inside the female polyp (internal) or on the surface of the colony between the polyps (external) (Alino & Coll 1989, Benayahu 1991, Schleyer et al. 1997).

1.1.3 Classification and taxonomy

Octocorallia is a subclass of Anthozoa (Phylum Cnidaria) comprised of three orders: Alcyonacea Lamouroux, 1812 (soft corals), Pennatulacea Verrill, 1865 (sea pens) and Helioporacea (blue corals) (Bayer et al. 1983). Alcyonacea are further subdivided into six subordinal groups wherein gorgonians (sea fans and sea whips) are organized into (1) Scleraxonia, (2) Holaxonia and (3) Calcaxonia depending on the structure of the supporting axis. The three remaining groups (4) Protoalcyonaria, (5) Stolonifera, and (6) Alcyoniina, lack internal axes (Grasshoff 1999). Taxonomic assignments are conventionally based on similarities in colony morphology and sclerites according to the classification of Bayer (1981). Sclerites are the most widely used diagnostic characters in octocoral taxonomy where classifications are based on their shape, distribution and arrangement in the colony (Bayer 1981, Bayer et al. 1983, Fabricius & Alderslade 2001). This traditional approach in octocorals traditionally follows biological (Mayr 1982) and morphological (Cronquist 1978) species concepts, the definitions of which are further discussed in Aldhebiani (2017) (See Chapter 5 for more details of Alcyonacea classification and taxonomy).

1.1.4 Molecular markers and systematics

The concept of a species-delimiting genetic marker that can be universally applied across taxa is known as a DNA barcode (Hebert et al. 2003). A DNA barcode ideally has high degrees of disparity between intra- and interspecific sequence variation, known as a barcode gap (Hajibabaei et al. 2007). A region of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) was proposed as a standard barcode for metazoan taxa whereby *COI* sequences from unknown specimens can be compared to a repository of *COI* sequences from known species for identification (Hebert & Gregory 2005, Ratnasingham & Hebert 2007, 2013). However, the effectiveness of DNA barcoding in many marine taxa is widely contested (Gao et al. 2010, Bucklin et al. 2011, McFadden, Brown, et al. 2014) and is limited in Octocorallia by the slow evolution of the mitochondrial genome compared with its nuclear counterpart (Shearer et al. 2002). Few regions of the octocoral mitochondrial genome exhibit sufficient variation to delineate congeneric species or distinguish populations of conspecifics (France & Hoover 2002, Shearer et al. 2002, Fukami & Knowlton 2005, Concepcion et al. 2006, McFadden et al. 2011). However, a *MutS* homolog, a mitochondrial gene exclusively found in octocorals (Pont-Kingdon et al. 1998, France & Hoover 2002), has been widely applied in systematic and phylogenetic studies providing substantial taxonomic insight (McFadden, France, et al. 2006b, McFadden et al. 2009, McFadden & van Ofwegen 2012a, McFadden & van Ofwegen 2013b, Reijnen et al. 2014, Bryce et al. 2015).

There is considerable interest in nuclear markers with a high sequence variation that can be amplified across a wide range of species (Jarman et al. 2002, Hellberg 2006, Concepcion et al. 2006, McFadden et al. 2011). Nuclear markers such as signal recognition particle 54kDa (*SRP54*), rRNA internal transcribed spacers (*ITS*s), adenosine triphosphate (ATP) synthetase subunit (*ATPS*) and 28S ribosomal RNA gene (*28S rRNA*) have been investigated in different Alcyonacea taxa with varied rates of success (Benayahu et al. 2012, McFadden & van Ofwegen 2012a, Haverkort-Yeh et al. 2013, Stemmer et al. 2013, Reijnen et al. 2014, Benayahu, McFadden, Shoham, et al. 2017, McFadden et al. 2017). Whole genome sequencing technologies can be used for the detection of taxonomically informative nuclear loci and the development of suitable markers for studies in octocorals (McFadden et al. 2017). However, these technologies are currently thwarted by the presence of the symbiont genome (*Symbiodinium* Freudenthal, 1962) in zooxanthellate Alcyonacea (McFadden et al. 2010). The absence of a reference genome sequence and lack of *de novo* assembly approaches are problematic for studies of these non-model organisms (McFadden et al. 2010, Quattrini et al. 2017). (Chapters 2 to 4 provide more details on molecular markers and their applications in studies of soft corals).

1.1.5 Study area and its significance

Southern African reef corals display a continuum of connectivity from southern Mozambique to northern KwaZulu-Natal (Benayahu et al. 2003, Schleyer & Celliers 2003b, a, Macdonald et al. 2008, 2011). The coral assemblages in this study lie in the northern region of the KwaZulu-Natal province, on the east coast of South Africa (Figure 1.2) at a high latitude and fall outside of the tropical reef-building range (Riegl et al. 1995, Guinotte et al. 2003). The existence of these subtropical reefs and coral communities is due to the influence of the warm Agulhas current and they represent the southernmost range limit for the majority of tropical coral species (Riegl 2003, McClanahan et al. 2014). These reefs are not the typically accretive coral reefs that form limestone reef structures (van Oppen & Gates 2006). They are, instead, comprised of beach rock and fossilized sand dunes (aeolionites) from the late Pleistocene age that have been colonised by a thin veneer of corals (Ramsay 1994) and form semi continuous assemblages that run parallel to the coastline (Ramsay & Mason 1990, Riegl et al. 1995, Benayahu et al. 2003).

These unique, marginal communities are situated within a world heritage site, the iSimangaliso Wetland Park, meaning "miracle and wonder". The iSimangaliso Wetland Park (iSWP) spans 220km of protected coastline from Maphelane (south of St. Lucia estuary) to the Kosi Bay river mouth near the Mozambique border (Figure 1.2). The iSWP incorporates the Maputaland and St. Lucia marine reserves (Figure 1.2) (Schleyer & Celliers 2005, Tunley 2009). These reefs lie in the Delagoa bioregion- a marine biogeographic region influenced by the Agulhas current that has long been recognized as an area with high levels of endemism (Schleyer & Celliers 2003a). A wealth of coral biodiversity has been reported along these reefs where the majority of the faunal component is comprised of diverse Alcyonacea as opposed to Scleractinia (Williams 1989a, b, 1993). Soft corals constitute up to two thirds of the community composition and are essential components of reef structure and ecology (Williams 1993).

High latitudinal marginal reefs are considered the most suitable candidates for the study of survival and dynamics of coral reefs (Beger, Selkoe, et al. 2014, Beger, Sommer, et al. 2014, von der Heyden et al. 2014). Published forecasts of the latitudinal

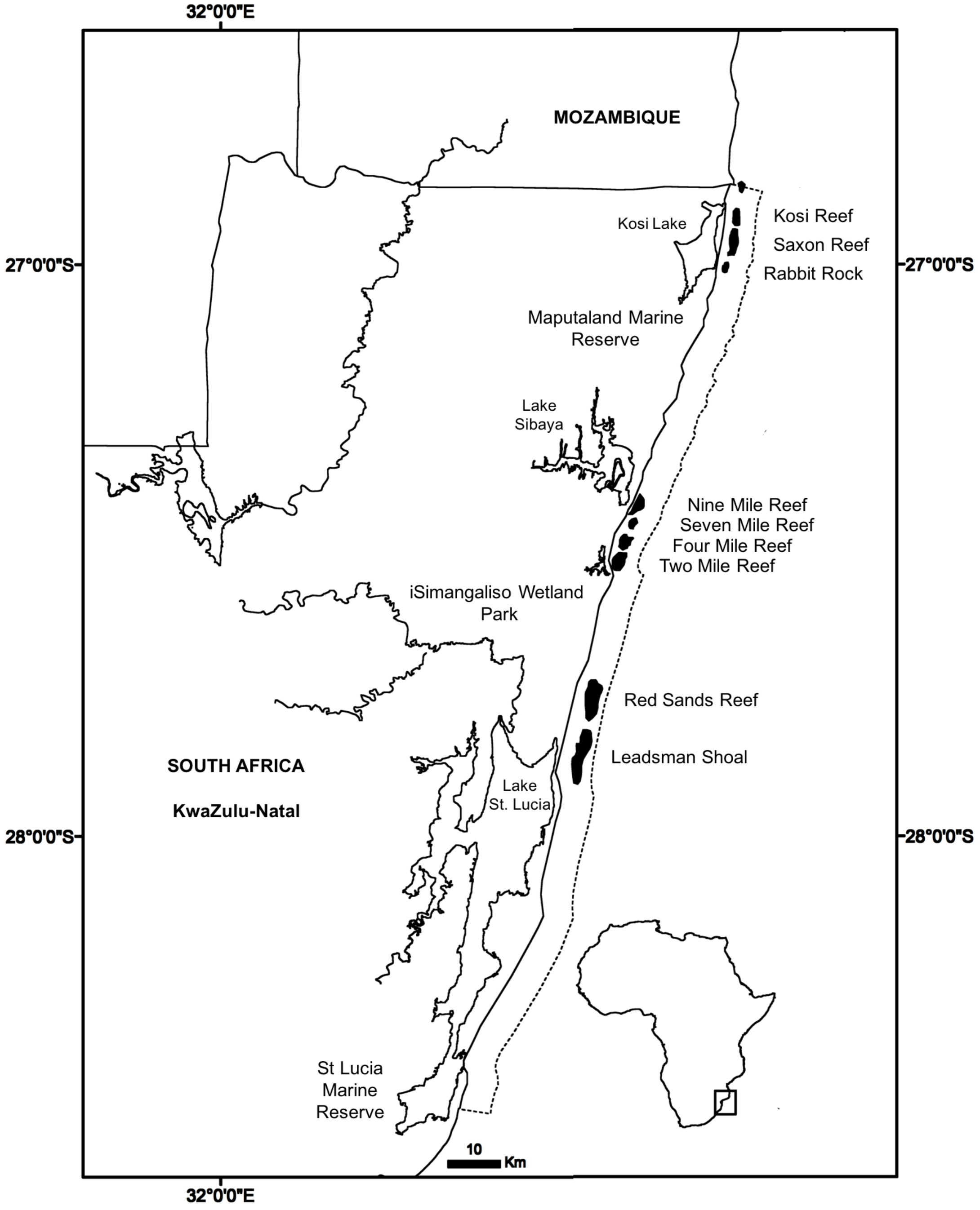

Figure 1.2 The high latitudinal reefs of the iSimangaliso Wetland Park, northern Kwazulu-Natal, South Africa. The dashed region indicates the marine protected area of iSWP.

effects of climate change predict an increase in the marginalization of existing reefs because of climate change (Guinotte et al. 2003, Riegl 2003, McClanahan et al. 2011, Freeman et al. 2013). They represent a valuable research opportunity for understanding the factors that build and maintain coral reef biodiversity and resilience.

Though these coral refuges are protected within the iSWP, they have been subject to bleaching events (Celliers & Schleyer 2002) and gradual temperature changes (Schleyer et al. 2008, Schleyer & Benayahu 2010, Porter & Schleyer 2017). The impact of climate change is progressively worsening and long term monitoring studies have reported temperature-related changes in community structure (Celliers & Schleyer 2002, Riegl 2003) resulting in a decline in Alcyonacea coverage (Porter & Schleyer 2017).

1.2 Problem statement

Alcyonacea in the iSimangaliso Wetland Park remain largely understudied and unknown despite their ecological significance in these high latitude reefs. The systematics of many Alcyonacea remains controversial and the understanding of the processes that led to their diversification is largely unexplored. Their ecology is confounded by an inconclusive knowledge of their taxonomy; poorly understood species boundaries, hybridization, and phenotypic plasticity that oftentimes render them indistinguishable in the field (Benayahu 1993, McFadden, France, et al. 2006, McFadden et al. 2011). There is insufficient taxonomic expertise globally for this important group and a complete lack of national expertise for this data deficient community in South Africa (Gibbons et al. 1999, Griffiths et al. 2010). DNA barcoding in soft corals has been handicapped by the slow rates of evolution in the octocoral mitochondrial genome and a lack of alternative markers that are suitable for species delineations (van Oppen et al. 1999, Shearer et al. 2002, Palumbi et al. 2012, Ament-Velásquez et al. 2016). Furthermore, molecular systematics often exhibit conflicts with traditional morphological taxonomy at the family, genus and species levels (France & Hoover 2002, McFadden et al. 2009, 2011, Benayahu et al. 2012).

1.3 Rationale and justification for the research

Surveys of the ecological status of coral reefs have indicated the ongoing degradation of these ecosystems; they are highly threatened and in decline worldwide (Souter et al. 2000, Hoeksema & van van Ofwegen 2008). Species richness and biodiversity contained in reef ecosystems may not regenerate once destroyed and the conservation of coral reefs has become a major concern (von der Heyden et al. 2014). Marginal coral communities may be the site of evolutionary divergence, local adaptation and speciation as a result of their high latitudinal distribution (Schleyer & Benayahu 2008, Beger, Sommer, et al. 2014, McClanahan et al. 2014). Therefore, a better understanding of the soft coral taxa (Alcyonacea) that constitute these communities is important because they provide insights into factors controlling coral abundance, diversity, distribution and resilience (Perry & Larcombe 2003, Riegl 2003, Pavoine & Bonsall 2011). Extrapolation of this information may be used to forecast the responses to environmental (climate change), biological and anthropogenic forces on the population dynamics of these systems (Munday et al. 2009, McClanahan et al. 2011, Ateweberhan et al. 2013, Selkoe et al. 2016).

Implicitly, without accurate biodiversity estimates and species data, it is not possible to fully assess or manage biodiversity, which is a key factor for the survival of these reef communities (Souter et al. 2000, Shearer et al. 2009, Jones et al. 2011, Huang & Roy 2015).

1.4 Aims and objectives

An assessment of the biodiversity of Alcyonacea corals from the world heritage site, the iSimangaliso Wetland Park (iSWP) will be carried out using integrative taxonomic approaches. A systematic review of soft corals from this region will be conducted using a combination of molecular data, basic taxonomy, and *in situ* photographs. Mitochondrial (*COI* and *MutS*) and nuclear (*28S rRNA*) loci will be utilized, both individually and concatenated to infer species identifications based on phylogenetic and species delimitations methods. These results can be consolidated with previously published records to compile a national species list of Alcyonacea in South Africa in order to update existing data for the marine protected iSWP. Furthermore, the taxonomic resolution of these markers and their utility as DNA barcodes are assessed for studies of Alcyonacea.

1.5 Outline of thesis

This thesis is written as a series of papers submitted to scientific journals. Each chapter

(Chapter 2 to 5) is self-contained and represents a separate paper comprised of an abstract, literature review, materials and methods, results, discussion and conclusion. The thesis is subdivided into 6 chapters wherein Chapter 1 serves as a general introduction to the thesis topic.

Chapter 2, *Phylogenetic investigation into several alcyoniid genera*, focuses on a phylogenetics-based approach that addresses genus boundaries in three morphologically similar genera and necessitates the reassignment of some of these taxa to a new genus.

Chapter 3, *Sinularia of the iSimangaliso Wetland Park, South Africa: genetic proxies provide estimates of species richness*, is devoted to the genus *Sinularia*, which is the single largest contributor to the reef biota in this region. It applies a previously described classification system with pre-defined phylogenetic clades that facilitate identifications of *Sinularia* species and requires basic taxonomic expertise.

Chapter 4, *Biodiversity of the Alcyonacea (Cnidaria: Octocorallia) of northern KwaZulu-Natal: a molecular and morphological synopsis*, is an expansion of chapters 2 and 3. Integrated taxonomic approaches were applied to all Alcyonacea collected from the iSWP in order to identify taxa to the genus and species level; and update biodiversity estimates for this region.

Chapter 5, *A checklist for soft coral (Alcyonacea) in South Africa*, provides an updated national species list of Alcyonacea in South Africa, with particular attention to species recorded from the iSWP. Biodiversity estimates, new genus and species records from the three preceding chapters were incorporated into these results.

The final chapter, Chapter 6, integrates the research and summarizes some general conclusions about the importance of taxonomy as an essential pre-requisite to any study of a given taxa.

1.6 References

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CHAPTER 2: A PHYLOGENETIC INVESTIGATION INTO SEVERAL ALCYONIID GENERA (PAPER 1)

2.1 Abstract

The addition of phylogenetic analyses and DNA barcoding to traditional taxonomic approaches has advanced systematic studies of soft corals (Octocorallia: Alcyonacea). However, few molecular markers are suitable for studies of Alcyonacea as they are predominantly mitochondrial and evolve significantly slower in octocorals than their nuclear counterparts. Multilocus DNA barcoding was used to investigate the generic boundaries of the phylogenetically overlapping genera: *Cladiella* Gray, 1869; *Aldersladum* Benayahu & McFadden, 2011 and *Klyxum* Alderslade, 2000. Previous work on these taxa showed that their morphologies are incongruent with their phylogenies. Furthermore, the taxonomic resolution of the nuclear marker ribosomal gene (*28S rRNA*) was assessed in addition to known mitochondrial markers cytochrome *c* oxidase subunit I (*COI*) and bacterial MutS homologue (*MutS*). Morphological evidence and DNA barcoding (*COI*+*MutS*+*28S*) supports the monophyly of *Aldersladum sodwanum* and its genetic distinction from *Cladiella* and *Klyxum*, though the ditypic genus itself is not monophyletic. Results also indicate that *Cladiella* is polyphyletic, a number of morphologically aberrant species display significant genetic distance from the genus and should be reassigned. These taxa are closely associated with the genus *Klyxum,* with an average genetic distance of 0.6%, (uncorrected *p*) across all gene regions. However, these taxa display significantly different morphologies to *Klyxum* that do not warrant their taxonomic placement in this genus. It is recommended that the polyphyletic and morphologically aberrant species of *Cladiella* be reassigned to a new genus that is characterised by an absence of platelet sclerites in the polyp region and the presence of double heads with complex warts in the coenenchyme.

Keywords: 28S rRNA, corals, genus, sclerites, phylogenetics, taxonomy

2.2 Introduction

The reef communities of northern KwaZulu-Natal, South Africa, exhibit high levels of marine biodiversity and feature conditions that promote endemism (Williams 1989, 1993, Schleyer & Celliers 2003). Alcyonacea (Octocorallia), dominate the faunal component of these reefs with members of the family Alcyoniidae in particular (Riegl et al. 1995). Alcyoniid taxa endemic to this region include *Cladiella kashmani* Benayahu & Schleyer, 1996 and *Aldersladum sodwanum* Benayahu, 1993 (Benayahu & Schleyer 1996, Benayahu & McFadden 2011). Other species of *Cladiella* Gray, 1869 that have been recorded in this region are *Cladiella australis* Macfadyen, 1936 and an undescribed species of *Cladiella*; which has only been documented from shallow intertidal rock pools approximately 300km further south of this region (Pople 1960, Williams 1992). *Aldersladum sodwanum* is the only species of this ditypic genus that is recorded in the northern KwaZulu-Natal region (Williams 1992, Benayahu & Schleyer 1996).

Soft corals are taxonomically classified according to their morphological features and in particular, their sclerites (Fabricius & Alderslade 2001). Sclerites are calcium carbonate spicules embedded in colony tissues that vary in size, structure and distribution within taxa (Bayer 1981). The figure-eight shaped sclerites (also known as platelets) in *Aldersladum* closely resemble those found in the polyp tissues of the genera *Cladiella* and *Klyxum* Alderslade, 2000 and is therefore considered a close relative of these two genera (Benayahu & McFadden 2011). However, *Aldersladum*, *Klyxum* and *Cladiella* are morphologically distinct monomorphic genera (Alderslade 2000, Fabricius & Alderslade 2001, Benayahu & McFadden 2011). *Aldersladum* typically possesses only minute figure-eight shaped sclerites throughout the entire colony which is a distinguishing factor from all other alcyoniid genera (Figure 2.1) (Benayahu & McFadden 2011). The genus *Klyxum* features sclerites that are characteristically in the form of spindles with large cone-shaped processes in the colony interior and flat rods and platelets in the polyps (Figure 2.1) (Alderslade 2000, Fabricius & Alderslade 2001). The major taxonomic features of the genus *Cladiella* is the presence of platelet-type or figure eight sclerites in the polyps and double heads with cone-shaped prominences in the colony surface and interior (Figure 2.1) (Tixier-Durivault 1941, Fabricius & Alderslade 2001).

Cladiella colonies rarely exceeds 30cm in diameter and are noted for their small sclerites (<0.1mm) (Fabricius & Alderslade 2001). Contrary to the typical genus characteristics of *Cladiella*, *Cladiella australis* and *Cladiella kashmani* colonies reach a span of up to 2-3m and are distinguished by an absence of figure-eight sclerites and the presence of large double heads (0.15-0.3mm) with complex tubercules (warts) in the coenenchyme (Benayahu & Schleyer 1996). Moreover, these two species have the largest sclerites recorded in the genus and include the presence of numerous elongated sclerites and long tuberculate spindles in the surface lobes, unlike other species within the genus (Benayahu & Schleyer 1996). Octocoral taxonomic expert, Dr. PN Alderslade, who for a long time had been aware of the morphological differences between these two species and most other species of *Cladiella*, recommended these taxa become a special focus for molecular analyses (personal communication, July 8, 2015). Though *C. australis* and *C. kashmani* are clearly

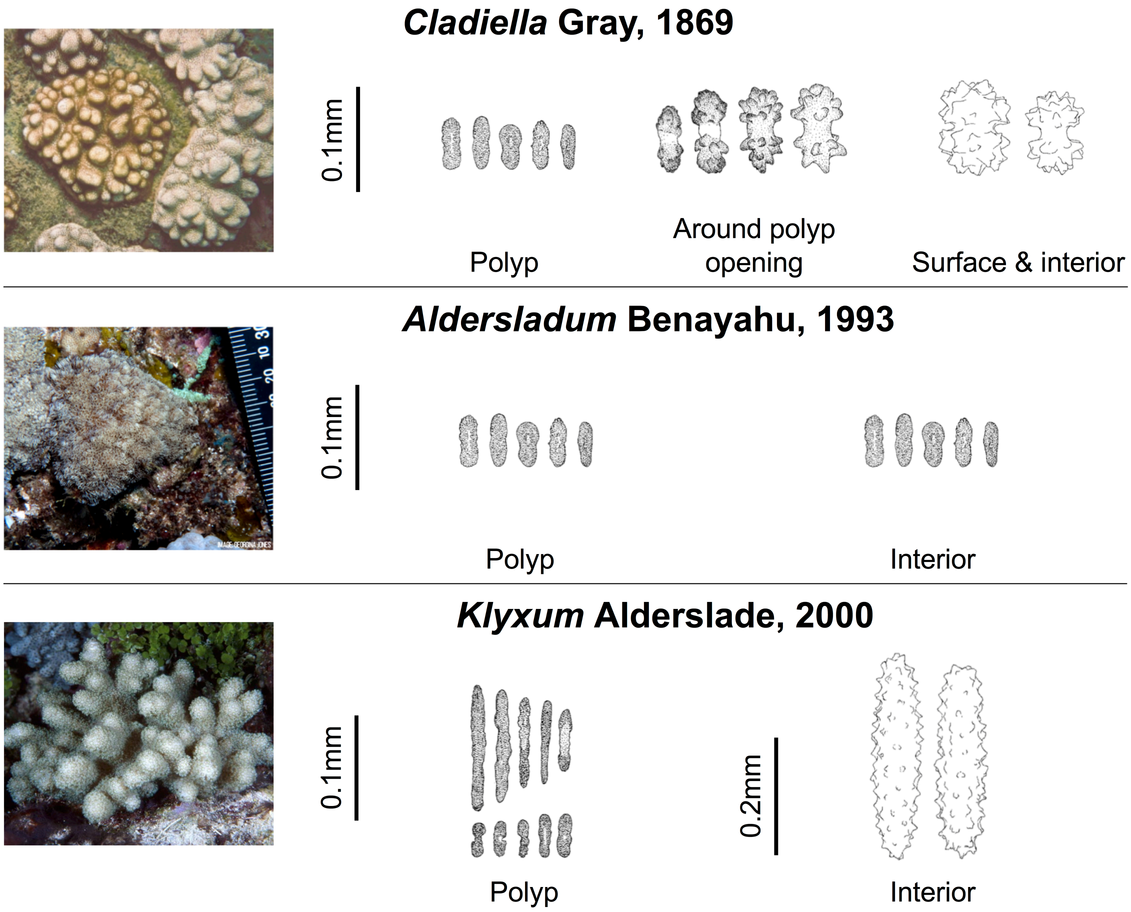

Figure 2.1 Sclerite compositions characteristic of the genera *Cladiella*, *Aldersladum* and *Klyxum* respectively. *In situ* colony photographs and sclerite images adapted from Fabricius & Alderslade (2001) and Benayahu et al. (2004).

delineated species based on their morphological features, their allocation to the genus *Cladiella* is questionable. Veron (2013) attributes these problematic assignments to the binomial nomenclature system that taxonomists are compelled to follow. A species must be assigned to the most appropriate genus, though not all genera are easily distinct. The consequence of these ambiguous taxonomic decisions supports the necessity of a revision of the genus *Cladiella*.

It has become standard practice to supplement taxonomic decisions that are based on morphological approaches with molecular data due to phenotypic plasticity and homoplasy (Concepcion et al. 2007, Páll-Gergely 2017). However, phylogenetic relationships and species boundaries are poorly understood in Alcyonacea and are predominantly based on mitochondrial sequences that evolve significantly slower than their nuclear counterparts, which limits the standard traditional barcode *COI* (France & Hoover 2002, McFadden et al. 2006, 2011). Molecular studies have shown that *Aldersladum*, *Cladiella* and *Klyxum* are closely related genera that form a paraphyletic assemblage and that their morphologies are incongruent with their phylogenetic history (Benayahu et al. 2012). Molecular analyses, with the inclusion of a nuclear locus, can be used to increase taxonomic resolution and elucidate taxonomic boundaries in alcyoniid taxa (McFadden, Brown, et al. 2014). Of the few known nuclear markers available for Alcyonacea, ribosomal *28S rRNA* has exhibited high variation and is easily amplified across a broad range of taxa (Bilewitch et al. 2010, McFadden & van Ofwegen 2012a). Therefore, a multilocus DNA barcode (*COI*+*MutS*+*28S*) was used to investigate the phylogenetic relationships of closely related genera; *Cladiella*, *Aldersladum* and *Klyxum*. Furthermore, phylogenetic analyses were used to ascertain whether current taxonomic assignments of morphologically aberrant species of *Cladiella* (*C. kashmani* and *C. australis*) should remain in the genus *Cladiella*.

2.3 Materials and methods

2.3.1 Sample collection

Specimens of *Cladiella* and *Aldersladum* were collected using SCUBA from the iSimangaliso Wetland Park (iSWP), South Africa, and snorkel-diving at Isipingo Rocks (Durban, South Africa) (Figure 2.2). *Klyxum* is not recorded in South Africa, therefore only sequences sourced from GenBank were included in phylogenetic analyses.

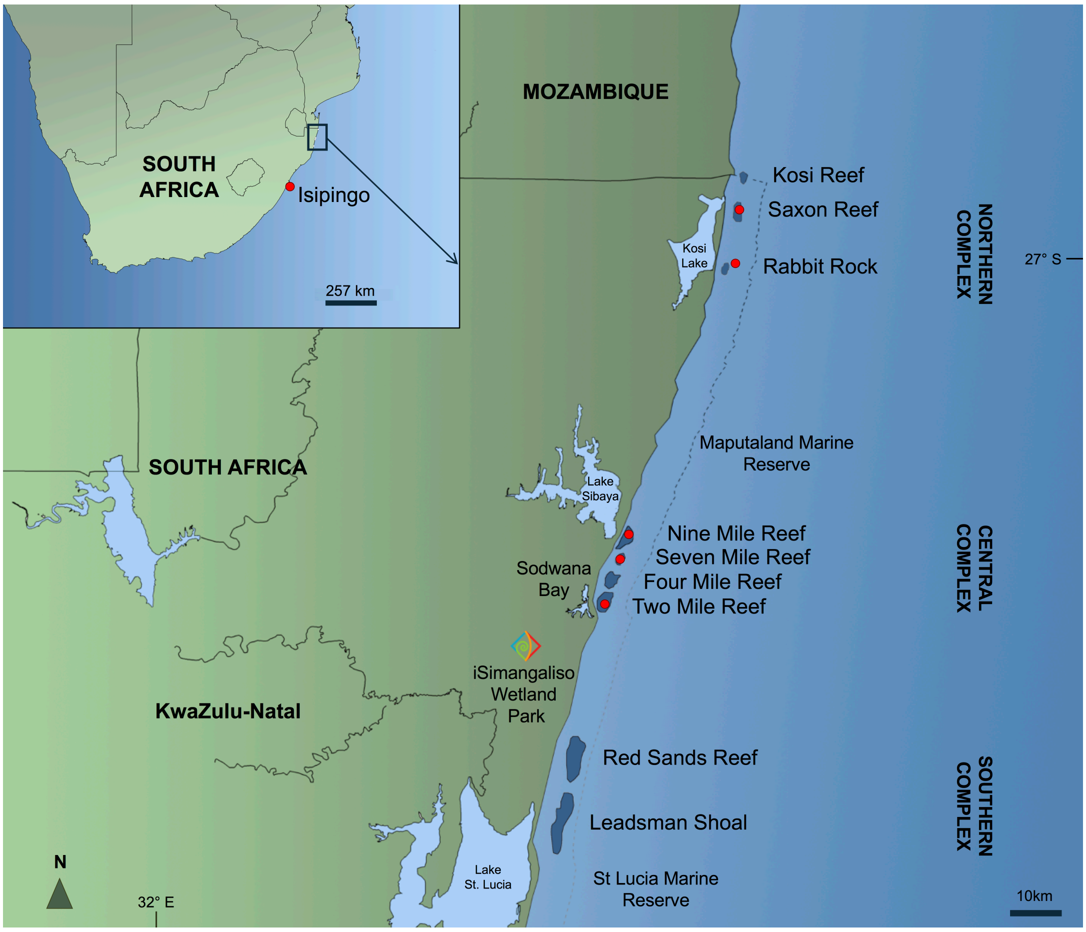

Figure 2.2 The KwaZulu-Natal coastline, South Africa, with collection sites (denoted by red circles) of specimens from the iSimangaliso Wetland Park and Isipingo Rocks (inset). The dotted line indicates the marine reserve.

Twenty-eight specimens were collected at a depth range of 1-20m, at least three meters apart from one another and in a random manner to circumvent the collection of clones (Appendix A, Table 7.1). The locations in this study lay within marine protected areas and the necessary permits were obtained for collection (permit numbers RES2013/13, RES 2014/06 and RES2015/70; Department of Agriculture, Forestry and Fisheries). Following collection, the colonies were fixed in 70% ethanol and stored at room temperature for morphological investigation. The samples have yet to be deposited at the Iziko South African Museum as they are currently being described by a taxonomic expert. Tissue subsamples were excised from the lobe of each collected specimen (prior to fixation) for subsequent genetic analyses. The tissue subsample (approximately 1cm^3) was placed directly into a cryofuge tube for storage at -80 °C.

2.3.2 Morphological analyses

Generic identifications were carried out for each specimen based on morphological characteristics such as colony shape and size, colour and sclerite forms (Benayahu & Schleyer 1996, Fabricius & Alderslade 2001). Gross morphology of the preserved colonies was inspected using a combination of *in situ* photographs and a stereomicroscope (Z730 Stereo Series; Unitron) at 20X power. For sclerite analyses, tissues were taken from the polyp, surface and interior of the preserved colonies and dissolved in 12% sodium hypochlorite for subsequent examination under a compound microscope (40-100X) with a digital camera (Eclipse 80i; Nikon). Preliminary taxonomic assignments for all relevant samples were sent for verification to octocoral taxonomic expert, Dr. PN Alderslade (Table 2.1). Specimens of *Cladiella* that did not feature sclerites in the polyp region and possessed double heads with complex tubercules were allocated to a new genus. For convenience, Beta was selected as an unofficial label for this new genus and any of its associated species. Beta is not the proposed generic name but a temporary 'placeholder' label (in accordance with ICZN rules and practices) as the taxonomic description is currently in preparation by Dr. PN Alderslade and K. Etsebeth.

Table 2.1 Sampling information for Alcyoniidae collected for this study and corresponding GenBank accession numbers for sequences of cytochrome *c* oxidase subunit I (*COI*), bacterial MutS homologue (*MutS*) and nuclear 28S ribosomal RNA (*28S rRNA*).

Sample	Scientific name	Site	GenBank accession numbers		
			COI	***MutS***	***28S rRNA***
Ro1	*Aldersladum sodwanum*	Isipingo Rocks	KX430071	KY511000	KX430099
Ro2	*Aldersladum sodwanum*	Isipingo Rocks	KX430072	KY511001	KX430100
Ro3	*Aldersladum sodwanum*	Isipingo Rocks	KX430073	KY511002	KX430101
Ro4	*Aldersladum sodwanum*	Isipingo Rocks	KX430074	KY511003	KX430102
Ri1	*Cladiella* sp.	Isipingo Rocks	KX430068	KY510997	KX430096
Ri2	*Cladiella* sp.	Isipingo Rocks	KX430069	KY510998	KX430097
Ri3	*Cladiella* sp.	Isipingo Rocks	KX430070	KY510999	KX430098

Table 2.1 continued...

Sample	**Scientific name**	**Site**	**GenBank accession numbers**		
			COI	***MutS***	***28S rRNA***
61	Beta sp.	Saxon Reef	KX430047	KY510976	KX430075
79	Beta sp.	Leadsman Shoal	KX430048	KY510977	KX430076
84	Beta sp.	Leadsman Shoal	KX430049	KY510978	KX430077
112	Beta sp.	Two Mile reef	KX430050	KY510979	KX430078
113	Beta sp.	Two Mile reef	KX430051	KY510980	KX430079
134	Beta sp.	Kosi Bay	KX430052	KY510981	KX430080
135	Beta sp.	Kosi Bay	KX430053	KY510982	KX430081
142	Beta sp.	Kosi Bay	KX430054	KY510983	KX430082
157	Beta sp.	Kosi Bay	KX430055	KY510984	KX430083
160	Beta sp.	Kosi Bay	KX430056	KY510985	KX430084
165	Beta sp.	Two mile reef	KX430057	KY510986	KX430085
192	Beta sp.	Seven Mile Reef	KX430058	KY510987	KX430086
194	Beta sp.	Seven Mile Reef	KX430059	KY510988	KX430087
195	Beta sp.	Seven Mile Reef	KX430060	KY510989	KX430088
198	Beta sp.	Nine Mile Reef	KX430061	KY510990	KX430089
209	Beta sp.	Nine Mile Reef	KX430062	KY510991	KX430090
215	Beta sp.	Nine Mile Reef	KX430063	KY510992	KX430091
220	Beta *kashmani*	Nine Mile Reef	KX430064	KY510993	KX430092
221	Beta sp.	Nine Mile Reef	KX430065	KY510994	KX430093
236	*Aldersladum sodwanum*	Two Mile reef	-	MF817826	MF817947
248	Beta sp.	Two Mile reef	KX430066	KY510995	KX430094
252	Beta sp.	Two Mile reef	KX430067	KY510996	KX430095

“-“ No amplicon obtained for specimen.

2.3.3 Genomic extraction

Prior to extraction, approximately 5 mm^3 of tissue was ground in liquid nitrogen with a sterilized pestle and mortar. Whole genomic DNA was extracted from the homogenized material using the Zymo ZR Genomic DNA^{TM} Tissue MiniPrep kit according to the manufacturer’s protocol that included a prolonged digestion of up to 16 hours. DNA quantity and quality were subsequently measured using the NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA).

2.3.4 PCR amplification

Primers described by Sánchez et al. (2003) and McFadden et al. (2014) were used to amplify mitochondrial loci, *MutS* (735bp) and *COI* (930bp), as well as a third nuclear fragment, *28S rRNA* (750bp). Nested primers were designed for several Beta specimens for which amplification of the *MutS* region failed. Oligonucleotides were designed to amplify a 710bp fragment of *MutS* based on GenBank sequences of *Cladiella kashmani* using PRIMER3 (Rozen & Skaletsky 2000): *MutS*$_K$F (5'-ATGAATC AAATACCTGCGCAAT-3') and *MutS*$_K$R (5'-ACACGGCAAGTTGGTTAATACT-3').

Each PCR reaction consisted of 1X DreamTaq Green PCR Master Mix (Thermo Scientific^{TM}); 0,1 µM of forward and reverse primer; 10 mg/ml BSA; 50-100 ng template and nuclease-free water to a final volume of 25 µl. Target regions were amplified using a T100^{TM} Thermal cycler (Bio-Rad) with an initial denaturation at 95 °C for 2 min; 35 cycles of 95 °C for 20 s, 58 °C for 30 s, and 72 °C for 25 s; followed by a final extension of 72 °C for 5 min. A negative control (no template) was included to confirm successful amplification of the targeted regions.

PCR products were analysed using agarose gel electrophoresis with GeneRuler 100bp DNA Ladder (Thermo Scientific^{TM}). PCR amplicons were purified for sequencing using a Zymogen Zymoclean™ Gel DNA Recovery Kit, according to the manufacturer's instructions. The amplicons were outsourced to Inqaba Biotechnical Industries (Pty) Ltd for Sanger sequencing, the samples were prepared using an ABI V3.1 sequencing kit and sequenced on an ABI 3500XL gene sequencer.

2.3.5 Sequence analyses

Raw sequences were checked for accuracy using BLASTN (Altschul et al. 1990) and aligned with GenBank reference data for each taxon in this study. The sequences were manually edited in BioEdit v 7.2.5 and subsequently uploaded to GenBank (Table 2.1).

2.3.6 Phylogenetic and distance analyses

A total of 21 Beta spp., 5 *A. sodwanum* and 4 *Cladiella* sp. sequences were obtained for two mitochondrial (*COI*, *MutS*) and one nuclear region (*28S rRNA*). Previously published GenBank sequences from vouchered specimens of *Aldersladum*, *Cladiella*, Beta and *Klyxum* were included in phylogenetic reconstructions to increase the representation of Alcyoniidae genera (Appendix B Table 7.1). However, few sequences of similar sequence lengths were available for taxa from each genus. A total of 45 *COI* sequences (930bp) were used in phylogenetic analyses: 16 GenBank sequences; 28 sequences from specimens collected in this study (Table 2.1); and the outgroup *Briareum hamrum* (GU355975.1) as per McFadden et al. (2011). For *MutS* analyses, 47 sequences (583bp) were used: 16 GenBank sequences; 29 sequences from taxa in this study (Table 2.1); and two outgroups (*Sinularia verseveldti* KC542861.1 and *Briareum hamrum* GU356010.1) as per McFadden et al. (2011). A total of 47 sequences were used for analyses of the *28S* rRNA region (741bp). The data were comprised of 18 GenBank sequences; 29 sequences from taxa collected in this study (Table 2.1); and the outgroup *Briareum asbestinum* (JX203669.1), as per McFadden et al. (2011).

Nuclear data were phased using PHASE v 2.1.1 (Flot 2010) and different alleles were included in subsequent analyses. Multiple sequence alignments were carried out for each dataset using MUSCLE (Edgar 2004) in MEGA v 6.06 (Tamura et al. 2013). Haplotypes were calculated for each dataset in DnaSP v 5.10.1 (Librado & Rozas 2009) in which gap regions were considered due to a high number of polymorphisms and the presence of indels. Alleles that were recovered from phased nuclear data shared the same haplotype per individual and were therefore not indicated in the resultant phylogenies. Nucleotide and haplotype diversities were calculated for each dataset in DnaSP v 5.10.1 (Librado & Rozas 2009). MEGA v 6.06 (Tamura et al. 2013) was used to calculate pairwise genetic distances (uncorrected *p*) and obtain estimates of intra and inter-generic distances among taxa for each gene region (Appendix C Table 6.6).

Best-fit substitution models were calculated in jModelTest v 2.1.10 (Guindon & Gascuel 2003, Darriba et al. 2012). The models were selected using the Akaike information criterion (AIC) (Akaike 1973) and applied to each dataset (*COI*: HKY+I; *MutS*: GTR+I and *28S rRNA*: HKY+G) for subsequent phylogenetic reconstructions.

Bayesian inference and maximum likelihood analyses were implemented in MrBayes v 3.2.6 (Ronquist & Huelsenbeck 2003) and Randomized Axelerated maximum likelihood (RAxML) (Stamatakis 2014) respectively. Bayesian analyses were run for each gene region using four chains for 10,000,000 generations with a burn-in value set at 10%. Tracer v 1.6 (Rambaut et al. 2018) was used to confirm that effective sample size (ESS) values were greater than 200 and to verify Markov Chain Monte Carlo (MCMC) convergence. Maximum likelihood analyses were performed using the web server RAxML Black Box[7] (Stamatakis et al. 2008) with appropriate substitution models, 2000 bootstrap replicates, and the remaining parameters set at default. Trees were rooted with outgroups used in Benayahu et al., 2014.

Additionally, a concatenated dataset (2292bp) for 39 specimens, consisting of all three gene regions (*COI*+*MutS*+*28S*), was phylogenetically reconstructed using partitioned substitution models relative to each gene region. Only ten GenBank sequences were included due to a lack of representatives for all three gene regions for the same voucher specimens. Maximum likelihood and Bayesian topologies were highly congruent for all data sets and differed only in support values. The most likely tree was edited in FigTree v 1.4 (Rambaut 2012) and presented with mapped bootstrap support and posterior probabilities.

2.4 Results

2.4.1 Morphological analyses and taxonomy

The alcyoniid specimens collected in this study belonged to one of three genera: *Cladiella, Aldersladum* and a proposed new genus, Beta, based on established morphological characters (Figure 2.5).

Species identifications were possible for certain colonies of Beta *kashmani* and *Aldersladum sodwanum*, based on the original species descriptions by Benayahu & Schleyer (1996) and Benayahu & McFadden (2011) respectively. Specimens of Beta *kashmani* and several unidentified morphospecies from the iSWP, that were previously assigned to the genus *Cladiella*, were reassigned to the genus Beta where their taxonomic affiliation is based on a lack of figure eight platelets in the polyps and the

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[7] http://www.genome.jp/tools/raxml/

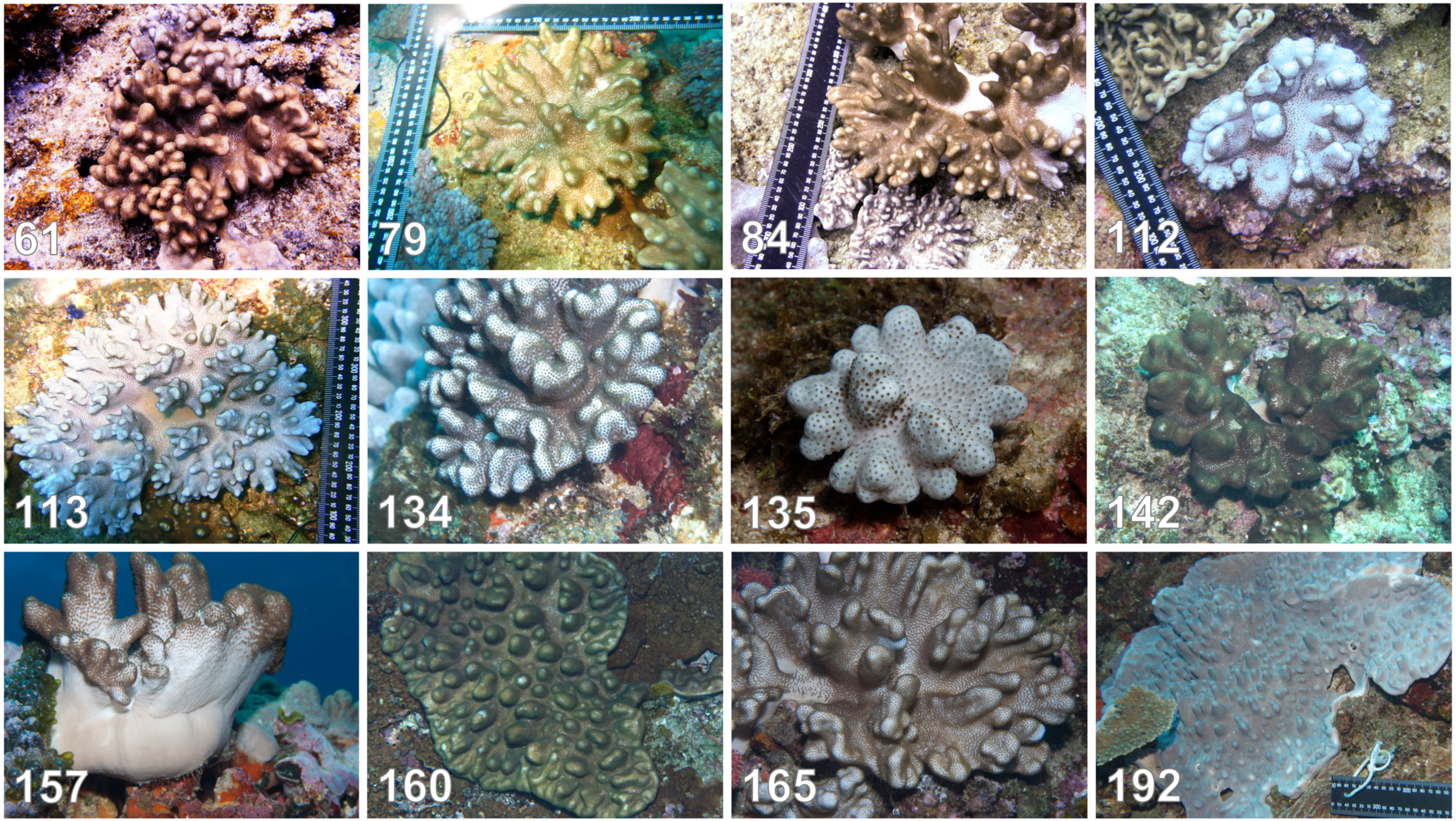

Figure 2.3 Beta colonies from the iSimangaliso Wetland Park and their sample identifications. Taxonomic expertise is required for identification to the species-level. Images: G. Jones.

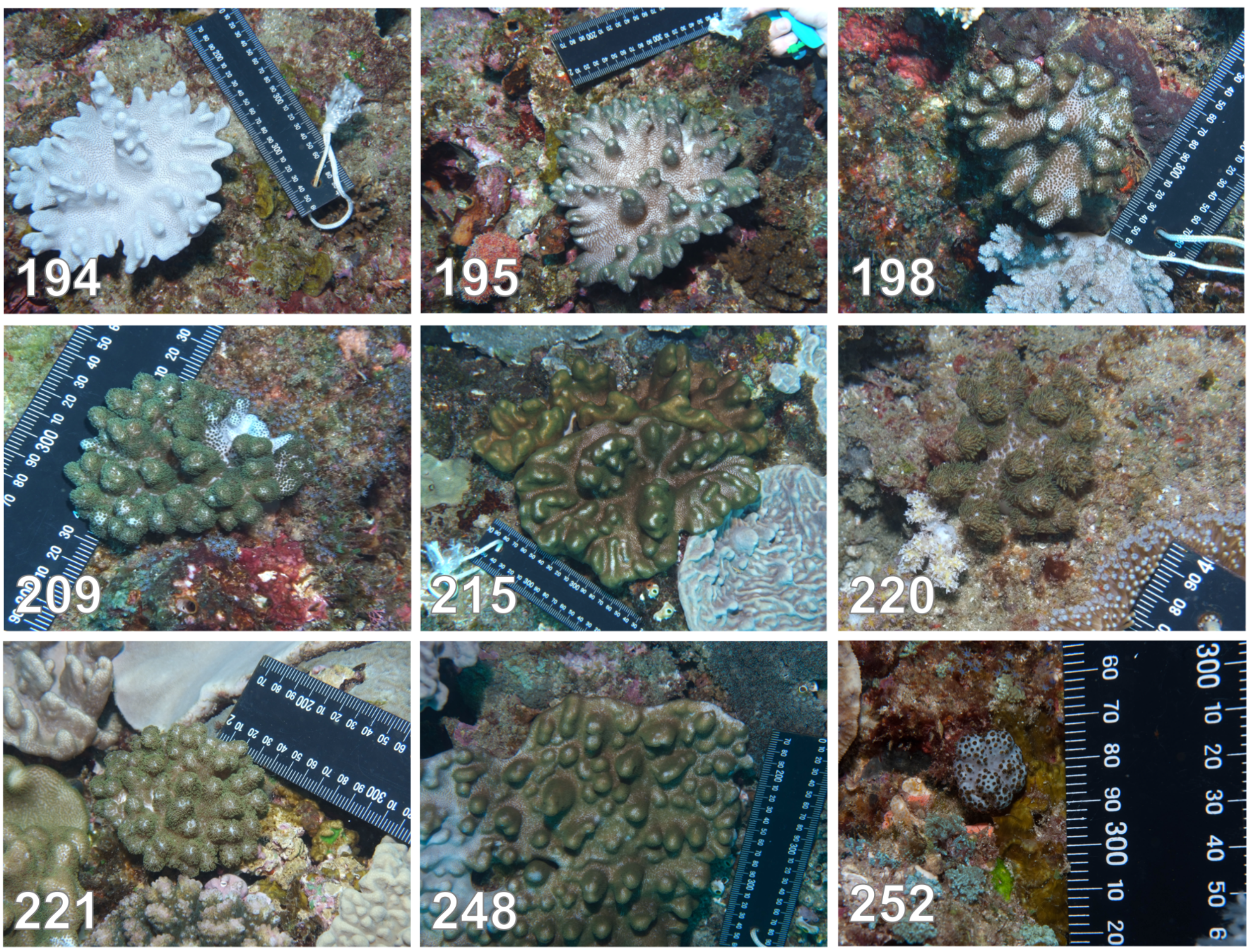

Figure 2.4 Beta colonies from the iSimangaliso Wetland Park and their sample identifications. Colony 220 is Beta *kashmani*. The remaining colonies are morphotypes of Beta that require taxonomic expertise for identification. Images: G. Jones.

presence of double heads with complex warts in the coenenchyme (Figure 2.5). Beta specimens were grouped according to their degree of resemblance to each species, B. *kashmani* and B. *australis*, based on sclerite examination. However, it was not possible to differentiate whether these taxa are merely morphotypes of a single species or entirely separate species without comprehensive taxonomic revision. Additional features of Beta that were inconsistent with *Cladiella* included the presence of notably larger sclerites (≥0.15 mm) and colonies that attain up to 3m in diameter. Beta colony forms were lobate and varied from encrusting to erect, similar to that of *Cladiella* (Figure 2.3 and Figure 2.4). Generic identifications of *Cladiella* were carried out according to the descriptions by Fabricius & Alderslade (2001) (Figure 2.5).

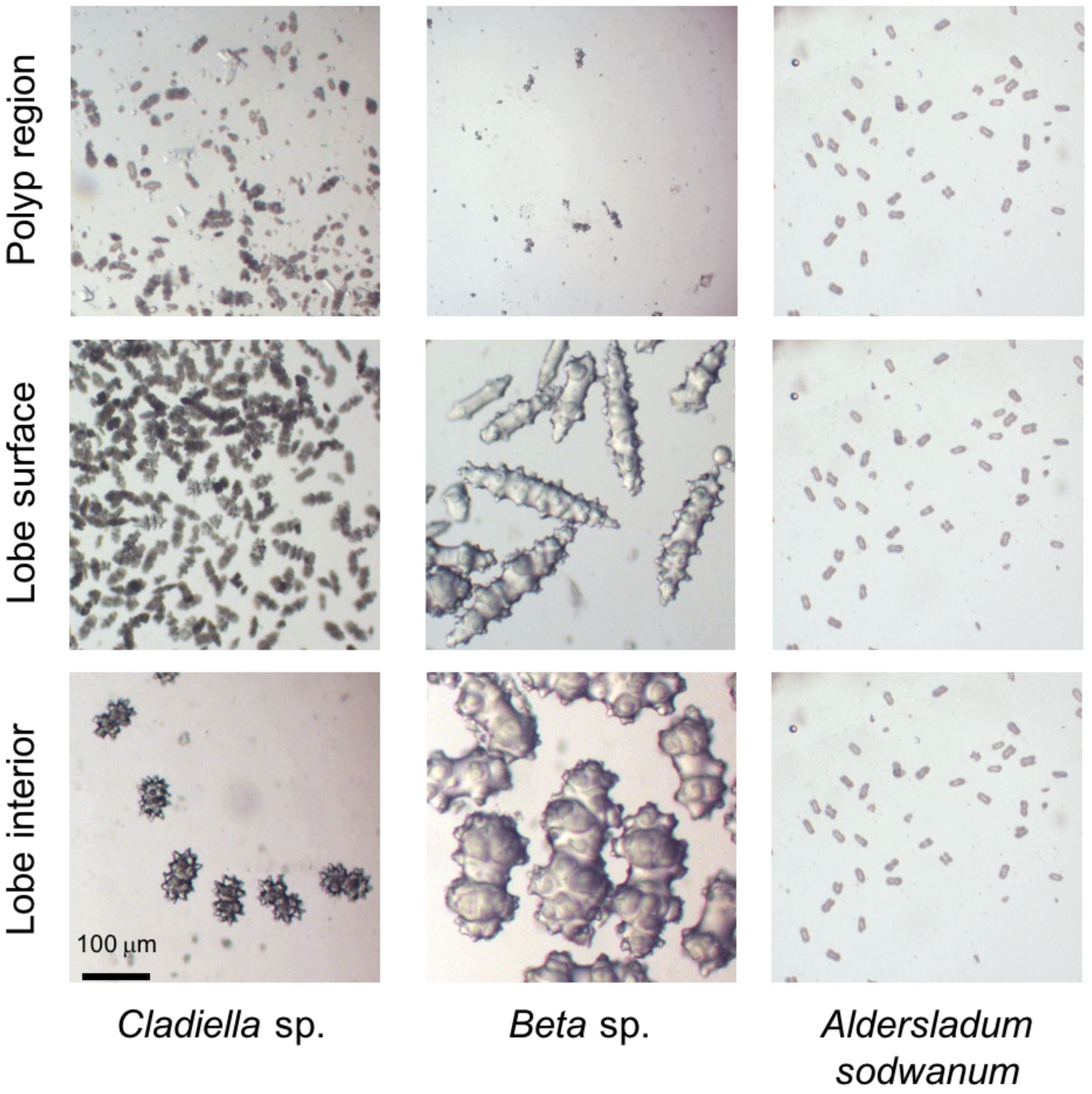

Figure 2.5 Sclerite compositions of comparable regions taken from *Cladiella*, Beta and *Aldersladum* from South Africa. No sclerites are present in the polyp tissues of Beta. The bar (bottom left) represents a scale of 100 µm.

No colonies of 'true' *Cladiella* were located within the iSWP and the specimens were recently discovered in the intertidal pools of the Durban area, approximately 380km south of the iSWP. This is indeed the first record of 'true' *Cladiella* in South Africa. The collected specimens of *Cladiella* were identified as conspecifics based on sclerite examination, however, species-level identification was not possible at the time without a revision of the genus.

Aldersladum colonies were distinguished by the exclusive presence of figure-eight shaped platelets throughout the entire colony as described by Benayahu & McFadden (2011) (Figure 2.5). Only one species of *Aldersladum*, *A. sodwanum*, is known from South Africa and was recorded in both the North and the South of KwaZulu-Natal in this study. As the namesake suggests, *A. sodwanum* is known for its occurrence in Sodwana Bay, iSWP (Benayahu & McFadden 2011), therefore this is the first record of these taxa outside this distribution and as far south as Durban.

2.4.2 Genetic diversity and phylogenetic analyses

Estimates of average evolutionary divergence (D) over all sequence pairs, between genera and within genera for loci are shown in Table 2.2. The D values in Table 2.2 indicate the overall mean pairwise genetic distance (uncorrected *p*) for each gene region. The multiple alignment of 45 *COI* sequences (970bp) revealed 116 variable sites (12%) of which 31 were parsimony-informative (Table 2.2). Forty-seven *MutS* sequences (583bp) yielded only 8 parsimony informative sites (5%) of the 143 variable sites (Table 2.2). Forty-seven sequences of *28S rRNA* (741bp) yielded 70 parsimony informative sites out of 128 variable sites (17%). Thirty-nine aligned and trimmed sequences (2292bp) of the concatenated gene regions, *COI*+*MutS*+*28S*, yielded 274 variable sites of which 96 were parsimony informative (35%) (Table 2.2).

COI and *MutS* displayed the lowest average intrageneric divergence of all loci (0.1%; Table 2.2) although *COI* displayed higher intergeneric distance (uncorrected *p*) than *MutS.* Nuclear *28S rRNA* exhibited more parsimony informative sites, a higher haplotype diversity and recovered more species than *COI* or *MutS* alone (Figures 2.6 and 2.7). The nuclear marker exhibited the most variation of the individual loci and was comparable to the multi-locus barcode, *28S rRNA* exhibited the greatest average intergeneric variation of all markers used (Table 2.2).

Table 2.2 Diversity estimates for the genetic barcodes, including genetic distance values (uncorrected *p*) for the sequences used in this study.

Gene region	nt	H	Hd	π	k	S	S_p	D	D(b)	D(w)
COI	970	22	0.937	0.018	14.77	116	31	0.018	0.017	0.001
MutS	583	14	0.778	0.018	4.62	143	8	0.013	0.012	0.001

Table 2.2 continued…

Gene region	**nt**	**H**	**Hd**	π	k	**S**	S_p	**D**	**D(b)**	**D(w)**
28S rRNA	741	33	3.942	0.036	26.25	128	70	0.037	0.029	0.008
COI+MutS+28S	2292	33	0.993	0.022	39.48	274	96	0.022	0.015	0.008

H= number of haplotypes; Hd= haplotype diversity; π= nucleotide diversity per site; k= average number of nucleotide differences; S= number of variable sites; Sp= number of parsimony-informative sites; D= estimates of average evolutionary divergence for all genera, between (b) and within (w) genera.

The phylogenies recovered for all gene regions (*COI*, *MutS*, *28S rRNA* and *COI+MutS+28S*) were congruent with previous studies of these taxa (McFadden et al. 2011, Benayahu et al. 2012) (Figure 2.6, Figure 2.7). Both mitochondrial phylogenies recovered supported clades that separated *Cladiella* and *Aldersladum* from Beta and *Klyxum*, however, the *Aldersladum* and *Cladiella* clade was unresolved with *COI* and the Beta and *Klyxum* clade was unresolved with *MutS* (Figure 2.6). The *COI* dataset recovered a supported, monophyletic *Klyxum* clade nested in a clade of Beta. Conversely, the *MutS* dataset recovered a paraphyletic clade of *Klyxum* and Beta that exhibited shared haplotypes. *Cladiella* and *Aldersladum* were recovered in well-supported monophyletic clades with the *MutS* dataset whereas both genera were paraphyletic with the *COI* marker.

The average intergeneric pairwise distances (uncorrected *p*) for the *COI* dataset showed significant distances between *Klyxum* and *Cladiella* (3.8%), Beta with *Cladiella* (3.7%), and Beta and *Aldersladum* (3.6%); whereas no notable distinction was found between genera *Cladiella* and *Aldersladum* (0.1%) nor between Beta and *Klyxum* (0.2%). *C. tuberosa* and *A. sodwanum* formed a paraphyletic clade within the *Cladiella* and *Aldersladum* group (Figure 2.6). Species were not delineated in that different species often shared identical haplotypes i.e. *C. tuberculoides* with *C. bottai* and *K. utinomii* with *K. adii* (Figure 2.6).

Phylogenetic analysis of the *MutS* region resolved *Cladiella* and *A. sodwanum* as monophyletic taxa (Figure 2.6). However, all *Klyxum* sequences shared a haplotype with several GenBank sequences of Beta *kashmani* and Beta spp. from this study (South Africa). Consequently, *Klyxum* and Beta displayed the equivalent genetic distance of conspecific taxa. The greatest intergeneric distance was found between *Cladiella* and *Klyxum* with Beta (2.8%), followed by *Aldersladum* and *Klyxum* with Beta (2.3%) and *Cladiella* with *Aldersladum* (0.5%). No species were distinguished within any of the genera wherein identical haplotypes were often shared between several different species i.e. *C. tuberculoides*, *C. pachyclados*, *C sphaerophora* and *K. utinomii*, *K. adii* and B. *kashmani* (Figure 2.6).

The nuclear (*28S rRNA*) and the concatenated (*COI+MutS+28S*) phylogenies were largely congruent with one another and recovered well-supported monophyletic genera for *Cladiella*, *Aldersladum* and *Klyxum*. The nuclear and concatenated datasets revealed greater levels of intrageneric resolution and species delimitations in several genera (Figure 2.7). However, the major distinction between the two phylogenies recovered showed Beta as a polyphyletic clade with *28S rRNA* and Beta as a monophyletic clade with the multi-locus barcode (Figure 2.7).

For the nuclear dataset, Beta demonstrated an overall 0.6% intrageneric variation, wherein GenBank specimens of B. *australis* and Beta sp. from South Africa (that are likely conspecifics of B. *australis*) formed a well-supported clade. The remaining congeners of Beta were all recovered in a distinct clade separated by a genetic distance (uncorrected *p*) of 1.3% to the other Beta clade. The former clade contained species of *B. kashmani* and sequences of Beta from South Africa that resemble B. *kashmani* (Figure 2.7). *Klyxum* formed a monophyletic clade with an intrageneric distance of 0.9% and exhibited species delimitations (Figure 2.7). *Klyxum* and Beta exhibited the closest intergeneric distance (1.4%, uncorrected *p*). *A. sodwanum* formed a well-supported monophyletic clade nested within *Cladiella* and separated by an intergeneric distance of 1.6% (uncorrected *p*). Sequences of *Cladiella* were recovered in a well-supported monophyletic clade with an intrageneric variation of 1.5% (uncorrected *p*). Beta and *Cladiella* (6.6%) exhibited the greatest intergeneric distance (uncorrected *p*) followed by *Cladiella* and *Klyxum* (6.4%), Beta and *Aldersladum* (6.4%) and *Aldersladum* with *Klyxum* (6.2%). The presence of a shared haplotype (*C. tuberculoides* and *C. bottai*) and scattered conspecifics (*C. pachyclados* and B. *kashmani*) limited species delineations for *Cladiella* and Beta. However, unlike the mitochondrial datasets, most known species formed distinct haplotypes with *28S rRNA* (Figure 2.7). The single nuclear gene region provided greater resolution than the

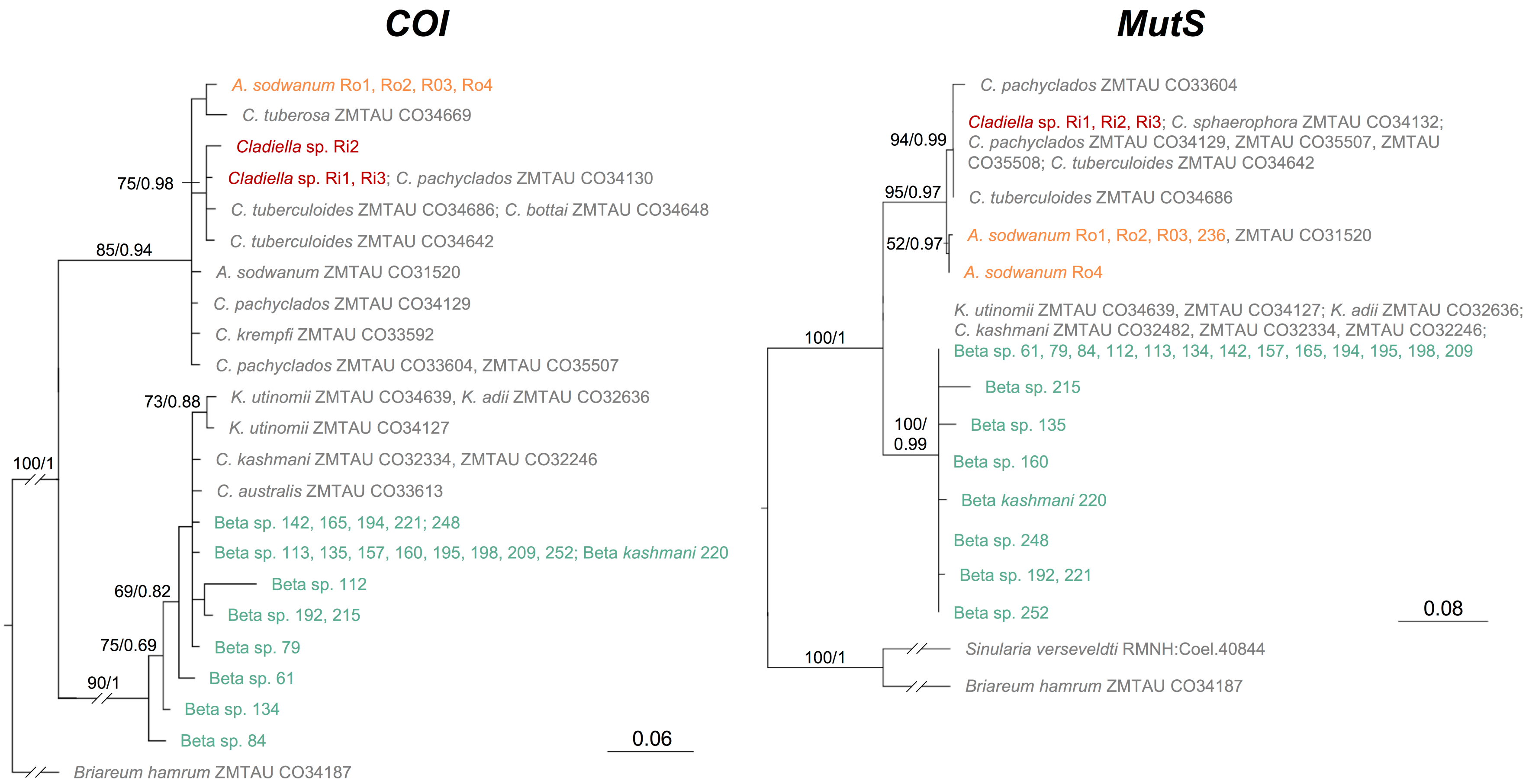

Figure 2.6 Maximum likelihood trees of Beta, *Cladiella*, *Aldersladum* and *Klyxum* based on cytochrome *c* oxidase subunit I (*COI*) and bacterial MutS homologue (*MutS*) sequence data. Branch length scales are given; branch labels indicate bootstrap support values and posterior probabilities respectively. GenBank sequences are denoted in grey and genera are colour-coded.

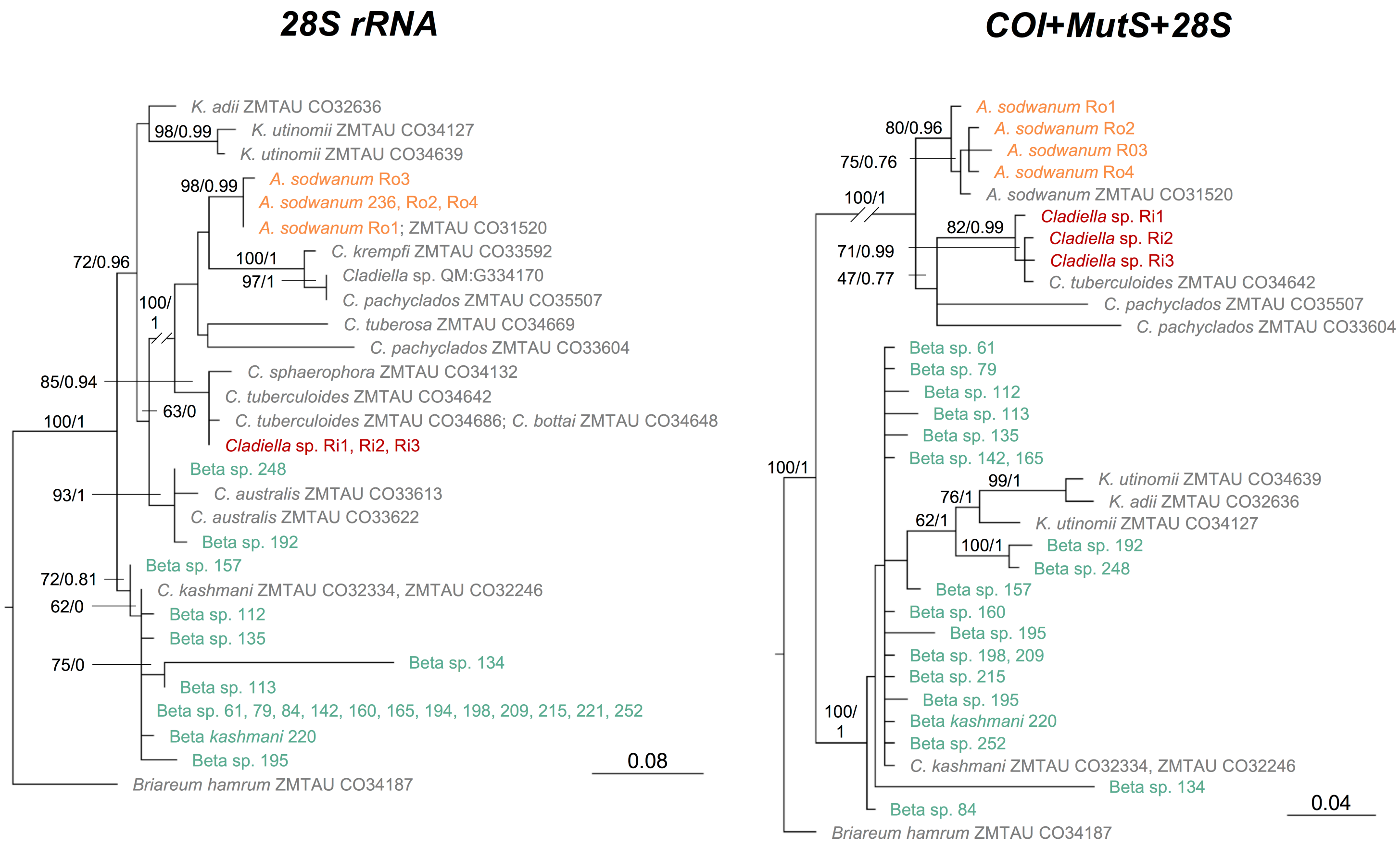

Figure 2.7 Maximum likelihood trees of Beta, *Cladiella*, *Aldersladum* and *Klyxum* based on nuclear 28S ribosomal RNA (*28S rRNA*) sequence data (left) and concatenated sequences (right) of cytochrome *c* oxidase subunit I (*COI*), bacterial MutS homologue (*MutS*) and *28S rRNA* (*COI+MutS+28S*). Branch length scales are given; branch labels indicate bootstrap support values and posterior probabilities respectively. GenBank sequences are denoted in grey and genera are colour-coded.

individual mitochondrial regions and enabled species delimitations for *Klyxum* and *Aldersladum* though showed limited interspecific variation for *Cladiella*.

Phylogenetic analysis using the concatenated barcode, *COI+MutS+28S,* recovered well-supported monophyletic clades of each genus and exhibited species delineations (Figure 2.7). The average pairwise genetic distance (uncorrected *p*) between Beta and *Aldersladum* were 4.6%, followed by *Klyxum* and *Aldersladum* (4.5%), *Cladiella* and *Klyxum*, Beta and *Cladiella* (3.6%), *Cladiella* and *Aldersladum* (1.7%) and Beta with *Klyxum* (0.6%). A distinct clade with significant bootstrap support was formed within the genus Beta wherein sequences of Beta sp. 192 and 248 (specimens that resemble *B. australis*) were separated from their congeners by 0.5%, similar to that for *28S rRNA* (Figure 2.7). Although most of the unidentified Beta specimens formed distinct haplotypes, further taxonomic expertise is required to ascertain whether these taxa are separate species to *B. kashmani* and B. *australis*.

2.5 Discussion

2.5.1 Molecular markers

The taxonomic resolution of the nuclear ribosomal gene (*28S rRNA*) was assessed in addition to known mitochondrial markers cytochrome *c* oxidase subunit I (*COI*) and bacterial MutS homologue (*MutS*). McFadden et al. (2005, 2011) established the limitations of *COI* and *MutS* for delimiting species of Alcyonacea due to the slow rates of mitochondrial evolution, therefore nuclear loci are used to compensate for the low substitution rate and sort taxa more effectively than mtDNA (Aguilar & Sánchez 2007, Sánchez 2007, McFadden et al. 2017, Soler-Hurtado et al. 2017). Consequently, phylogenetic resolution indicated that the nuclear region, *28S rRNA*, exhibited the greatest number of informative characters of the individual loci in this study (Table 2.2) and Figure 2.7). Likewise, Bilewitch et al. 2010, McFadden et al. (2014) also remarked on the phylogenetic utility of nuclear *28S rRNA* in the examination of inter- and intrageneric relationships of *Briareum* and stoloniferous taxa respectively. The inclusion of the nuclear marker in concatenation of the mitochondrial loci further potentiated the phylogenetic signal and resulted in the most informative phylogeny (Figure 2.7). However, this approach was limited by a lack of GenBank sequence representatives for all three loci. In addition, even with the combination of the three genes, variation at the genus and species level was insufficient for uncovering the relationships of some of these alcyoniid complexes.

2.5.2 Phylogenetic relationships and taxonomy

This generic complex has been afforded little phylogenetic and taxonomic attention. The most comprehensive phylogenetic analysis to date for these genera was carried out by Benayahu et al. (2012) and parallels the phylogenies recovered in this study. It must be noted, however, that sequences of *Aldersladum jengi* Benayahu & McFadden, 2011 were not incorporated into phylogenetic analyses in this study as there are no existing records for these taxa within the study region. It is widely accepted that *Aldersladum* is a paraphyletic genus as *A. jengi* was recovered amongst *Cladiella* sequences in previous studies (Benayahu & McFadden 2011, Benayahu et al. 2012) (although this may be an artefact of the representation of one specimen of *A. jengi*). Therefore, the monophyly of the ditypic genus *Aldersladum* is an artefact of the omission of *A. jengi,* for which sequences from only a single specimen are available. *Aldersladum* was consistently recovered as a sister taxon to *Cladiella* and mirrored support for the monophyly of *A. sodwanum* to that of previous studies (Benayahu & McFadden 2011, Benayahu et al. 2012). Notwithstanding the little genetic distinction shown between these two genera in this study and in those of previous reports (with or without the inclusion of *A. jengi*) (Benayahu et al. 2012), *Aldersladum* is a separate genus from *Cladiella* due to considerable morphological differences (Benayahu & McFadden 2011). However, these taxa require additional systematic investigation with greater taxon sampling in order to elucidate their phylogenetic relationships.

Similar to the results of this study, previous studies (Benayahu et al. 2012; Benayahu et al. 2014) have shown that B. *australis* and B. *kashmani* exhibited a greater phylogenetic affiliation with *Klyxum* than with their original designated genus, *Cladiella,* which would have otherwise been polyphyletic if not for the reassignment of the former taxa to the genus Beta. Furthermore, *Cladiella* was more closely associated with *Aldersladum* than any other alcyoniid genus (Benayahu et al. 2012). Phylogenetic comparisons of all loci (both individual and concatenated) consistently differentiated Beta from *Cladiella* (Figure 2.6 and Figure 2.7) with an average pairwise distance of 4.2% (uncorrected *p*). The genetic distance between these taxa is equivalent to the intergeneric distances between other Alcyoniidae genera (Appendix C: Tables 7.10- 7.12). Moreover, Beta and *Cladiella* exhibited a greater genetic distance than the intergeneric distance between *Cladiella* and *Aldersladum* (0.97%). Therefore, B. *australis*, *B. kashmani* and Beta spp. are not morphospecies nor congeners of *Cladiella*. The considerable genetic differentiation and substantial morphological discrepancies between Beta and *Cladiella* ratifies the establishment of Beta as a separate genus. Furthermore, taxonomic experts have formerly acknowledged that an entire taxonomic revision of the genus *Cladiella* is needed (eg. Benayahu & Chou 2010), as it is extremely difficult, if not impossible, to identify species of the genus using the available literature.

Beta was recovered as a monophyletic clade with the loci *COI* and *COI+MutS+28S* whereas the variable *28S rRNA* marker recovered Beta as a polyphyletic genus consisting of a clade of the B. *kashmani* group and a second clade of the B. *australis* group, both of which formed sister clades to *Klyxum* (Figure 2.6 and Figure 2.7). Additionally, the B. *australis* group was recovered in a distinct clade from the B. *kashmani* group with *COI+MutS+28S* (in which Beta was a monophyletic genus) which further supported the delineation of the two groups.

Although Beta was recovered in clades that were phylogenetically distinct from *Klyxum* in most phylogenies, there was little genetic distance between the two genera (0.6%, uncorrected *p*) across the gene regions. Whilst Beta was genetically and morphologically distinguished from its previously assigned genus, *Cladiella*, its close genetic distance to the genus *Klyxum* was noted in this study and in that by Benayahu et al. (2012). The results of which suggest that Beta, although forming a distinct clade, cannot be genetically distinguished from the *Klyxum* genus as the molecular evidence suggests that the two may be congeners.

However, Beta cannot be assigned to the genus *Klyxum* because of the established morphological differences between these two taxa (Benayahu & Schleyer 1996, Fabricius & Alderslade 2001) that do not warrant their taxonomic placement in *Klyxum*. Beta is characterised by an absence of platelet sclerites in the polyp region and the presence of double heads with complex warts in the coenenchyme whereas *Klyxum* is distinguished by flat rods and platelets in the polyp region and spindles with large cone-shaped processes in the coenenchyme (Alderslade 2000, Fabricius & Alderslade 2001). Therefore, Beta does not fit the diagnosis of the genus *Klyxum.*

Owing to the extensive morphological differences between the two taxa, it is implausible to revise the genus description of *Klyxum* to accommodate these differences as no common diagnostic morphological characters exists between the two taxa. Regardless of the extent of these disparities, morphological distinctions exhibited by Beta were not fully supported by genetics, which was also noted by Benayahu et al. (2012). In other alcyonacean taxa, Bilewitch et al. (2014) also noted a lack of correspondence between levels of genetic divergence and morphological diversity among Ellisellidae genera and Soler-Hurtado et al. (2017) found evidence of morphological differentiation with no apparent genetic differentiation in *Pacifigorgia.* Benayahu et al. (2012) noted no apparent generic distinction between *Aldersladum* and *Cladiella* when *A. jengi* was included in molecular analyses. Regardless of the limited phylogenetic distinction between *Cladiella* and *Aldersladum*, they remain separate genera because of their varied morphology, the same reasoning could be used to support Beta and *Klyxum* as separate genera.

For this study, the lack of genetic differentiation observed between the genera was based on two mtDNA markers and one nuclear marker, which may account for the apparent shallow difference between *Klyxum* and Beta. Apparent incongruences between morphological and molecular differentiation could be due to possible rapid speciation or ancient hybridisation (Soler-Hurtado et al. 2017) and anthozoan mtDNA may evolve at too slow a rate to corroborate these morphological distinctions (Shearer et al. 2002). Although the selected gene regions used in this study were successful in delimiting a wide range of octocoral genera in previous studies (Bilewitch et al. 2010, Aguilar-Hurtado et al. 2012, Benayahu et al. 2012, Haverkort-Yeh et al. 2013, Stemmer et al. 2013, McFadden, Brown, et al. 2014, McFadden, Reynolds, et al. 2014, Cairns & Wirshing 2015), intergeneric variation varies among closely related taxa and traditional loci used in octocoral systematics cannot resolve all generic boundaries i.e. *Rumphella*, *Pacifigorgia*, *Xenia*, *Bayerxenia* and *Heteroxenia* (Benayahu et al. 2012, Haverkort-Yeh et al. 2013, Stemmer et al. 2013, McFadden, Brown, et al. 2014, Vargas et al. 2014). Therefore, more nuclear loci are required to elucidate deeper-level relationships in Alcyonacea. Recent developments in the use of probes for targeted enrichment of ultraconserved element (UCE) loci (Faircloth et al. 2012) shows potential for resolving phylogenetic relationships in non-model organisms and recently diverged taxa (Quattrini et al. 2017).

2.6 Conclusion

Integrated taxonomy is necessary for the systematics of closely related genera within the family Alcyoniidae. The generic boundaries of polyphyletic and morphologically aberrant species of *Cladiella* did not conform to their taxonomic assignments. It is recommended that these taxa be reassigned and placed in a new genus, Beta. This genus features a unique combination of an absence of sclerites in the polyp region and the presence of double heads with complex warts in the coenenchyme, which distinguishes these taxa from other alcyoniid genera. Further investigation into the relationships between *Cladiella*, Beta, *Aldersladum* and *Klyxum* is required using a larger sample group from a widespread geographic range in addition to molecular approaches that target nuclear genomic elements.

2.7 Acknowledgements

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CHAPTER 3: *SINULARIA* OF THE ISIMANGALISO WETLAND PARK, SOUTH AFRICA: GENETIC PROXIES PROVIDE ESTIMATES OF SPECIES RICHNESS (PAPER 2)

3.1 Abstract

Sinularia May, 1898 (Octocorallia: Alcyonacea) is an ecologically important and diverse genus of soft coral distributed throughout the Indo Pacific. The high latitudinal coral assemblages of Northern KwaZulu-Natal, South Africa, all lie within the world heritage site, the iSimangaliso Wetland Park (iSWP) and is the southernmost distribution limit of *Sinularia* in the Indian Ocean. A recent systematic revision of this speciose genus revealed additional diagnostic characters that exhibited congruence with the phylogeny of the mitochondrial bacterial MutS homologue (*MutS*) region. The phylogenetic aspect of this classification system was utilized to facilitate identifications for 67 *Sinularia* specimens collected from the iSWP and assess species richness for the region. Species boundaries were tested with *MutS* using Automatic Barcode Gap Discovery (ABGD), Bayesian Poisson Tree Processes (bPTP) and Generalised Mixed Yule Coalescence (GMYC), although no barcoding gap was established. In addition, nuclear ribosomal 28S rRNA (*28S rRNA*) was used in concatenation with *MutS* to increase taxonomic resolution. However, certain allospecific taxa exhibited shared haplotypes. Forty-two of the 67 *Sinularia* specimens (63%) were identified to the species-level that represented six of the fourteen species recorded in the region, with a new record for *S. grandilobata* Verseveldt, 1980. The remaining 25 specimens were assigned to 16 putative species that require taxonomic expertise for identification. The significant genetic distance (9.3%, uncorrected *p*) between *S. brassica* May, 1898 and all other congeners implies that these taxa should exist as two separate genera. This is the first study to address this the data-deficient octocoral community in the iSWP using a phylogenetic approach.

Keywords: *phylogeny, MutS, 28S rRNA, species, Sinularia, South Africa*

3.2 Introduction

Sinularia May, 1898 is the most speciose genus of zooxanthellate soft coral with approximately 170-190 known species worldwide (van Ofwegen et al. 2016, Horton et al. 2017). This genus has an extensive distribution throughout the Indo-Pacific region from east Africa, the Red Sea to Polynesia and Hawaii; at a depth ranging from the shallow (<1m) to the mesophotic zone (>60m) though most known species occur at shallower depths (<35m) (Fabricius & Alderslade 2001, van Ofwegen 2002, Benayahu, McFadden, & Shoham 2017, Benayahu, McFadden, Shoham, et al. 2017). These monomorphic octocorals are dominant space occupiers in a range of habitat types and conditions (Riegl et al. 1995, Fabricius 1998, Samimi-Namin & van Ofwegen 2012). *Sinularia* have considerably diverse growth forms that vary in size from a few centimetres in diameter to massive encrusting colonies that span several meters (Benayahu et al. 1998, Fabricius 1998). These ecologically important taxa have markedly large coenenchymal sclerites that, in some species, form spiculite (fused sclerites masses) and are the only soft corals known to exhibit reef building properties (Fabricius & Alderslade 2001, Cornish & DiDonato 2004, Jeng et al. 2011).

The high latitude marginal reefs of Northern KwaZulu-Natal, South Africa, form the southernmost limit of *Sinularia*'s distribution in the Indian Ocean (Riegl et al. 1995, Schleyer 1999, Riegl 2003, Celliers & Schleyer 2007, Benayahu et al. 2012). These coral assemblages are found within the Delagoa Bioregion- a tropical to subtropical transition zone in which the UNESCO World Heritage site, the iSimangaliso Wetland Park (iSWP) was established (Williams et al. 1992, Schleyer & Celliers 2005). *Sinularia* are the most diverse alcyonacean group in South Africa and form the largest component of the reef biota in the iSWP (Benayahu 1993, Williams 1993, 2000, Schleyer et al. 2008). Studies of the effects of climate change in this region report a decline in *Sinularia* at a rate of 0.95% p.a. compared with an increase in their Scleractinian counterparts (Celliers & Schleyer 2007, Muthiga et al. 2008, Schleyer et al. 2008, Porter & Schleyer 2017).

Ecological and evolutionary studies of these taxa are challenged by their difficult taxonomy due to phenotypic plasticity and unreliable diagnostic morphological characters. *Sinularia* remains an understudied taxon in the region with only 14 recorded species and additional species predicted (Williams 1992, Williams et al. 1992, Benayahu 1993, Gibbons et al. 1999). Recorded taxa for this region include: *Sinularia abrubta* Tixier-Durivault, 1970; *Sinularia brassica* May, 1898; *Sinularia erecta* Tixier-Durivault, 1945; *Sinularia firma* Tixier-Durivault, 1970; *Sinularia gardineri* Pratt, 1930; *Sinularia gravis* Tixier-Durivault, 1970; *Sinularia heterospiculata* Verseveldt, 1970; *Sinularia hirta* Pratt, 1903; *Sinularia muralis* May, 1899; *Sinularia notanda* Tixier-Durivault, 1966; *Sinularia querciformis* Pratt, 1903; *Sinularia schleyeri* Benayahu, 1993 (endemic); *Sinularia triangula* Tixier-Durivault, 1970; and *Sinularia variabilis* Tixier-Durivault, 1945 (see Chapter 5, Table 5.1, for the basis of each record in the region).

It was previously thought that *Sinularia polydactyla* Ehrenberg, 1834; and *Sinularia leptoclados* Ehrenberg, 1834 were recorded from the iSWP but recent taxonomic revisions dispute the occurrence of these species in South Africa (van Ofwegen et al. 2013, 2016). *S. polydactyla* is considered endemic to the Red Sea whereas taxa formerly known as *S. polydactyla* that are recorded outside of this region, were reassigned to *S. ceramensis* or one or more unidentified species (van Ofwegen et al. 2016). Furthermore, specimens identified as *S. leptoclados* have been called into question since the taxonomic revision of this species by van Ofwegen et al. (2013) in which a number of taxa were reassigned to the species *S. australiensis*. It remains unclear as to whether records of *S. leptoclados* from the iSWP are still valid (van Ofwegen et al. 2013). These recent revisions further necessitate an update of the current species recorded in the iSWP.

The lack of information for these ecologically significant taxa is attributed to their difficult taxonomy. Until recently, species boundaries for these taxa were poorly understood due to outdated, inaccurate and ambiguous specimen descriptions (Benayahu et al. 1998, van Ofwegen 2008, van Ofwegen et al. 2016). Verseveldt (1980) established five main morphological groupings in his revision of *Sinularia* based on club sclerites in the surface tissues to help facilitate species identifications.

However, the inclusion of molecular phylogenies in systematic studies of *Sinularia* revealed no congruence between Verseveldt's groupings with sequence variation (McFadden et al. 2009, 2010). McFadden et al. (2009) re-examined over 80 species of *Sinularia* (that included 11 of the 14 recorded species from South Africa) and discovered additional characters of taxonomic importance by mapping them onto a molecular phylogeny. Previously overlooked morphological features included the presence of sclerites in the tentacle, collaret, and point regions of the polyps; the shape of the surface club sclerites and general colony form (McFadden et al. 2009). These morphological characters correlated to distinct phylogenetic clades and subclades recovered in the analysis of the mitochondrial *MutS* homolog gene (McFadden et al. 2009, van Ofwegen et al. 2016). This revised taxonomic approach provided a reference phylogeny in which the identity of specimen could be inferred from clades to which candidate species were assigned and required little taxonomic expertise. The key facilitated discrimination of clades within the genus for closely related taxa and even species boundaries in some cases. This approach has ameliorated cumbersome taxonomic identifications and a considerable number of *Sinularia* species have since been described from various regions and depths (van Ofwegen 2008, Dautova et al. 2010, Benayahu & van Ofwegen 2011, van Ofwegen & Benayahu 2012, van Ofwegen et al. 2016, Benayahu, McFadden, Shoham, et al. 2017).

However, the taxonomic utility of this systematic key is limited by the slow evolution of the mitochondrial genome in Octocorallia and the use of a single-locus (Bucklin et al. 2011, McFadden et al. 2011, 2014). Nevertheless, *MutS* is among the fastest evolving regions of the mitochondrial genome specific to Octocorallia, even compared to the standard barcode cytochrome *c* oxidase (*COI*) (Hebert & Gregory 2005), which lacks variation in Anthozoans (Culligan et al. 2000, France & Hoover 2002, Shearer et al. 2002, Bucklin et al. 2011). The limitations of the *MutS* gene region for *Sinularia* is that it recovers no clear distinction between levels of interspecific and intraspecific variation necessary to establish a barcoding gap (McFadden et al. 2006, 2009, 2014). McFadden et al. (2014) showed that *MutS* recovered clades of morphologically similar species of *Sinularia* and were able to determine species identifications for 96% of the specimens using a vouchered database of *MutS* sequences and comparisons with *in situ* photographs of colonies. Analyses of regions from more rapidly evolving nuclear regions, such as ribosomal RNA, revealed higher levels of interspecific variation for octocorals but also lacked the intrageneric variation to delineate between closely related species (McFadden et al. 2014).

For this study, a multilocus barcode of mitochondrial *MutS* and nuclear *28S rRNA* was used to infer species estimates of *Sinularia* from South Africa and investigate their evolutionary relationships in marginal reef habitats. The resolution and utility of mitochondrial and nuclear markers were assessed for their application in studies of *Sinularia*. This study was conducted to ease the identification process of taxa from this area and highlight potentially undescribed species that require taxonomic attention. The results may be used to highlight areas of concern for management in the marine reserve. Furthermore, biodiversity estimates and the availability of molecular data from this marginal community will help facilitate comparative studies with other localities in order to forecast their response to climate change.

3.3 Materials and methods

3.3.1 Specimen collection

Sixty-seven samples of *Sinularia* were collected via SCUBA from several reefs within the world heritage site, the iSimangaliso Wetland Park (iSWP) in KwaZulu-Natal, South Africa (Figure 3.1). Research and sampling permits (permit reference numbers RES2013/13, RES 2014/06 and RES2015/70) were granted by the Department of Agriculture, Forestry and Fisheries (DAFF). Samples were collected at a depth range of 8-20 m and a minimum of five meters apart from one another to avoid the collection of clones (Appendix A, Table 7.2). Photographs were taken of each respective colony *in situ* and a longitudinal slice was excised from each specimen for morphological examination and genetic analyses. The voucher samples were subsequently fixed in 70% ethanol, stored at room temperature and await museum deposition. For genetic analyses, tissue subsamples ($1cm^3$) were excised from the lobe region of each sample using a sterile scalpel, placed directly into cryofuge tubes and stored at -80°C.

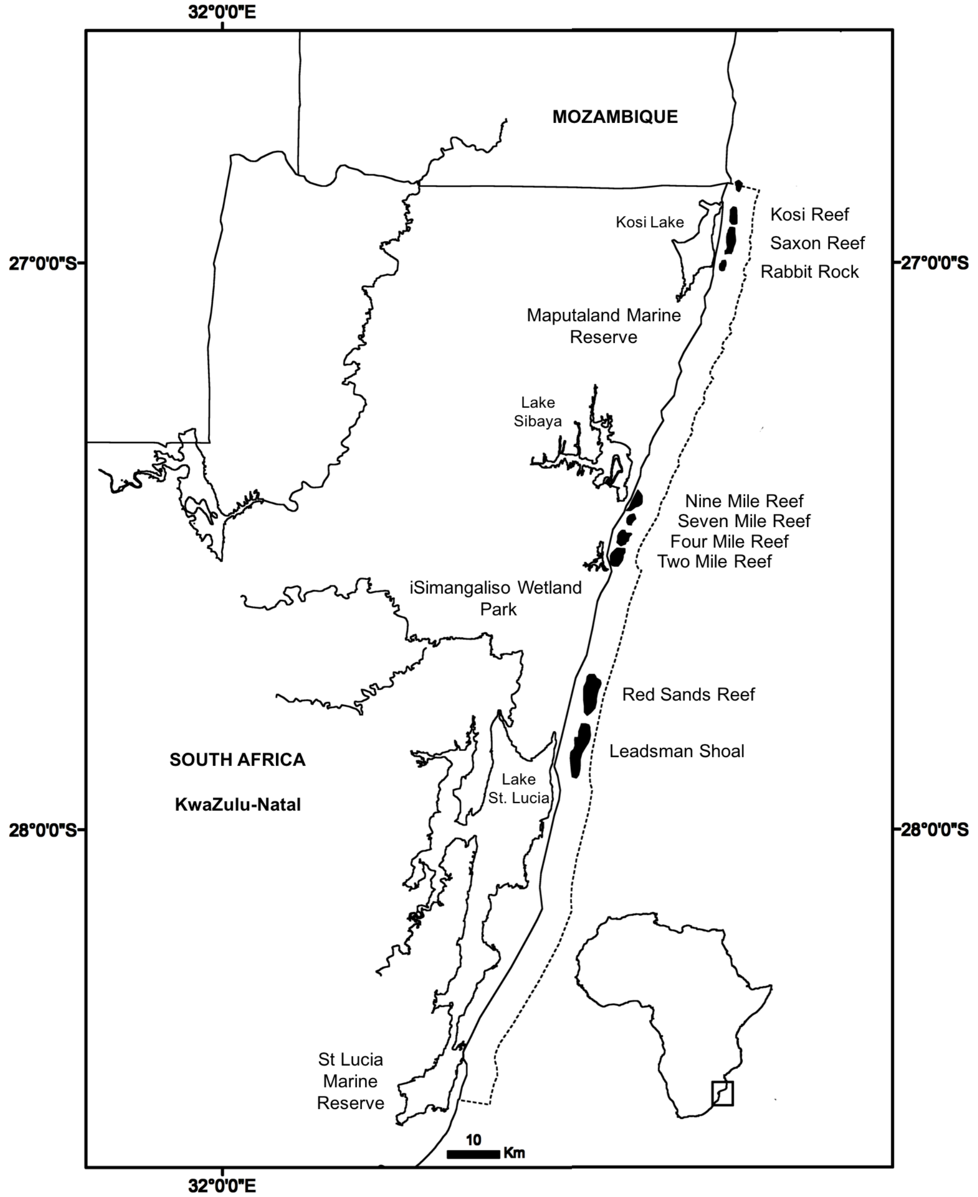

Figure 3.1 Collection sites of *Sinularia* from the iSimangaliso Wetland Park, South Africa. Coastal region under protection is indicated by the dotted line.

3.3.2 Morphological analyses

Preliminary generic assignments were carried out according to protocols and taxonomic features as described by Fabricius & Alderslade (2001) and Verseveldt (1980). Colony form was examined under a stereomicroscope (Z730 Stereo Series; Unitron) at 20X power. Tissue fragments were excised from the polyps, the surface and the interior of both the polypary and the base of the stalk. The fragments were subsequently dissolved in 12% sodium hypochlorite to free the sclerites for examination with a compound microscope (Eclipse 80i; Nikon) at 40 and 100X magnification.

3.3.3 DNA extraction and PCR amplification

Approximately 5mm^3 of coral tissue was ground in liquid nitrogen with a sterilized pestle and mortar. Genomic DNA was isolated from the homogenized material using a Zymogen ZR Genomic DNA^{TM} Tissue MiniPrep kit according to the manufacturer’s protocol and included the recommended 16hr overnight digestion step. DNA quantity and quality were subsequently measured using a NanoDrop 2000 (Thermo Scientific^{TM}, Wilmington, DE, USA).

A 735bp fragment of the mitochondrial *MutS* region and nuclear fragment, *28S rRNA* (750bp) were amplified using primers described by Sánchez et al. (2003) and McFadden et al. (2014) respectively. Each PCR reaction consisted of 1 X DreamTaq Green PCR Master Mix (Thermo Scientific^{TM}); 0,18 µM each of forward and reverse primer; 10mg/ml BSA; 50-150ng DNA template and nuclease-free water to a final volume of 25 µl. Amplifications were carried out using a T100^{TM} Thermal cycler (Bio-Rad) with an initial denaturation at 95 °C for 2 min; 35 cycles of 95 °C for 30s, 50 °C for 30s, and 72 °C for 60s; followed by a final extension of 72 °C for 5 min. The PCR products, along with a negative control and GeneRuler 100bp DNA Ladder (Thermo Scientific), were electrophoresed using a 1% agarose gel for 60 min and examined under a Molecular Imager® ChemiDoc^{TM} XRS (Bio-Rad) to confirm successful amplification of the targeted gene regions.

PCR amplicons were subsequently sent to the Central Analytical Facilities unit of Stellenbosch University for purification, quantification and Sanger dideoxy sequencing with an ABI 3730xl DNA analyser (Thermo Scientific^{TM}). Raw sequences of taxa from the study were examined by alignment with *MutS* and *28S rRNA* sequences of *Sinularia* taken from GenBank using BLASTN (Altschul et al. 1990). The sequences were manually edited and annotated in MEGA v 6.06 (Tamura et al. 2013) and subsequently uploaded to GenBank (Table 3.1).

3.3.4 Phylogenetic analyses and species identifications

Phylogenetic analyses comprised datasets of 126 *MutS* (735bp) sequences and 76 *MutS+28S* concatenated sequences (1494bp), of which 67 *MutS* and 53 *MutS+28S* sequences were from taxa collected in this study. Additional GenBank sequences of *Sinularia* were included in phylogenetic analyses to increase the representation of species (Appendix A). Preliminary phylogenetic analyses were conducted for *MutS* that included GenBank sequences of congeners for up to 55 different species of *Sinularia*. For convenience sake, the final analyses for *MutS* only included GenBank sequences that were pertinent to the clades in which the taxa collected in this study fell. These taxa comprised 58 *MutS* sequences from amongst 35 different species of vouchered specimens of *Sinularia* from GenBank. The GenBank sequences included representative species from each major clade and subclade (1, 2, 3, 4A, 4B, 4C, 4D, 5A, 5B, and 5C) as described in McFadden et al. (2009) and included taxa that have been known to occur in South Africa, in order to facilitate species inferences from the phylogenetic framework (Appendix B, Table 7.4). *Pterogorgia anceps* (GQ342500.1) was used to root the tree as per McFadden et al. (2009). Similarly, 38 of the GenBank *MutS* sequences that had corresponding *28S rRNA* sequences were included in the concatenated analysis with *Pterogorgia anceps* (JX203714.1) as an outgroup.

Nuclear data were phased using PHASE v 2.1.1 (Flot 2010) and multiple sequence alignments of *MutS* (n=126) and *MutS+28S* (n=76) were carried out respectively using the MUSCLE (Edgar 2004) function in MEGA v 6.06 (Tamura et al. 2013). DnaSP v 5.10.1 (Librado & Rozas 2009) was used to calculate haplotypes for each dataset. Gaps were taken into consideration for the calculation of haplotypes from the concatenated dataset to account for indels and high levels of polymorphism exhibited by the nuclear region. Sequences from diploid individuals with phased alleles shared the same haplotypes and were therefore not indicated in the resultant phylogenetic tree. In addition, nucleotide and haplotype diversities were calculated for each dataset in DnaSP v 5.10.1 (Librado & Rozas 2009). MEGA v 6.06 (Tamura et al. 2013) was used to calculate pairwise genetic distances (uncorrected *p*) among and within the 5 major clades and subclades for both *MutS* and the concatenated dataset (*MutS+28S*).

Substitution models were determined using jModelTest v 2.1.10 (Guindon & Gascuel 2003, Darriba et al. 2012) and selected using the Akaike information criterion (AIC) (Akaike 1973) for *MutS* (HKY+G) and *28S rRNA* (GTR+G) for implementation in subsequent phylogenetic analyses. Phylogenetic analyses were carried out for each dataset (*MutS*; *MutS+28S*) using both maximum likelihood and Bayesian inferences. Concatenated data were analysed using a partitioned substitution model (HKY+G; GTR+G).

Maximum likelihood analyses were carried out with Randomized Axelerated maximum likelihood (RAxML) (Stamatakis 2014) using the web server RAxML Black Box[8] (Stamatakis et al. 2008). Substitution models calculated in jModelTest v 2.1.10 (Guindon & Gascuel 2003, Darriba et al. 2012) and were implemented in analyses that consisted of 2000 bootstrap replicates and remaining parameters left at default. Bayesian inferences were carried out in MrBayes v 3.2.6 (Ronquist & Huelsenbeck 2003) using four chains; 10,000,000 generations and a burn-in of 10%. Tracer v 1.6 (Rambaut et al. 2018) was used to assess Markov Chain Monte Carlo (MCMC) convergence and verify that effective sample size (ESS) values were greater than 200.

Maximum likelihood and Bayesian phylogenetic trees displayed congruent topologies that differed only in branch support values. The most likely tree was edited in FigTree v 1.4 (Rambaut 2012) and displayed with bootstrap support values and posterior probabilities mapped onto branches. Taxonomic clades

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[8] http://www.genome.jp/tools/raxml/

were identified and assigned according to the key devised by McFadden et al. (2009). Species identifications were inferred from *MutS* phylogenetic clades exhibiting shared haplotypes with species known to have been recorded previously in South Africa, in combination with comparisons of *in situ* photographs to reference photographs in a curated online database[9].

3.3.5 Species delimitation

Delimitation analyses were run for *MutS* in order to facilitate species inferences from the phylogenetic framework. At least 35 different *Sinularia* species that were used in phylogenetic construction were included in delimitation analyses. *S. brassica* was analysed as a separate group due to the significant genetic distance from all other *Sinularia* species which may skew results. Several delimitation approaches that were used included: Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012), Bayesian Poisson Tree Processes (bPTP) (Zhang et al. 2013) and Generalized Mixed Yule Coalescent (GMYC) (Michonneau 2017).

ABGD is a distance-based method that partitions sequences into clusters of hypothetical species using a barcode gap based on measures between intraspecific and interspecific distances (Puillandre et al. 2012). ABGD analyses were conducted using an online server[10] with simple distance (uncorrected *p*), a relative gap width set at X= 0.5. and default values left for prior intraspecific divergence (P). The selected partitions were based on a combination of partition stability and the amount of known species included in the analyses.

Bayesian PTP is a phylogenetic-based method that uses coalescent theory to infer the number of putative species included in the input tree and plot posterior support for these delineations (Zhang et al. 2013). Analyses were run using an online service[11] (Zhang et al. 2013) with unrooted Bayesian trees as input. The input trees were generated in MrBayes v 3.2.6 implemented with 20,000,000

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[9] https://science.naturalis.nl/en/people/scientists/leen-van-ofwegen/#sinulariaimages

[10] http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html.

[11] http://species.h-its.org/

generations, 10% burn-in and substitution models from jModelTest v 2.1.10 (Guindon & Gascuel 2003, Darriba et al. 2012). MCMC convergence was verified in Tracer v 1.6 (Rambaut et al. 2018). PTP analyses were conducted using default parameters for burn-in (10%), thinning (100) and Seed (123); however, the number of MCMC generations was set at a maximum value of 500,000.

GMYC uses a likelihood approach to detect differences in phylogeny attributed to speciation or the intra-species coalescent process (Michonneau 2017). Ultrametric trees with a chain length of 20,000,000 were generated in BEAST v 2.4.7 (Drummond et al. 2003) and used as input for GMYC analyses. Three different sets of priors were tested in order to assess the most appropriate model for the data: Yule model with constant clock, Yule model with relaxed clock, and coalescent model with constant population size and constant clock model (Michonneau 2017). Tracer v 1.6 (Rambaut et al. 2018) was used to verify that ESS values were greater than 200 and assess MCMC convergence to ensure valid Bayesian support values of the species guide tree. The input trees generated in BEAST were subsequently analysed using GMYC in R v. 3.4.0 (R Core Team 2013) with packages ape v 5.1 (Paradis et al. 2004), paran v 1.5.1 (Dinno 2012), rncl v 0.8.2 (Michonneau et al. 2016) and splits v 1.0-19 (Ezard et al. 2014) to infer the number of species included in each tree and plot posterior support for putative species.

3.4 Results

3.4.1 MutS phylogeny and species inferences

The phylogenetic tree recovered with *MutS* (Figure 3.2) was congruent with the five major taxonomic clades and subclades: 1, 2, 3, 4A, 4B, 4C, 4D, 5A, 5B and 5C in McFadden et al. (2009). The majority of the 5 major clades demonstrated significant bootstrap support and posterior probabilities (≥70 and ≥0.95 respectively). Clades 1, 2, 3, 4 and 5 displayed an average genetic distance (uncorrected *p*) from one another of 9.3%, 7.4%, 5.6%, 6% and 6.6% respectively. Similar to previous studies, not all species were phylogenetically resolved (McFadden et al. 2009, 2014, Benayahu et al. 2012).

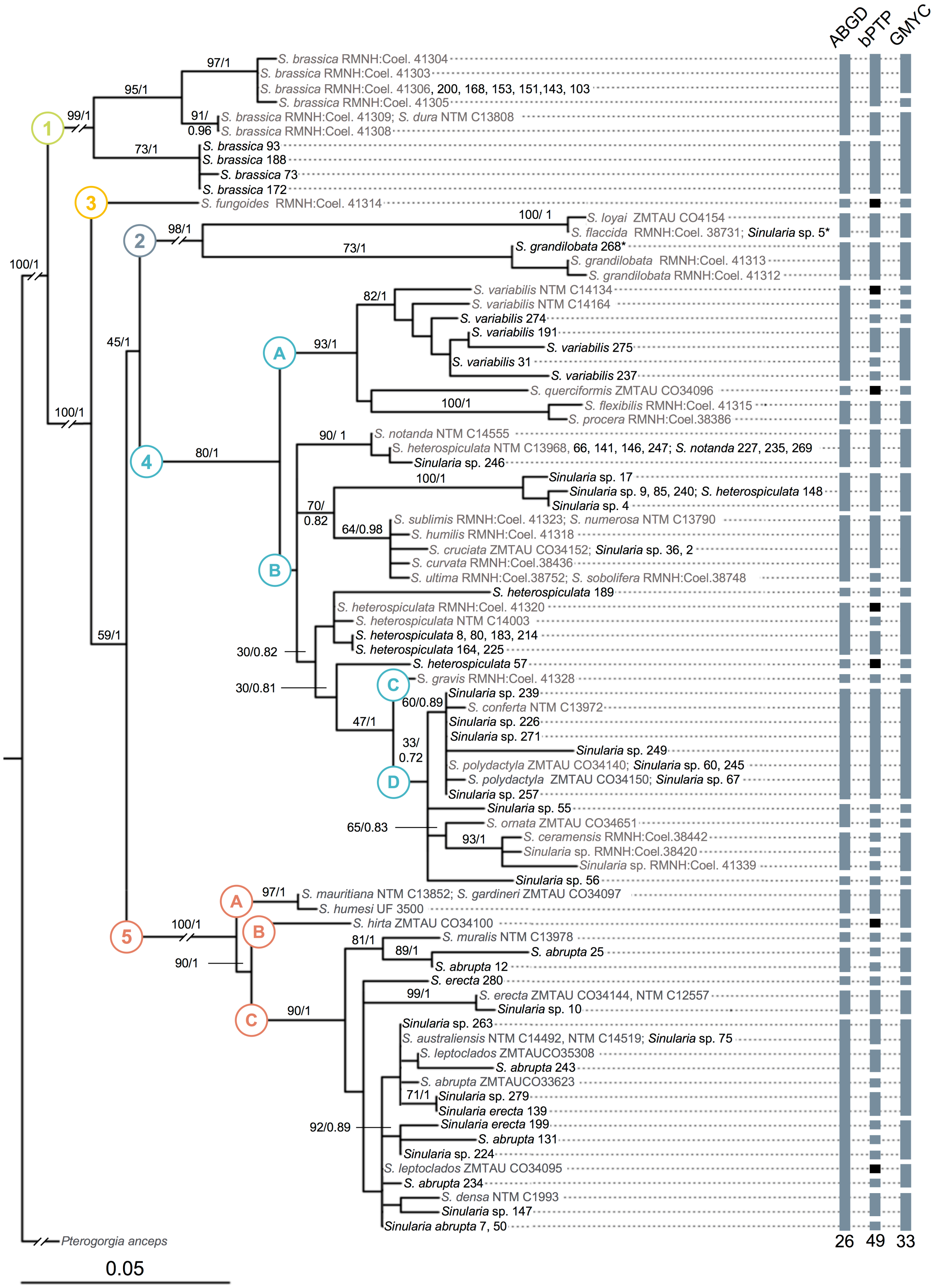

Figure 3.2 Maximum likelihood tree of bacterial MutS homologue (*MutS*) gene region of *Sinularia* indicating taxonomic clades defined by McFadden et al. (2009) (circled numbers). Branch length scale is given; branch labels indicate bootstrap support values and Bayesian posterior probabilities respectively. GenBank sequences are denoted in grey. Delimitation results for ABGD, bPTP and GMYC methods are represented by vertical bars to the right of the taxa with significant species clusters indicated in black.

Three well supported subclades were distinguished in clade 5 (5A-5C) and four subclades were recovered in clade 4 (4A-4D) in which only subclade 4A demonstrated significant support. Specimens collected in this study were recovered in clades 1, 2, 4A, 4B, 4D and 5C and absent in clades 3, 4C, 5A and 5B.

The total number of species clusters for ABGD, bPTP and GMYC (indicated by the vertical bars in Figure 3.2) totalled 26, 49 and 33 operational taxonomic units (OTU) respectively though the reference sequences from GenBank included up to 35 different species of *Sinularia*. No clear barcoding gap could be established for ABGD, which recovered the least amount of putative species; bPTP yielded the highest species estimate whereas the total estimate for GMYC closely corresponded to the total number of species included in the phylogeny from GenBank. The GMYC analysis that generated the closest match to the number of expected species was based on priors of a coalescent model with constant population size, constant clock. However, none of these methods consistently delineated species in congruence with how each species was recovered in the *MutS* phylogeny (Figure 3.2).

Six of the fourteen *Sinularia* species previously recorded in South Africa were identified for samples that were recovered from 6 out of the total 10 clades and subclades defined by McFadden et al. (2009) (Table 3.1). Forty-two of the 67 *Sinularia* specimens (63%) were identified to the species-level, with a new record for *S. grandilobata* Verseveldt, 1980. The remaining unidentified 25 specimens were assigned to 16 putative species (groups a-p in Table 3.1) based on their position in the clade (Figure 3.2), consensus of species delimitations and colony morphology (Figure 3.3 and Figure 3.6).

A combination of reference sequences and photographic information were lacking for two species recorded in South Africa: *S. triangula* (clade 3) and *S. schleyeri*. The possibility of specimens being identified as *S. triangula* was ruled out as no taxa from this study were recovered in clade 3. Furthermore, *S. schleyeri* was eliminated as a potential candidate as no specimens in the collection matched the colony description of this taxon which is devoid of lobes, cup shaped and has a distinct rim of 2-3cm (Benayahu 1993). Specimens with unique *MutS* haplotypes and colony morphologies that did not match any species previously recorded from South Africa or the reference set provided by McFadden et al. (2009) may represent new records or undescribed species that require taxonomic expertise.

Table 3.1 Species identifications, taxonomic groupings and accession numbers of *Sinularia* specimens from this study and their corresponding clade assignments based on McFadden et al. (2009) and Figure 3.2.

Species recorded from South Africa within their assigned clades		Specimen identifications	Species grouping	GenBank accession no.	
				MutS	***28SrRNA***
1	*S. brassica*	*S. brassica* 73	b1	MF817833	MF817911
		S. brassica 93		MF817827	MF817915
		S. brassica 172		MF817877	MF817926
		S. brassica 188		MF817875	MF817928
		S. brassica 103	b2	MF817891	MF817916
		S. brassica 143		MF817886	MF817919
		S. brassica 151		MF817882	MF817923
		S. brassica 153		MF817881	-
		S. brassica 168		MF817879	MF817925
		S. brassica 200		MF817870	MF817931
2	None	*Sinularia* sp.* 5	a	MF817841	MF817895
		*S. grandilobata** 268	n/a	MF817851	MF817944
3	*S. triangula*	none	n/a	n/a	n/a
4A	*S. variabilis*	*S. variabilis* 31	n/a	MF817844	MF817903
	S. flexibilis	*S. variabilis* 191		MF817873	MF817930
	S. querciformis	*S. variabilis* 237		MF817862	-
		S. variabilis 274		MF817848	-
		S. variabilis 275		MF817847	-

Table 3.1 continued...

Species recorded from South Africa within their assigned clades		**Specimen identifications**	**Species grouping**	**GenBank accession no.**	
				MutS	***28SrRNA***
4B	*S. heterospiculata*	*Sinularia* sp. 2	b	MF817871	MF817893
	S. notanda	*Sinularia* sp. 36		MF817843	MF817904
		Sinularia sp. 4	c	MF817842	MF817894
		Sinularia sp. 9		MF817828	MF817898
		Sinularia sp. 17		MF817878	MF817901
		Sinularia sp. 85		MF817829	MF817914
		S. heterospiculata 8	n/a	MF817831	MF817897
		S. heterospiculata 57		MF817837	MF817907
		S. heterospiculata 66		MF817835	MF817909
		S. heterospiculata 80		MF817830	MF817913
		S. heterospiculata 141		MF817887	-
		S. heterospiculata 146		MF817885	MF817920
		S. heterospiculata 148		MF817883	MF817922
		S. heterospiculata 164		MF817880	-
		S. heterospiculata 183		MF817876	MF817927
		S. heterospiculata 189		MF817874	MF817929
		S. heterospiculata 214		MF817869	-
		S. heterospiculata 225		MF817867	-
		S. heterospiculata 247		MF817856	MF817940
		S. notanda 227	n/a	MF817865	MF817933
		S. notanda 235		MF817863	MF817935
		S. notanda 269		MF817850	MF817945
		Sinularia sp. 240	d	MF817860	MF817937
		Sinularia sp. 246	e	MF817857	-
4C	*S. gravis*	none	n/a	n/a	n/a
4D	*S. polydactylal*	*Sinularia* sp. 55	f	MF817839	MF817906

Table 3.1 continued...

Species recorded from South Africa within their assigned clades		**Specimen identifications**	**Species grouping**	**GenBank accession no.**	
				MutS	***28SrRNA***
	S. ceramensis/ Sinularia spp.	*Sinularia* sp. 56		MF817838	-
		Sinularia sp. 60	g	MF817836	MF817908
		Sinularia sp. 67		MF817834	MF817910
		Sinularia sp. 226	h	MF817866	MF817932
		Sinularia sp. 239	i	MF817861	MF817936
		Sinularia sp. 245	j	MF817858	MF817939
		Sinularia sp. 249		MF817855	MF817941
		Sinularia sp. 257	k	MF817853	MF817942
		Sinularia sp. 271		MF817849	-
5A	*S. gardineri*	none	n/a	n/a	n/a
5B	*S. hirta*	none	n/a	n/a	n/a
5C	*S. abrupta*	*Sinularia* sp. 10	l	MF817892	MF817899
	S. erecta	*S. abrupta* 7	n/a	-	MF817896
	S. firma	*S. abrupta* 12		MF817890	MF817900
	S. muralis	*S. abrupta* 25		MF817854	MF817902
	S. leptoclados/ S. australiensis/ Sinularia spp.	*S. abrupta* 50		MF817840	MF817905
		S. abrupta 131		MF817889	MF817917
		S. abrupta 234		MF817864	MF817934
		S. abrupta 243		MF817859	MF817938
		Sinularia sp. 147	m	MF817884	MF817921
		Sinularia sp. 224	n	MF817868	-
		Sinularia sp. 75	o	MF817832	MF817912
		Sinularia sp. 263		MF817852	MF817943
		Sinularia sp. 279	p	MF817846	MF817946
		S. erecta 139	n/a	MF817888	MF817918
		S. erecta 199		MF817872	-

Table 3.1 continued...

Species recorded from South Africa within their assigned clades	**Specimen identifications**	**Species grouping**	**GenBank accession no.**	
			MutS	***28SrRNA***
	S. erecta 280		MF817845	-

"-" Amplicon failed; ** Indicates taxa from this study that fall within clades that did not previously contain any known species from South Africa. Species names in colour denote taxa that have recently been revised and therefore their former records in South Africa are disputed, therefore, specimen identifications could fall within either one of three species assignments.

Clade 1, consisting of only one species, *S. brassica*, formed a distinct sister clade to the remaining 4 major clades with a significant average genetic distance (uncorrected *p*) of 9.3%. Clade 1 formed a monophyletic clade of *S. brassica* that was further differentiated into two separate subclades (Figure 3.2).

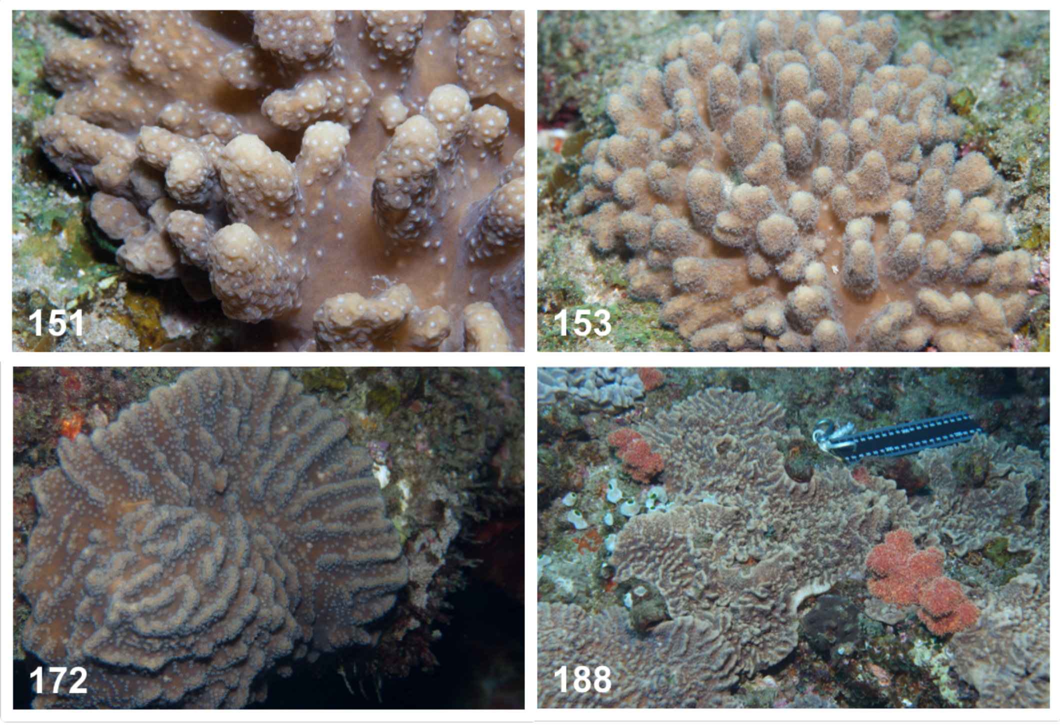

Figure 3.3 *In situ* photos of two morphotypes of *S. brassica* May, 1898 from South Africa. Colonies exhibited either short and digitate forms (specimens 151 and 153) or flat, encrusting, lobate growth forms (specimens 172 and 188).

Two highly supported monophyletic clades with species delimitations were recovered that corresponded to two morphologically differentiated colony forms of *S. brassica* from South Africa. These taxa exhibited distinct colony growth forms that were either digitate and shared identical haplotypes (specimens 200, 168, 153, 151, 143 and 103) or lobate and encrusting with individual haplotypes (specimens 73, 93, 172 and 188) (Figure 3.3). The well supported clusters, congruent with colony growth form, may be indicative of two species.

MutS sequences for two specimens collected in this study were recovered in clade 2 (Figure 3.2). Clade 2 did not contain any taxa previously recorded from South Africa and therefore these taxa constitute two new records: *Sinularia grandilobata* Verseveldt, 1980 and specimen *Sinularia* sp. 5 (Figure 3.4 a), the latter requiring taxonomic expertise for identification. *S. grandilobata* 268 was recovered in a monophyletic clade with conspecifics from GenBank and shared a consensus species cluster whereas *Sinularia* sp. 5 shared a haplotype with *S. flaccida*, which was recovered in a clade with *S. loyai*, all three of which shared a species cluster.

Clade 4 was the largest clade in the genus and was subdivided into 4 subclades that varied in support values as was also the case in McFadden et al. (2009) (Figure 3.2). Subclade 4A was well supported and notably distinct from the other subclades in which *Sinularia variabilis* was recovered in a monophyletic clade amongst other conspecifics from GenBank. The remaining subclades (4B-4D) were poorly supported phylogenetically and with unreliable species delimitations. *Sinularia notanda* and *S. heterospiculata* were the only two species with records from South Africa that formed part of clade 4B. These morphologically and genetically similar taxa formed polytomies wherein *S. notanda* specimens from South Africa and *S. heterospiculata* (GenBank and South African specimens) shared identical haplotypes with one another, thereby making molecular species inferences challenging. *S. heterospiculata* was recovered throughout subclade 4B and was polyphyletic whilst *S. notanda* displayed paraphyly (Figure 3.2). Taxa from this study that fell within subclade 4B (Figure 3.2) and without species assignments are considered new records: *Sinularia* sp. 2 and 36 (Figure 3.4 b) were considered conspecifics based on

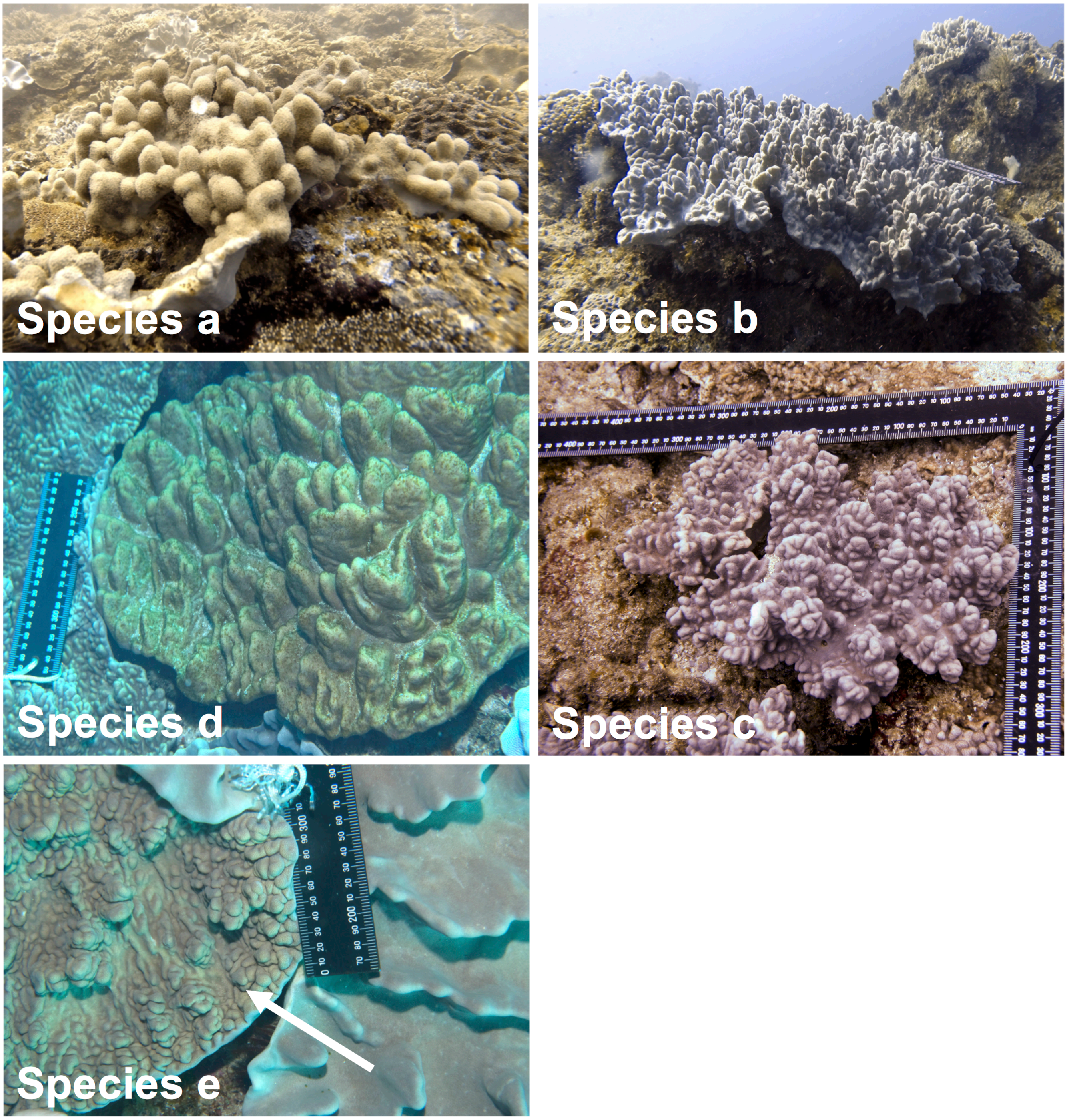

Figure 3.4 *In situ* photos of several unidentified *Sinularia* species from South Africa with their assigned species groupings and phylogenetic clades. Species a was recovered in clade 2 whereas putative species b-e were recovered in subclade 4B (Figure 3.2). Images: K. Etsebeth.

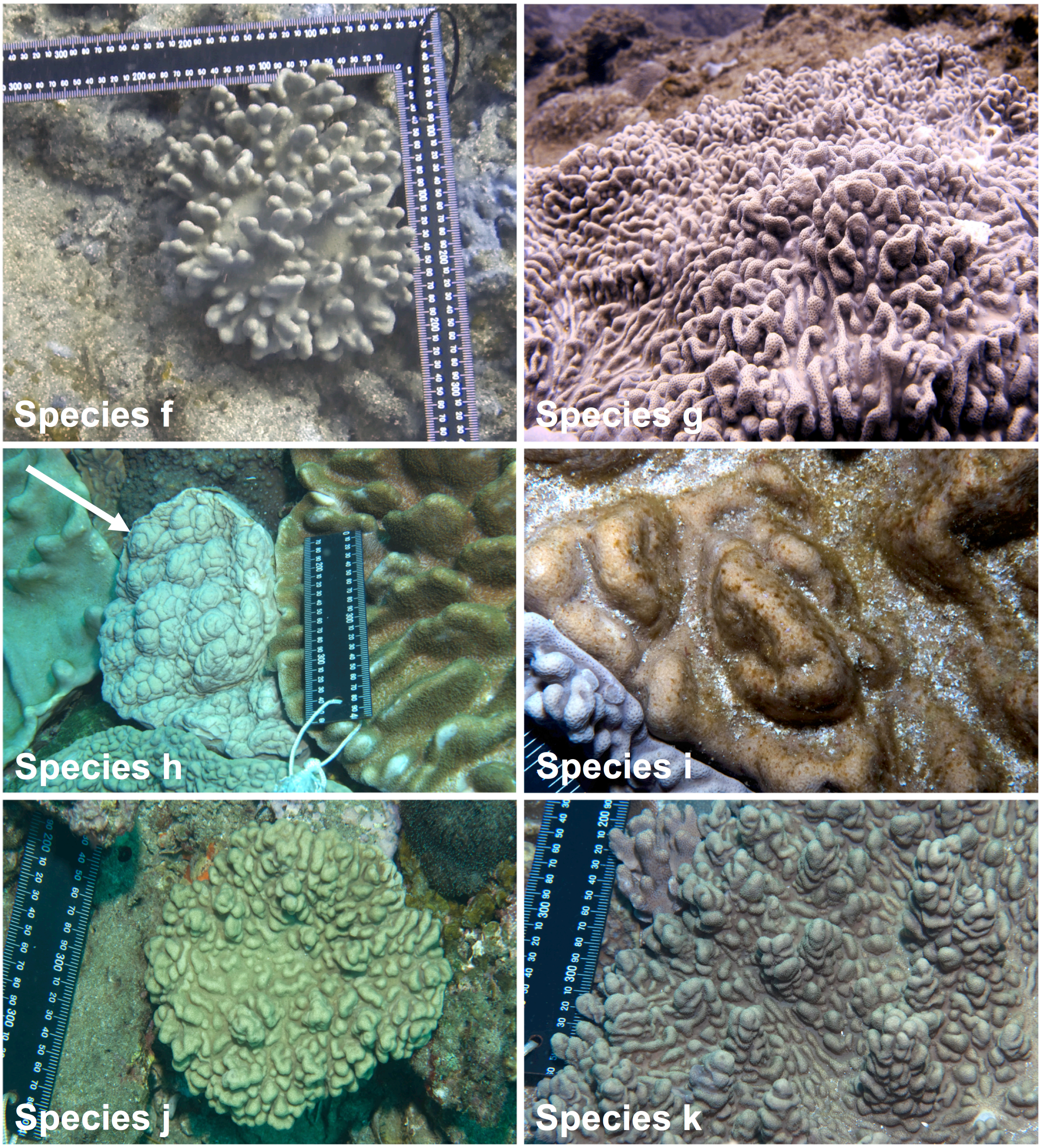

Figure 3.5 ***In situ*** **photos of several unidentified** ***Sinularia*** **species from South Africa and their assigned species groupings.** Species types f- k were recovered in subclade 4D (Figure 3.2). Images: K. Etsebeth & G. Jones.

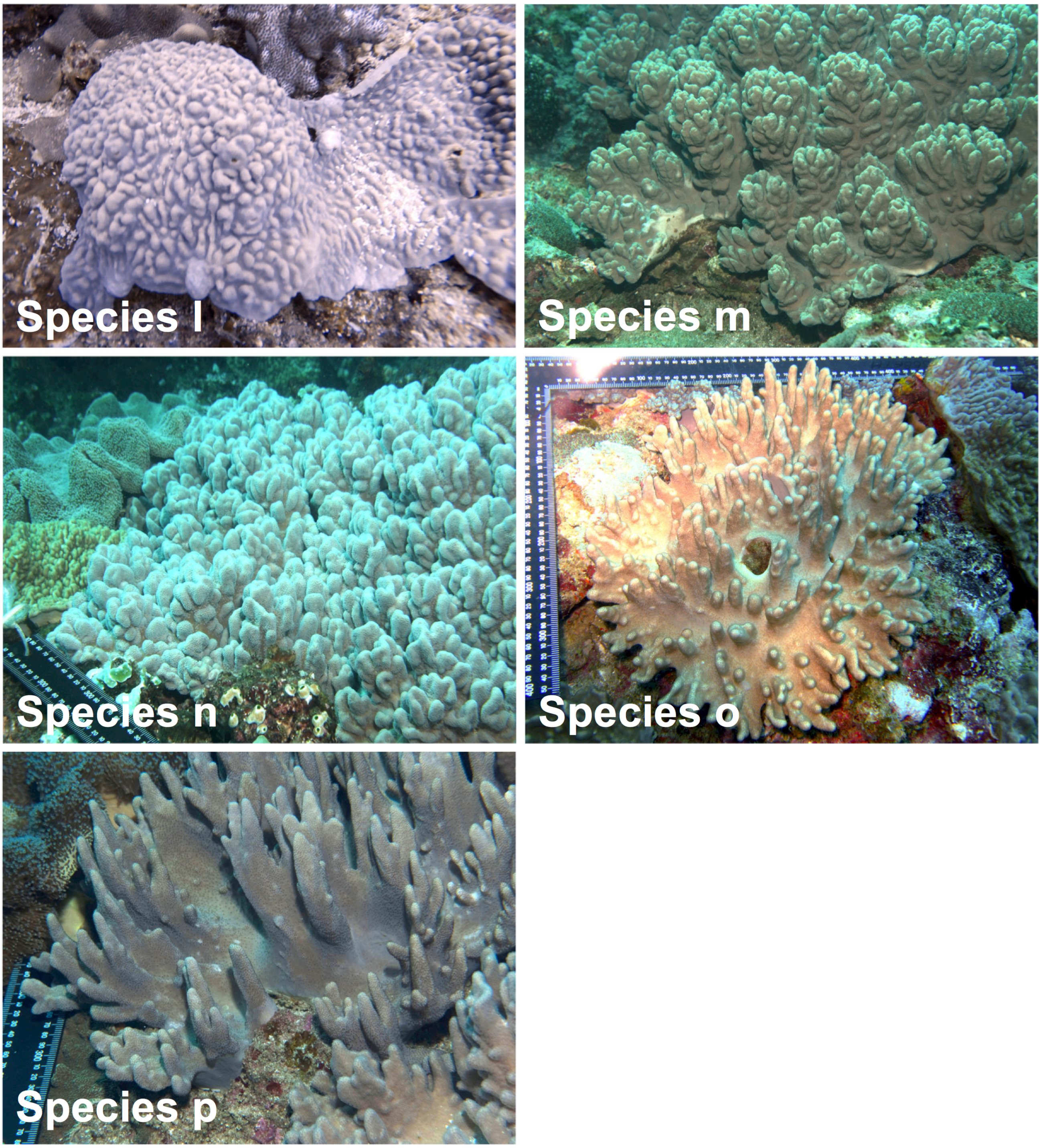

Figure 3.6 *In situ* photos of several unidentified *Sinularia* species from South Africa and their assigned species groupings. Species types l-m were recovered in subclade 5C (Figure 3.2). Images: K. Etsebeth & G. Jones.

their phylogenetic placement and comparable colony morphologies. These taxa shared identical haplotypes with *S. cruciata* whilst the remaining unidentified taxa (Figure 3.4 c-e; Table 3.1) were recovered in a highly supported clade with *S. heterospiculata* 148.

Similarly, clade 4B and subclade 4D remained a largely unresolved polytomy of several species, thereby rendering species inferences impossible for closely related congeners. A species recorded from South Africa that was formerly identified as *S. polydactyla* is the only taxon predicted for this subclade (McFadden et al. 2009). The unresolved taxa *Sinularia* sp. 55 and 56 formed two putative species with distinct haplotypes. However, both taxa exhibited gross morphologies that resembled *S. polydactyla* and require further taxonomic investigation (Figure 3.5 f) (Table 3.1). The possibility of true *S. polydactyla* occurring in South Africa is therefore considered. *Sinularia* sp. 60 and 245; and *Sinularia* sp. 67 displayed identical haplotypes to two sequences of *S. polydactyla* respectively but exhibited no colony resemblance to that of *S. polydactyla* (Figure 3.5 g & j). The remaining unidentified *Sinularia* specimens from this study within subclade 4D require further taxonomic investigation.

Similar to previous studies, subclade 5C was poorly resolved and formed polytomies (McFadden et al. 2009, 2014, Benayahu et al. 2012). ABGD, bPTP and GMYC analyses showed limited species delineations in subclade 5C and were incongruent with species positions in the *MutS* phylogeny: allospecific taxa shared haplotypes, lineages and clusters. Species of *Sinularia* with previous records in South Africa that fall within subclade 5C include: *S. abrupta*, *S. erecta*, *S. firma* and *S. muralis*. Specimens recorded from this region that were previously identified as *S. leptoclados* also fell within subclade 5C. Sequences for *S. erecta* and *S. abrupta* specimens from South Africa were polyphyletic and distributed throughout the subclade, none of which shared haplotypes with their respective conspecific sequences from GenBank. *Sinularia* sp. 75 shared identical haplotypes with two sequences of *S. australiensis* (GenBank). These taxa, along with *Sinularia* sp. 263 (due to its position in the clade relative to *S. australiensis* in Figure 3.2), exhibited a strong colony resemblance to *S. australiensis* (Figure 3.6 o) and may potentially form a new record. Consequently, the possibility of true S*. leptoclados* not occurring in South Africa is considered. The remaining taxa in subclade 5C included *Sinularia* sp. 10, 224, 147, 279 that formed distinct haplotypes and exhibited colony forms (Figure 3.6 l-p) that could not be identified to the species-level without taxonomic expertise (Table 3.1).

3.4.2 Sinularia MutS+28S rRNA phylogeny

The multilocus barcode, *MutS+28S*, recovered a phylogeny (Figure 3.7) comparable to *MutS* (Figure 3.2) and included the major clades discussed in McFadden et al. (2009). However, the limited availability of nuclear reference sequences from GenBank and that of taxa from this study resulted in a tree from which clade 3 and subclade 4C were absent. The average genetic distance (uncorrected *p*) between each major clade (1, 2, 4 and 5) recovered was 7.8%, 5.4%, 5.3% and 5.4% respectively. Moreover, the lack of nuclear sequences limited comparative species inferences between the two phylogenies and did not further resolve ambiguous species identifications. Phylogenetic inconsistencies were apparent between *MutS* and concatenated *MutS+28S* (Figure 3.2 and Figure 3.7) for clade 2 and subclade 4D.

Clade 1 recovered with *MutS+28S* (Figure 3.7) was consistent with clade 1 recovered with *MutS* (Figure 3.2). The concatenated phylogeny reaffirmed that *S. brassica* specimens from South Africa showed evidence of a species complex in clade 1 and corresponded to two distinct colony growth forms (Figure 3.3). Clade 2 was recovered within clade 4 with *MutS+28S* (Figure 3.7) unlike that for *MutS* (Figure 3.2). Unlike with *MutS*, *Sinularia* sp. 5 was nested in *S. grandilobata*. However, both phylogenies recovered *S. grandilobata* from South Africa separately from conspecific sequences sourced from GenBank.

Of the 5 major clades recovered with *MutS+28S*, clade 4 itself was phylogenetically unresolved as a result of polytomies and shared haplotypes between allospecific taxa (Figure 3.7). Subclades 4A and 4B were recovered amongst members of subclade 4D where taxa from the latter subclade were distributed throughout clade 4. In subclade 4A, *S. variabilis* did not form a monophyletic clade as in *MutS*. Furthermore, *S. querciformis* and *S. variabilis* sequences from GenBank shared a haplotype, which was inconsistent with the results of the *MutS* phylogeny. Subclade 4B recovered three clades that corresponded to those recovered with *MutS*. Likewise, *S. notanda* demonstrated paraphyly and *S. heterospiculata* was polyphyletic for the concatenated barcode. Taxa that formed polytomies in clade 4 (*Sinularia* sp. 55, 67, 226, 239, 245,

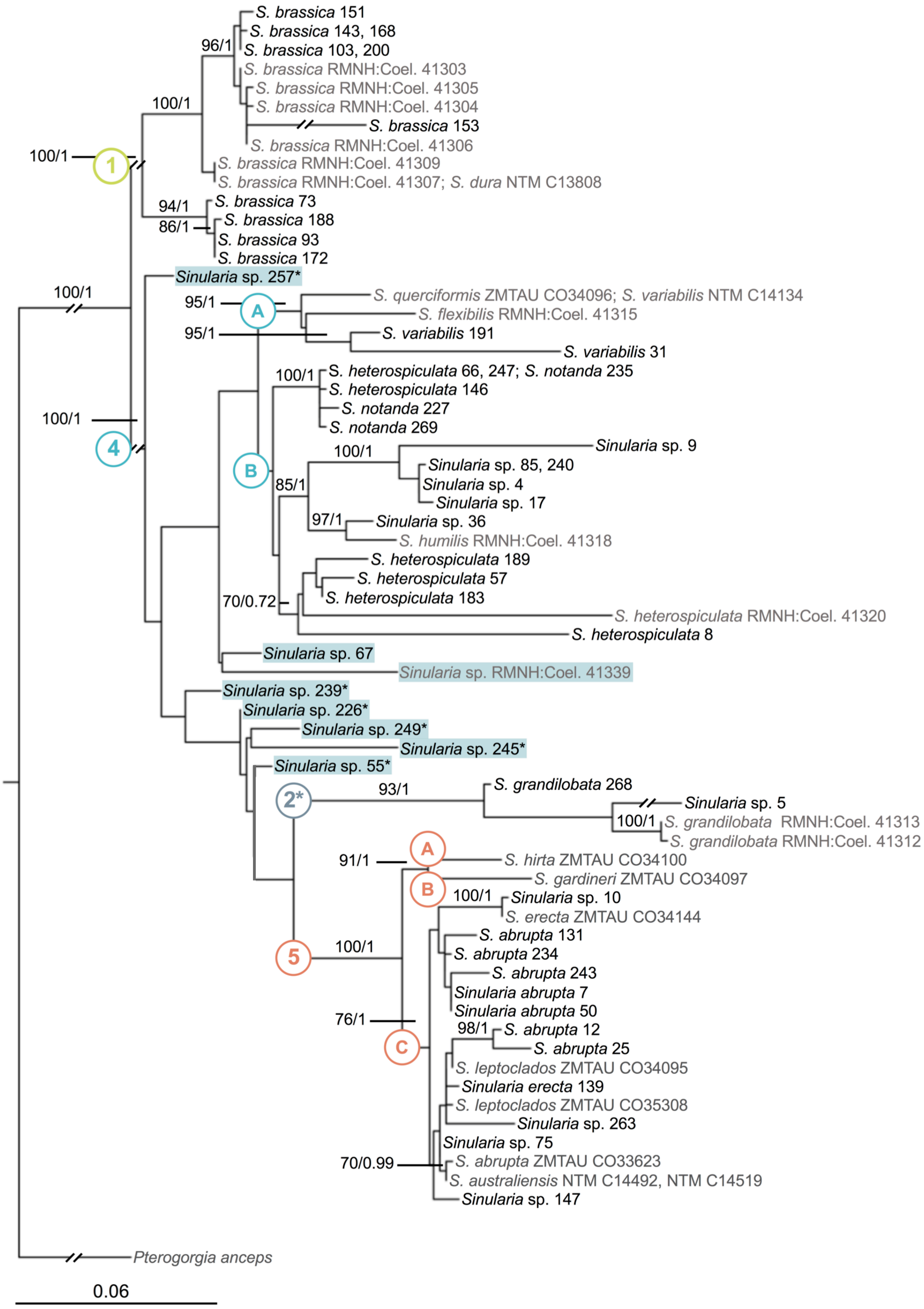

Figure 3.7 Maximum likelihood tree of concatenated bacterial MutS homologue (*MutS*) and 28S ribosomal RNA (*28S rRNA*) of *Sinularia* with taxonomic clades defined by McFadden et al. (2009) (circled numbers). Branch length scale is given; branch labels indicate bootstrap support values and Bayesian posterior probabilities respectively. GenBank sequences are denoted in grey. Taxa highlighted in blue belong to subclade 4D whereas taxa highlighted in grey belong to subclade 4B. Asterisks signify taxa or clades that do not correspond to their relative position in the *MutS* phylogeny (Figure 3.2).

249 and 257) (Figure 3.7), were previously identified as members of subclade 4D with *MutS* (Figure 3.2) and were distributed throughout clade 4.

Clade 5 formed three subclades and no taxa from this study were recovered in subclades 5A and 5B (Figure 3.7). Similar to *MutS*, subclade 5C was largely unresolved with no distinct species boundaries in that *S. erecta* and *S. abrupta* were both polyphyletic and *S. leptoclados* exhibited paraphyly. No haplotypes were shared between any sequences in this subclade let alone for conspecific taxa.

3.4.3 Genetic variation and distance

Haplotype diversity, nucleotide diversity and estimates of average evolutionary divergence (D) for *MutS* and *MutS+28S* are presented in Table 3.2. Evolutionary divergence (D) over all sequence pairs is equivalent to the mean pairwise genetic distance (uncorrected *p*) for *MutS* and *MutS+28S* respectively.

Table 3.2 Diversity estimates and mean evolutionary distance for genetic barcodes used in this study of *Sinularia* from South Africa.

Gene region	nt	H	Hd	π	S	S_p	D
MutS	735	45	0.951	0.04942	209	85	0.058
MutS+28S	1494	52	0.984	0.04799	239	134	0.048

H= number of haplotypes; Hd= haplotype diversity; π= nucleotide diversity; S= number of variable sites; S_p= number of parsimony-informative sites; D= estimates of average evolutionary divergence.

The addition of *28S rRNA* resulted in an increased haplotype diversity and more parsimony informative sites. However, the concatenated barcode displayed a lower nucleotide diversity and average evolutionary divergence despite its increased sequence length, which is likely an artefact of the fewer available sequences for the multi-locus *MutS+28S*.

Evolutionary divergence estimates between each phylogenetic clade for *Sinularia* from this study are shown in Table 3.3. *MutS* pairwise genetic distances (uncorrected *p*) between clades 1, 2, 4A, 4B, 4D and 5C ranged from 2.3-10.3% whereas the inter-clade variation for *MutS+28S* (uncorrected *p*) ranged from 2.2-7.6%. Clade 1 showed the greatest divergence from the other clades for both markers and ranged from 9.4%-10.3% for *MutS* and 3.9%-7.6% for *MutS+28S*. These distances were comparable to the intergeneric distances (uncorrected *p*) between genera that are closely related to *Sinularia*: *Lobophytum* and *Sarcophyton* exhibited 7.5% and 5.8% for *MutS* and *MutS+28S* respectively (Appendix C, Tables 7.10 and 7.12).

Table 3.3 Genetic distance (uncorrected *p*) between clades for bacterial MutS homologue (*MutS*) (above the diagonal) and concatenated *MutS* and 28S ribosomal RNA (*28S rRNA*) (below the diagonal) for taxa collected in this study.

Clade	1	2	4A	4B	4D	5C
1		0.101	0.097	0.096	0.094	0.103
2	0.073		0.068	0.057	0.058	0.075
4A	0.075	0.045		0.023	0.023	0.065
4B	0.039	0.037	0.026		0.019	0.056
4D	0.073	0.034	0.027	0.022		0.047
5C	0.076	0.039	0.045	0.037	0.027	

3.5 Discussion

Up to two thirds of the specimens collected in this study were identified to the species level using the phylogenetic classification system described in McFadden et al. (2009) and *in situ* colony photographs. A new record of *S. grandilobata* was reported for the iSWP which increased the total *Sinularia* species recorded in this region to 15. Upon further taxonomic investigation, an additional 16 *Sinularia* species are forecast that would raise the total number of species in this region to 31.

The significant genetic distance between *S. brassica* with other congeners for this study has been paralleled in other studies (McFadden et al. 2009) where pairwise distances also equated to the intergeneric distances displayed by other Alcyoniidae. Analyses that include additional alcyoniid genera would be required to test whether *S. brassica* would warrant a separate generic status and a consequent revision of the genus would be required. Moreover, *S. brassica* presented as two putative sympatric species in this study that require further taxonomic attention. *Sinularia dura* Pratt, 1903 and *S. brassica* were previously regarded as separate species that were primarily distinguished by the shape of their capitula where *S. dura* was distinctly cup-shaped and *S. brassica* primarily lobed (Verseveldt 1974, 1980). Benayahu (1993) noted the occurrence of both taxa in Sodwana Bay (South Africa) although no known records for this region reflect the descriptions of colonies with cup-like growth forms and no such colony growth forms were recovered in this study either. Benayahu et al. (1998) synonymized the two species on the basis of considerable similarities between the sclerites of the two taxa and colonies comprising a spectrum of growth forms from cup-shaped to lobed. However, McFadden et al. (2009, 2014) provided evidence of molecular discrepancies within *S. brassica* across different gene regions that were congruent with two distinct growth forms and parallels the outcomes of this study. The similarities between the diagnostic sclerites of the two taxa may be the outcome of convergent evolution (homoplasy) where identical morphological characters have evolved along separate lineages (McFadden et al. 2014) or the result of a recent speciation event (Prada et al. 2008). The presence of two variants of *S. brassica* indicates that the taxon can be divided into two species. The diagnostic features that determine the species boundaries between these two taxa warrant further investigation by a taxonomic expert.

It is known that *MutS* lacks the resolution to delimit closely-related species (McFadden et al. 2011) albeit useful in reducing the number of possible species identifications to a few candidates (McFadden et al. 2009, 2010). Fewer species identifications were inferred for taxa in this study that were recovered in clades 2-5 (Figure 3.2) compared to previous studies. Furthermore, most of the collected specimens were distributed amongst largely unresolved subclades (4B, 4D and 5C) which rendered species inferences more challenging. Sixty-two percent of the *Sinularia* sequences from this study were assigned molecular-based identifications whereas other studies reported identifications for up to 95% of the collection (McFadden et al. 2009, Benayahu et al. 2012). According to McFadden et al. (2014), 81% of the specimen sequences conformed to their predicted morphological identifications. The disparity between the success rates of molecular identifications in this study and that of others may be accredited to the lack of available representative sequences for each *Sinularia* species. Furthermore, the reference sequences represent taxa from distant tropical reefs in the Coral Triangle and the Red Sea (van Ofwegen & McFadden 2009, Benayahu et al. 2012, van Ofwegen et al. 2013, 2016, McFadden et al. 2014), as opposed to the isolated, subtropical high latitudinal communities in this study.

As expected, sequences of the collected taxa exhibited few shared haplotypes with conspecific representatives, notwithstanding monophyletic taxa i.e. *S. brassica*, *S. grandilobata* and *S. variabilis* (Figure 3.2). However, this also applied within several reference sequences such as *S. phenghuensis*, *S. wanannensis*, *S. ornata* (Benayahu et al. 2012); and *S. variabilis*, *S. brassica*, *S. erecta* (McFadden et al. 2009). It stands to reason that geographic scale could account for the genetic differences between conspecifics and may extend to cases of paraphyly (*S. notanda*) and polyphyly (*S. abrupta*) exhibited by conspecifics from South Africa (Figure 3.2) to taxa from other locations. Whether these taxa form distinct populations or incipient species is indeterminate due to the limited sequences available from this region for phylogeographic comparison. In addition, corals in marginal habitats at high latitudes may evolve differently compared to their conspecifics in tropical conditions at low latitudes (Beger, Sommer, et al. 2014). Cases have been reported of hard and soft corals adopting alternative reproductive strategies in an attempt to prevail in suboptimal conditions at high latitudes reefs (Schleyer et al. 1997, 2004, St. Gelais et al. 2016).

3.5.1 Mitochondrial and nuclear discord

Fewer nuclear *28S rRNA* sequences were available for *Sinularia* which limited the utility of the concatenated *MutS+28S* phylogeny for additional species inferences and comparisons between the two gene regions. The lack of availability of nuclear loci and sequence data thwarts attempts to resolve polytomies where mitochondrial markers lack resolution. Nonetheless, the interspecific resolution of the nuclear and concatenated phylogenies in this study did not differ significantly from the *MutS* phylogeny, whereas in previous studies the addition of the *28S rRNA* locus reportedly exhibited marginally greater distances between species (McFadden et al. 2014). Even so, both loci recovered shared haplotypes for allospecific taxa in this study which has also been demonstrated in previous studies (Benayahu et al. 2012, McFadden et al. 2014) and indicates a lack of interspecific variation for both gene regions.

Incongruities were evident between *MutS* and *MutS+28S* phylogenies which consequently obscurred species boundaries further. Several reasons are proposed for these observed inconsistencies. Some hypotheses suggest that mitochondrial and nuclear inconsistencies may have arisen from events such as incomplete lineage assortment in recently diverged species or as a result of sequence paralogy (Radulovici et al. 2010, van Ofwegen et al. 2013, Ament-Velásquez et al. 2016), all of which may result in established ancestral polymorphisms (McFadden et al. 2011). However, additional loci are needed to test sequence homology in order to eliminate convergence.

Alternatively, discordance between mitochondrial and nuclear markers may have resulted from hybridization events as hybrids cannot be differentiated from their maternal ancestors in mitochondrial lineages (Neigel et al. 2007, Radulovici et al. 2010). As a result, nuclear markers are incorporated into molecular studies to corroborate barcode analyses (Bucklin et al. 2011, McFadden et al. 2014, Bryce et al. 2015). However, evaluating hybridization in non-model taxa such as octocorals is challenging as they cannot be crossed *in vivo* and much information is needed to verify assumptions (McFadden et al. 2010). Although little is known about hybridization events in octocorals, studies of scleractinia have indicated that hybridization and introgression are likely to occur between congenerics that are morphologically similar (Willis et al. 1997, Richards et al. 2008)- a concept that may likely explain the lack of genetic resolution exhibited in Clades 4B, 4D and 5C. *Sinularia* are broadcast spawners, a reproductive strategy which increases the likelihood of hybridization (Alino & Coll 1989, Willis et al. 1997, 2006). Consequently, hybridization has been recorded in several taxa of *Sinularia* (van Ofwegen et al. 2013) and hybrid vigour has even been reported for *S. polydactyla* and *S. maxima* (Slattery et al. 2008).

Furthermore, hard and soft corals have been shown to adopt alternative reproductive strategies (Schleyer et al. 1997, 2008) in an attempt to prevail in suboptimal conditions at high latitudinal reefs that would resultantly increase the likelihood of hybridization events. Marginal reefs, like that of the iSWP, have been shown to have an effect on scleractinian species diversity and behaviour (Willis et al. 2006, Richards et al. 2008) though the octocoral community remains understudied. In addition, Budd & Pandolfi (2010) reported that evolutionary novelties occur at the distribution limits of

Scleractinia species distributions where introgressive hybridization contributes toward adaptive radiation, this theory may well apply to their octocoral counterparts but would warrant further study.

3.6 Conclusion

This integrated tool refined species assignments and facilitated express identifications with basic traditional taxonomy. This is the first data from South Africa for *Sinularia* and has demonstrated a degree of divergence with congeners from other geographic locations. This is the first step in establishing a reference sequence and data repository for South African Alcyonacea in order to establish a basis for comparative and ecological studies. Further expert taxonomic examination of the unidentified taxa from this study will refine and ameliorate the identification process using phylogenetics in future studies of these ecologically important taxa from this region. This contribution is anticipated to assist management and conservation plans of the iSimangaliso Wetland Park.

3.7 Acknowledgements

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CHAPTER 4: BIODIVERSITY OF THE ALCYONACEA (CNIDARIA: OCTOCORALLIA) OF NORTHERN KWAZULU-NATAL: A MOLECULAR AND MORPHOLOGICAL SYNOPSIS (PAPER 3)

4.1 Abstract

The iSimangaliso Wetland Park, a world heritage site in Northern KwaZulu-Natal, South Africa, features the southernmost coral assemblages and sublittoral reefs in East Africa. The coral assemblages are dominated by shallow water soft corals (Octocorallia: Alcyonacea), many of which reach their distribution limit in the Indian Ocean. The study of the ecology of these significant reef assemblages is impeded by inconclusive taxonomic knowledge. Two hundred and thirty-nine colonies of Alcyonacea, distributed among 23 genera from within 8 families, were collected from several reefs located in the iSimangaliso Wetland Park. The biodiversity and systematics of these shallow-water genera were investigated using phylogenetic analyses with mitochondrial bacterial MutS homologue (*MutS*) and nuclear ribosomal 28S RNA (*28S rRNA*) gene regions. Divergence estimates (uncorrected *p*) and phylogenies supported the monophyly of several genera and species but was limited in closely related taxa. Nonetheless, phylogenetic analyses facilitated specimen identifications via elimination to fewer candidate species. Nineteen known species were inferred from phylogenies and traditional taxonomic characters. New records were reported for genera *Chironephthya* Studer, 1887; *Scleronephthya* Studer, 1887; *Paraplexaura* Kuekenthal, 1909; and the species *Sinularia grandilobata* Verseveldt, 1980. Revised biodiversity estimates for the iSimangaliso Wetland Park, as an outcome of this study and consolidated literature records, increased from 37 to 81 known species (including 12 endemic species and 30 unidentified, putative species), 11 to 47 genera and 4 to 17 families. This collection will yield an additional ~33 species once the remaining material has been taxonomically identified. This study presents the first ever phylogenetic analysis of the Alcyonacea community from this marine reserve and provides updated estimates of species richness that will facilitate comparative studies, taxonomic descriptions and management decisions.

Keywords: *octocorals, systematics, MutS, 28S rRNA, South Africa*

4.2 Introduction

A wealth of coral biodiversity and endemism has been reported along the southernmost east African reefs in Northern Kwa-Zulu Natal, South Africa (Williams et al. 1992, Williams 2000, Celliers & Schleyer 2007, Tunley 2009). These unique assemblages of hard and soft corals occur at high latitudes and in subtropical conditions (Ramsay & Mason 1990, Riegl et al. 1995). Marginal reefs such as these are targeted for modelling the forecast effects of climate change as these systems have evolved to prevail in suboptimal conditions for shallow reef corals (Schleyer & Celliers 2003b, a, Beger et al. 2014). Furthermore, climate change may influence community composition and result in spatial shifts from hard to soft corals (Inoue et al. 2013). These coral communities lie in the Delagoa bioregion and are situated in the iSimangaliso Wetland Park (iSWP), a world heritage site that spans 220 km of protected coastline and incorporates the St. Lucia and Maputaland marine reserves (Riegl et al. 1995, Schleyer & Celliers 2003a, 2005).

Unlike many true coral reefs, soft corals (Order Alcyonacea) are the dominant space occupiers of these benthic communities with almost two-thirds coverage (Williams 1989a, b, 1993). Alcyoniidae are the most speciose family in this region with a reportedly wider latitudinal distribution compared with other families (Benayahu et al. 2003). Alcyoniid genera that are key contributors to the reef biota include *Sinularia* May, 1898; *Lobophytum* Marenzeller, 1886 and *Sarcophyton* Lesson, 1834; all of which are found at the southernmost limit of their faunal distribution in the Indian Ocean (Williams 1993, Riegl et al. 1995, Schleyer 1999, Celliers & Schleyer 2007, Schleyer & Benayahu 2008, Benayahu et al. 2012). Long term monitoring studies have shown that these shallow water taxa are in decline in this region (Porter & Schleyer 2017), they have a slow population turnover (Fabricius 1995) and are vulnerable to bleaching events due to their algal symbionts (Riegl 2003, Celliers & Schleyer 2007, Schleyer & Benayahu 2008, Porter & Schleyer 2017).

The Delagoa bioregion has long been acknowledged as an area of high endemism (Schleyer & Celliers 2003a, Celliers & Schleyer 2007, Schleyer et al. 2008). Taxonomists have noted that a significant number of octocoral species remain unidentified in this region, many of which are believed to be endemic and necessitate further taxonomic investigation (Williams 1989b, Benayahu 1993). The east coast is an understudied region of interest within South Africa and has the lowest distribution of marine invertebrate sampling (Griffiths et al. 2010) with consequently few studies of Alcyonacea. In 1992, Williams (1992a, d) recorded 26 alcyonacean species from the iSWP within 16 genera and 10 families, however, this estimate was later updated to 38 species by Benayahu (1993). A revised list of Alcyonacea recorded from the iSWP is presented in Table 4.1 and includes the basis of record and taxonomic authority. The species list is a consolidated, updated compilation of published literature totalling 77 species (including 12 endemic species and 27 unidentified species), 43 genera and 17 families.

Alcyonacea classification and identification is traditionally based on characters such as colony morphology and sclerite characterization (Fabricius & Alderslade 2001). Species assignments based on these approaches require great taxonomic expertise as identification is complex since species and even genus boundaries are poorly understood in many taxa (McFadden, France, et al. 2006, McFadden et al. 2010, 2011, Ament-Velásquez et al. 2016). Furthermore, the number of octocoral taxonomists worldwide are considerably scarce (Gibbons et al. 1999). In addition to traditional taxonomic approaches, molecular markers are valuable tools for biodiversity and systematic studies in corals and are fast becoming a prerequisite in the identification process (Severance & Karl 2006, Vollmer & Palumbi 2007, Vargas et al. 2014, McFadden, Reynolds, et al. 2014, McFadden et al. 2017). However, the anthozoan mitochondrial genome evolves considerably slower than its nuclear counterpart which has resulted in a lack of suitable markers for corals (France & Hoover 2002, Shearer et al. 2002, Fukami & Knowlton 2005, Concepcion et al. 2006, McFadden et al. 2011). Traditional DNA barcodes, such as *COI*, can therefore not be applied widely across taxa as it lacks sufficient variation to distinguish species and even genera in certain families (Huang et al. 2008, McFadden et al. 2010, 2011). Mitochondrial *MutS*, a gene region exclusive to the octocoral genome (Pont-Kingdon et al. 1998), has been extensively applied in phylogenetic studies of these taxa and has been useful in elucidating taxonomic relationships to a degree (McFadden, Alderslade, et al. 2006, McFadden et al. 2009, Bryce et al. 2018). However, the addition of nuclear loci are required to further understand taxonomic boundaries between closely related taxa (McFadden et al. 2011) in order to provide rapid screening of identifications where taxonomic expertise is lacking. Multilocus barcodes have been useful in phylogenetic

Table 4.1 Octocorals of the order Alcyonacea Lamouroux, 1812 recorded from Northern KwaZulu-Natal from Leven point to the Mozambique border, including the iSimangaliso Wetland Park.

Family	Genus	Scientific name	Authority	Basis of record
Acanthogorgiidae	*Anthogorgia*	*Anthogorgia* sp.	Verrill, 1868	Williams 1992b
Acrophytidae	*Acrophytum*	*Acrophytum claviger* (e)	Hickson, 1900	Williams 1992a
Alcyoniidae	*Alcyonium*	*Alcyonium* sp.	Linnaeus, 1758	Williams 1992a
	Aldersladum	*Aldersladum sodwanum* (e)	(Benayahu, 1993)	Benayahu 1993
	Beta*	Beta *australis**	(Macfadyen, 1936)	Benayahu & Schleyer 1996
		Beta *kashmani** (e)	(Benayahu & Schleyer, 1996)	Benayahu & Schleyer 1996
	Lobophytum	*Lobophytum crassum*	von Marenzeller, 1886	Williams 1992a
		Lobophytum depressum	Tixier-Durivault, 1966	Benayahu 1993
		Lobophytum latilobatum	Verseveldt, 1971	Benayahu 1993
		Lobophytum patulum	Tixier-Durivault, 1956	Benayahu 1993
		Lobophytum sp.	von Marenzeller, 1886	Williams 1992a
		Lobophytum venustum	Tixier-Durivault, 1957	Benayahu 1993
	Paraminabea	*Paraminabea kosiensis*	Tixier-Durivault, 1946	Williams 1992c
	Sarcophyton	*Sarcophyton crassum*	(von Marenzeller, 1886)	Benayahu 1993
		Sarcophyton ehrenbergi	Tixier-Durivault, 1966	Benayahu 1993
		Sarcophyton flexuosum	(Quoy & Gaimard, 1833)	Benayahu 1993
		Sarcophyton glaucum	Tixier-Durivault, 1958	Benayahu 1993
		Sarcophyton infundibuliforme	Lesson, 1834	Benayahu 1993
		Sarcophyton spp.	von Marenzeller, 1886	Williams 1992a
		Sarcophyton trocheliophorum	von Marenzeller, 1886	Williams 1992a
	Sinularia	*Sinularia abrubta*	Tixier-Durivault, 1970	Benayahu 1993
		Sinularia brassica	May, 1898	Benayahu 1993

Table 4.1 continued...

Family	**Genus**	**Scientific name**	**Authority**	**Basis of record**
		Sinularia erecta	Tixier-Durivault, 1945	Benayahu 1993
		Sinularia firma	Tixier-Durivault, 1970	Benayahu 1993
		Sinularia gardineri	(Pratt, 1903)	Benayahu 1993
		Sinularia gravis	Tixier-Durivault, 1970	Benayahu 1993
		Sinularia heterospiculata	Verseveldt, 1970	Benayahu 1993
		Sinularia hirta	(Pratt, 1903)	Benayahu 1993
		Sinularia muralis	(May, 1899)	Benayahu 1993
		Sinularia notanda	Tixier-Durivault, 1966	Benayahu 1993
		Sinularia querciformis	(Pratt, 1903)	Benayahu 1993
		Sinularia schleyeri (e)	Benayahu, 1993	Benayahu 1993
		Sinularia spp.	May, 1898	Williams 1992a, Benayahu 1993
		Sinularia triangula	Tixier-Durivault, 1970	Benayahu 1993
		Sinularia variabilis	Tixier-Durivault, 1945	Benayahu 1993
Chrysogorgiidae	*Chrysogorgia*	*Chrysogorgia* sp.	Duchassaing & Michelotti, 1864	Williams 1992b
	Helicogorgia	*Helicogorgia squamifera* (e)	(Kuekenthal, 1919)	Williams 1992d
	Radicipes	*Radicipes* sp.	Stearns, 1883	Williams 1992b
	Bathytelesto	*Bathytelesto tubuliporoides*	Williams, 1989	Williams 1989d
	Carijoa	*Carijoa* sp.	F. Mueller, 1867	McFadden & van Ofwegen 2012a
Clavulariidae	*Clavularia*	*Clavularia* sp.	Blainville, 1830	Williams 1989b
	Scyphopodium	*Scyphopodium* sp.	Bayer, 1981	Williams 1989c
Ellisellidae	*Junceella*	*Junceella* sp.	Valenciennes, 1855	Williams 1992b
	Verrucella	*Verrucella bicolor*	Nutting, 1908	Thomson 1917
Gorgoniidae	*Eunicella*	*Eunicella papillosa*	(Esper, 1797)	Williams 1992b
	Leptogorgia	*Leptogorgia* sp.	Milne Edwards, 1857	Williams 1992b
	Rumphella	*Rumphella* sp.	Bayer, 1955	Williams 1992b

Table 4.1 continued...

Family	Genus	Scientific name	Authority	Basis of record
Isididae	*Acanella*	*Acanella* sp.	Gray, 1870	Williams 1992b
	Chathamisis	*Chathamisis ramosa*	(Hickson, 1904)	Williams 1992b
	Keratoisis	*Keratoisis* sp.	Wright, 1869	Williams 1992b
Melithaeidae	*Melithaea*	*Melithaea* sp.	Milne Edwards, 1857	Reijnen et al. 2014
Nephtheidae	*Dendronephthya*	*Dendronephthya* spp.	Kuekenthal, 1905	Williams 1992a
	Eunephthya	*Eunephthya thyrsoidea* (e)	Verrill, 1869	Williams 1992a
	Leptophyton	*Leptophyton benayahui*	van Ofwegen & Schleyer, 1997	van Ofwegen and Schleyer 1997
	Litophyton	*Litophyton* sp.	Forskål, 1775	Williams 1992a
	Stereonephthya	*Stereonephthya* spp.	Kuekenthal, 1905	Riegl et al. 1995
Nidaliidae	*Siphonogorgia*	*Siphonogorgia* sp.	Koelliker, 1874	Williams 1992a
Parasphaerascleridae	*Parasphaerasclera*	*Parasphaerasclera aurea* (e)	(Benayahu & Schleyer, 1995)	Benayahu & Schleyer 1995
		Parasphaerasclera morifera	(Tixier-Durivault, 1954)	Williams (2003)
		Parasphaerasclera rotifera (e)	(Thomson, 1910)	Verseveldt & Bayer 1988
		Parasphaerasclera valdiviae (e)	(Kuekenthal, 1906)	Williams 1992a
Plexauridae	*Euplexaura*	*Euplexaura* sp.	Verrill, 1869	Williams 1992b
	Menella	*Menella* sp.	Gray, 1870	Williams 1992b
Primnoidae	*Calyptrophora*	*Calyptrophora* sp.	Gray, 1866	Williams 1992b
	Narella	*Narella gilchristi*	(Thomson, 1911)	Williams 1992b
	Primnoeides	*Primnoeides* sp.	Studer & Wright, 1887	Williams 1992b
Spongiodermidae	*Homophyton*	*Homophyton verrucosum* (e)	(Moebius, 1861)	Williams 1992b
Tubiporidae	*Tubipora*	*Tubipora* sp. (nt)	Linnaeus, 1758	Benayahu 1993
Xeniidae	*Anthelia*	*Anthelia* sp.	Lamarck, 1816	Williams 1992a
		Anthelia glauca	Lamarck, 1816	Williams 1992a
	Heteroxenia	*Heteroxenia fuscescens*	(Ehrenberg, 1834)	Benayahu 1993
	Sansibia	*Sansibia flava*	(May, 1899)	Williams 1992a

Table 4.1 continued...

Family	Genus	Scientific name	Authority	Basis of record
	Sympodium	*Sympodium caeruleum*	Ehrenberg, 1834	Benayahu 1993
	Xenia	*Xenia* sp.	Lamarck, 1816	Williams 1992a
		Xenia crassa	Schenk, 1896	Benayahu 1993
		Xenia garciae	Bourne, 1894	Benayahu 1993
		Xenia kuekenthali	Roxas, 1933	Benayahu 1993

Authority names in brackets indicate species that were originally in different genera when first described (in accordance with the International Code of Zoological Nomenclature, ICZN).

(n) New zoogeographical record from this study,

(e) Endemic species,

(nt) 'Near threatened' status. The IUCN Red List of Threatened Species^{TM},

* Two nominal species of *Cladiella* are assigned to a new genus and the name Beta has been selected as a placeholder for that genus while the full taxonomic description is being prepared for publication.

studies of octocorals that have utilized nuclear markers in addition to mitochondrial markers (Benayahu et al. 2012, McFadden & van Ofwegen 2013a, Haverkort-Yeh et al. 2013, McFadden, Brown, et al. 2014, Mcfadden & van Ofwegen 2017, McFadden et al. 2017). Nuclear gene regions: signal recognition particle 54 (*SRP54*)*,* internal transcribed spacer (*ITS*)*,* 18S ribosomal RNA (*18S rRNA*)*,* 28S ribosomal RNA (*28S rRNA*)*,* adenosine triphosphate α and β (*ATPS* α*, ATPS* β; and mitochondrial loci: bacterial MutS homologue (*MutS*), cytochrome *c* oxidase I (*COI*), cytochrome *c* oxidase I intergenic region (*COI+igr),* NADH dehydrogenase subunits 2, 3 and 6 (*ND2, ND3, ND6*) and 16S ribosomal RNA *(16S rRNA*)*;* have been investigated in octocorals in previous studies (Sánchez et al. 2003, McFadden et al. 2005, McFadden, France, et al. 2006, Hellberg 2006, Concepcion et al. 2007, McFadden & van Ofwegen 2012, Stemmer et al. 2013, Vargas et al. 2014, Wirshing & Baker 2015). *MutS* and *28S rRNA* were amplified across a range of diverse taxa compared with other loci, however, a barcoding gap could not be established for all taxa (Benayahu et al. 2012, McFadden, Reynolds, et al. 2014).

The systematics and phylogenetic relationships of Alcyonacea from the iSWP were investigated using mitochondrial and nuclear loci (*MutS* and *28S rRNA* respectively) and basic taxonomy. This is the first study of its kind for Alcyonacea in this understudied region of interest and serves to assess species richness, provide insight into species complexes and identify taxa requiring further taxonomic attention. The results of which will facilitate biodiversity studies that can be utilized in conservation strategies.

4.3 Method and materials

4.3.1 Specimen collection

Samples of Alcyonacea (n= 239) were collected by SCUBA across 7 reefs within the world heritage site, iSimangaliso Wetland Park (iSWP), KwaZulu-Natal, South Africa between 2014 and 2015 (Figure 4.1). Additional samples were collected from Durban, ~300km south of iSWP (inset) by snorkel-diving. Specimen samples were collected at a depth range of 1-40m, a minimum of five meters apart from one another to prevent the collection of clones (Table 4.2). The locations in this study lay within marine protected areas with sampling permits, RES2013/13, RES 2014/06 and RES2015/70, authorised by the Department of Agriculture, Forestry and Fisheries. Photographs were taken of each colony *in situ* and a longitudinal slice or branch was excised from each specimen with a scalpel. Following collection, the colonies were fixed in 70% ethanol and stored at room temperature for morphological investigation. The specimen samples have yet to be deposited at the Iziko South African Museum.

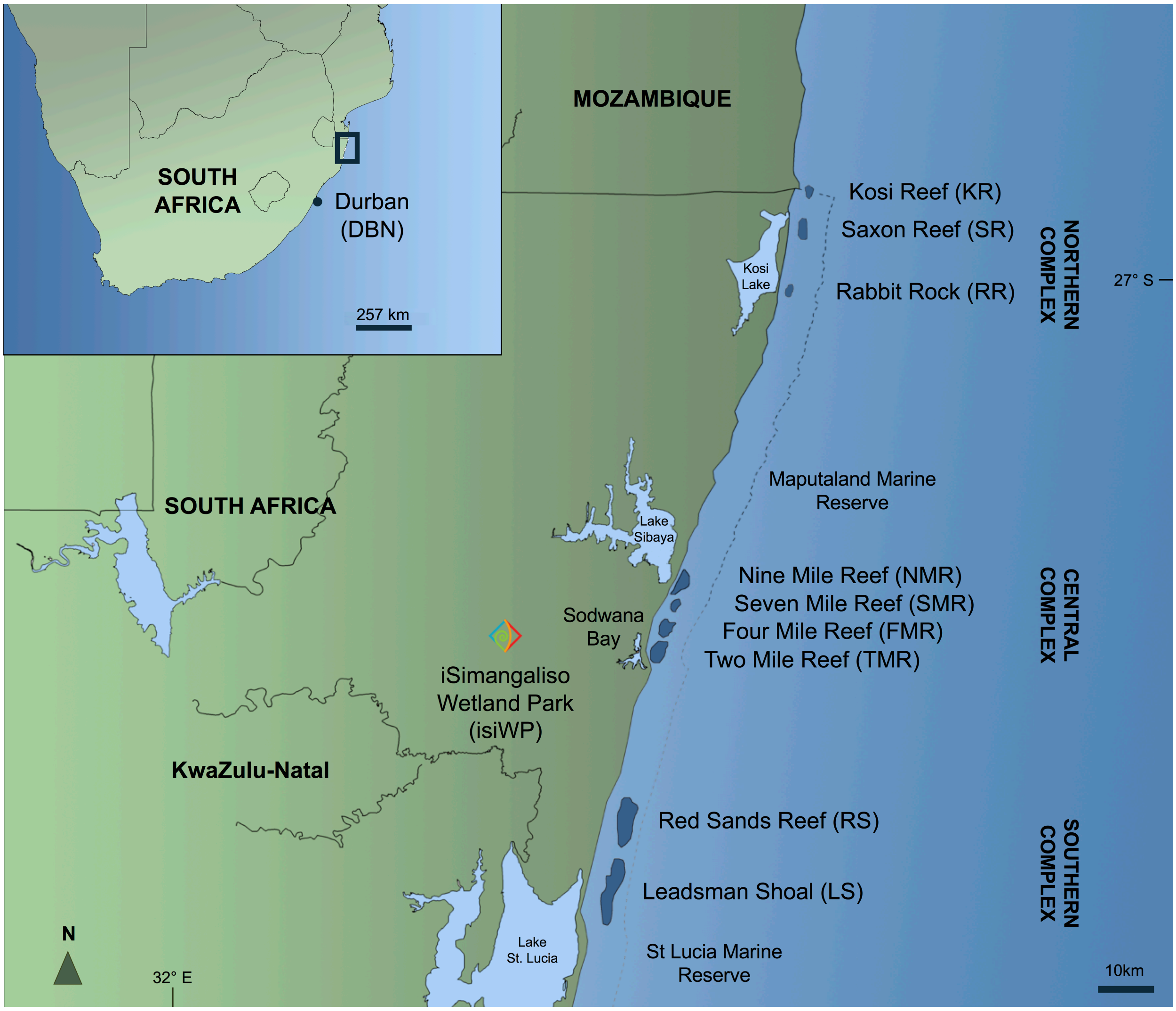

Figure 4.1 Reefs within the iSimangaliso Wetland Park and Durban (inset), KwaZulu-Natal, South Africa, from which Alcyonacea specimens were collected.

Table 4.2 Collection, taxonomic information and GenBank accession numbers for octocoral taxa collected for this study.

Sample	Site	Latitude (S)	Longitude (E)	Depth (m)	Family	Genus	Species	*MutS*	*28S rRNA*
236	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Aldersladum*	*sodwanum*	MF817826	MF817947
Ro1 (RJ)	IR	30.00716	30.91482	1	Alcyoniidae	*Aldersladum*	*sodwanum*	KY511000	KX430099
Ro2 (RJ)	IR	30.00716	30.91482	1	Alcyoniidae	*Aldersladum*	*sodwanum*	KY511001	KX430100
Ro3 (RJ)	IR	30.00716	30.91482	1	Alcyoniidae	*Aldersladum*	*sodwanum*	KY511002	KX430101
Ro4 (RJ)	IR	30.00716	30.91482	1	Alcyoniidae	*Aldersladum*	*sodwanum*	KY511003	KX430102
Ri1 (RJ)	IR	30.00716	30.91482	1	Alcyoniidae	*Cladiella*	sp.	KY510997	KX430096
Ri2 (RJ)	IR	30.00716	30.91482	1	Alcyoniidae	*Cladiella*	sp.	KY510998	KX430097
Ri3 (RJ)	IR	30.00716	30.91482	1	Alcyoniidae	*Cladiella*	sp.	KY510999	KX430098
61	SR	26.92642	32.88772	11	Alcyoniidae	Beta	sp.	KY510976	KX430075
79	LS	27.87455	32.60642	19	Alcyoniidae	Beta	sp.	KY510977	KX430076
84	LS	27.87465	32.60798	11	Alcyoniidae	Beta	sp.	KY510978	KX430077
112	TMR	27.51815	32.68987	18	Alcyoniidae	Beta	sp.	KY510979	KX430078
113	TMR	27.52223	32.68668	11	Alcyoniidae	Beta	sp.	KY510980	KX430079
134	KR	26.92825	32.88568	9.5	Alcyoniidae	Beta	sp.	KY510981	KX430080
135	KR	26.92825	32.88568	9.5	Alcyoniidae	Beta	sp.	KY510982	KX430081
142	KR	26.92825	32.88568	9.5	Alcyoniidae	Beta	sp.	KY510983	KX430082
157	KR	26.92807	32.8875	14.3	Alcyoniidae	Beta	sp.	KY510984	KX430083
160	KR	26.92807	32.8875	14.3	Alcyoniidae	Beta	sp.	KY510985	KX430084
165	TMR	27.5278	32.68482	15.9	Alcyoniidae	Beta	sp.	KY510986	KX430085
192	SMR	27.4515	32.7118	20.7	Alcyoniidae	Beta	sp.	KY510987	KX430086
194	SMR	27.4515	32.7118	17.6	Alcyoniidae	Beta	sp.	KY510988	KX430087
195	SMR	27.4515	32.7118	17.6	Alcyoniidae	Beta	sp.	KY510989	KX430088
198	NMR	27.4148	32.72662	17.6	Alcyoniidae	Beta	sp.	KY510990	KX430089
209	NMR	27.4148	32.72662	17.6	Alcyoniidae	Beta	sp.	KY510991	KX430090

Table 4.2 continued…

Sample	Site	Latitude (S)	Longitude (E)	Depth (m)	Family	Genus	Species	***MutS***	***28S rRNA***
215	NMR	27.4148	32.72662	17.6	Alcyoniidae	Beta	sp.	KY510992	KX430091
220	NMR	27.4148	32.72662	16.8	Alcyoniidae	Beta	*kashmani*	KY510993	KX430092
221	NMR	27.4148	32.72662	16.8	Alcyoniidae	Beta	sp.	KY510994	KX430093
248	TMR	27.52105	32.6867	12.8	Alcyoniidae	Beta	sp.	KY510995	KX430094
252	TMR	27.52105	32.6867	12.8	Alcyoniidae	Beta	sp.	KY510996	KX430095
2	LS	27 52.513	32 36.370	18	Alcyoniidae	*Sinularia*	sp.	MF817871	MF817893
4	LS	27 52.513	32 36.370	18	Alcyoniidae	*Sinularia*	sp.	MF817842	MF817894
5	LS	27 52.513	32 36.370	18	Alcyoniidae	*Sinularia*	sp.	MF817841	MF817895
7	LS	27 52.513	32 36.370	18	Alcyoniidae	*Sinularia*	*abrupta*	-	MF817896
8	LS	27 52.513	32 36.370	18	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817831	MF817897
9	LS	27 52.513	32 36.370	18	Alcyoniidae	*Sinularia*	sp.	MF817828	MF817898
10	LS	27 52.513	32 36.370	18	Alcyoniidae	*Sinularia*	sp.	MF817892	MF817899
12	LS	27 52.350	32 36.354	18	Alcyoniidae	*Sinularia*	*abrupta*	MF817890	MF817900
17	LS	27 52.350	32 36.354	18	Alcyoniidae	*Sinularia*	sp.	MF817878	MF817901
25	LS	27 52.328	32 36.340	18	Alcyoniidae	*Sinularia*	*abrupta*	MF817854	MF817902
31	NMR	27 24.818	32 43.647	18	Alcyoniidae	*Sinularia*	*variabilis*	MF817844	MF817903
36	NMR	27 24.818	32 43.647	18	Alcyoniidae	*Sinularia*	sp.	MF817843	MF817904
50	TMR	27 31.257	32 41.384	18	Alcyoniidae	*Sinularia*	*abrupta*	MF817840	MF817905
55	TMR	27 31.257	32 41.384	18	Alcyoniidae	*Sinularia*	sp.	MF817839	MF817906
56	TMR	27 31.257	32 41.384	18	Alcyoniidae	*Sinularia*	sp.	MF817838	-
57	TMR	27 31.257	32 41.384	18	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817837	MF817907
60	SR	26.92641	32.88771	11	Alcyoniidae	*Sinularia*	sp.	MF817836	MF817908
66	SR	26.92642	32.88772	11	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817835	MF817909
67	SR	26.92643	32.88773	11	Alcyoniidae	*Sinularia*	sp.	MF817834	MF817910
73	NMR	27.41491	32.72666	12	Alcyoniidae	*Sinularia*	*brassica*	MF817833	MF817911
75	LS	27.87455	32.60641	19	Alcyoniidae	*Sinularia*	sp.	MF817832	MF817912

Table 4.2 continued…

Sample	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth (m)**	**Family**	**Genus**	**Species**	***MutS***	***28S rRNA***
80	LS	27.87455	32.60643	19	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817830	MF817913
85	LS	27.87465	32.6079	11	Alcyoniidae	*Sinularia*	sp.	MF817829	MF817914
93	LS	27.87465	32.6079	11	Alcyoniidae	*Sinularia*	*brassica*	MF817827	MF817915
103	TMR	27.51815	32.68986	18	Alcyoniidae	*Sinularia*	*brassica*	MF817891	MF817916
131	SR	26.92825	32.8856	9.5	Alcyoniidae	*Sinularia*	*abrupta*	MF817889	MF817917
139	SR	26.92825	32.8856	9.5	Alcyoniidae	*Sinularia*	*erecta*	MF817888	MF817918
141	SR	26.92825	32.8856	9.5	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817887	-
143	SR	26.92825	32.8856	9.5	Alcyoniidae	*Sinularia*	*brassica*	MF817886	MF817919
146	SR	26.92825	32.8856	14.3	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817885	MF817920
147	KR	26.92806	32.8875	14.3	Alcyoniidae	*Sinularia*	sp.	MF817884	MF817921
148	KR	26.92806	32.8875	14.3	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817883	-
151	KR	26.92806	32.8875	14.3	Alcyoniidae	*Sinularia*	*brassica*	MF817882	MF817923
153	KR	26.92806	32.8875	14.3	Alcyoniidae	*Sinularia*	*brassica*	MF817881	-
164	TMR	27.5278	32.6848	14.3	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817880	-
168	TMR	27.5278	32.6848	17.9	Alcyoniidae	*Sinularia*	*brassica*	MF817879	MF817925
172	TMR	27.5278	32.6848	17.9	Alcyoniidae	*Sinularia*	*brassica*	MF817877	MF817926
183	SMR	27.4515	32.7118	20.7	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817876	MF817927
188	SMR	27.4515	32.7118	20.7	Alcyoniidae	*Sinularia*	*brassica*	MF817875	MF817928
189	SMR	27.4515	32.7118	20.7	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817874	MF817929
191	SMR	27.4515	32.7118	20.7	Alcyoniidae	*Sinularia*	*variabilis*	MF817873	MF817930
199	NMR	27.4148	32.72661	17.6	Alcyoniidae	*Sinularia*	*erecta*	MF817872	-
200	NMR	27.4148	32.72661	17.6	Alcyoniidae	*Sinularia*	*brassica*	MF817870	MF817931
214	NMR	27.4148	32.72661	17.6	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817869	-
224	NMR	27.41496	32.72675	16.8	Alcyoniidae	*Sinularia*	sp.	MF817868	-
225	NMR	27.41496	32.72675	16.8	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817867	-
226	NMR	27.41496	32.72675	16.8	Alcyoniidae	*Sinularia*	sp.	MF817866	MF817932

Table 4.2 continued…

Sample	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth (m)**	**Family**	**Genus**	**Species**	***MutS***	***28S rRNA***
227	NMR	27.41496	32.72675	12.8	Alcyoniidae	*Sinularia*	*notanda*	MF817865	MF817933
234	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	*abrupta*	MF817864	MF817934
235	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	*notanda*	MF817863	MF817935
237	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	*variabilis*	MF817862	-
239	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	sp.	MF817861	MF817936
240	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	sp.	MF817860	MF817937
243	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	*abrupta*	MF817859	MF817938
245	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	sp.	MF817858	MF817939
246	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	sp.	MF817857	-
247	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	*heterospiculata*	MF817856	MF817940
249	TMR	27.52105	32.6867	12.8	Alcyoniidae	*Sinularia*	sp.	MF817855	MF817941
257	TMR	27.52105	32.6867	16.8	Alcyoniidae	*Sinularia*	sp.	MF817853	MF817942
263	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Sinularia*	sp.	MF817852	MF817943
268	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Sinularia*	*grandilobata*	MF817851	MF817944
269	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Sinularia*	*notanda*	MF817850	MF817945
271	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Sinularia*	sp.	MF817849	-
274	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Sinularia*	*variabilis*	MF817848	-
275	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Sinularia*	*variabilis*	MF817847	-
279	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Sinularia*	sp.	MF817846	-
280	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Sinularia*	*erecta*	MF817845	-
65	SR	26.92641	32.88772	11	Alcyoniidae	*Sarcophyton*	sp.	MG583665	MG583658
89	LS	27.87465	32.60798	11	Alcyoniidae	*Sarcophyton*	sp.	MG583666	MG583659
90	LS	27.87465	32.60798	11	Alcyoniidae	*Sarcophyton*	sp.	MG583667	MG583660
98	TMR	27.51815	32.68987	18	Alcyoniidae	*Sarcophyton*	sp.	MG583668	MG583661
138	KR	26.92825	32.88568	9.5	Alcyoniidae	*Sarcophyton*	sp.	MG583669	MG583662
256	TMR	27.52105	32.6867	16.8	Alcyoniidae	*Sarcophyton*	sp.	MG583670	MG583663

Table 4.2 continued…

Sample	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth (m)**	**Family**	**Genus**	**Species**	***MutS***	***28S rRNA***
262	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Sarcophyton*	sp.	MG583671	MG583664
S1	TMR	27 31.809	32 41.289	18	Alcyoniidae	*Sarcophyton*	sp.	KY511004	KY910760
S2	TMR	27 31.809	32 41.289	18	Alcyoniidae	*Sarcophyton*	sp.	KY511005	KY910761
S3	TMR	27 31.809	32 41.289	18	Alcyoniidae	*Sarcophyton*	sp.	KY511006	KY910762
S4	TMR	27 31.809	32 41.289	18	Alcyoniidae	*Sarcophyton*	sp.	KY511007	KY910763
S5	TMR	27 31.809	32 41.289	18	Alcyoniidae	*Sarcophyton*	sp.	KY511008	KY910764
S6	TMR	27 31.809	32 41.289	18	Alcyoniidae	*Sarcophyton*	sp.	KY511009	KY910765
S7	TMR	27 31.810	33 41.289	19	Alcyoniidae	*Sarcophyton*	sp.	KY511010	KY910766
S8	TMR	27 31.809	32 41.289	18	Alcyoniidae	*Sarcophyton*	sp.	KY511011	KY910767
S9	TMR	27 31.809	32 41.289	18	Alcyoniidae	*Sarcophyton*	sp.	KY511012	KY910768
S10	TMR	27 31.809	32 41.289	18	Alcyoniidae	*Sarcophyton*	sp.	KY511013	KY910769
S11	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511014	KY910770
S12	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511015	KY910771
S13	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511016	KY910772
S14	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511017	KY910773
S15	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511018	KY910774
S16	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511019	KY910775
S18	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511020	KY910776
S19	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511021	KY910777
S20	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511022	KY910778
S21	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511023	KY910779
S22	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511024	KY910780
S23	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511025	KY910781
S24	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511026	KY910782
S25	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511027	KY910783
S26	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511028	KY910784

Table 4.2 continued…

Sample	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth (m)**	**Family**	**Genus**	**Species**	***MutS***	***28S rRNA***
S27	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511029	KY910785
S28	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511030	KY910786
S29	TMR	27 31.642	32 41.205	18	Alcyoniidae	*Sarcophyton*	sp.	KY511031	KY910787
Sa1	TMR	27 31.005	32 41.425	14	Alcyoniidae	*Sarcophyton*	sp.	KY511032	KY910788
Sa2	TMR	27 31.005	32 41.425	14	Alcyoniidae	*Sarcophyton*	sp.	KY511033	KY910789
Sa3	TMR	27 31.005	32 41.425	14	Alcyoniidae	*Sarcophyton*	sp.	KY511034	KY910790
Sa4	TMR	27 31.005	32 41.425	14	Alcyoniidae	*Sarcophyton*	sp.	KY511035	KY910791
Sa5	TMR	27 31.005	32 41.425	14	Alcyoniidae	*Sarcophyton*	sp.	KY511036	KY910792
Sa6	TMR	27 31.005	32 41.425	14	Alcyoniidae	*Sarcophyton*	sp.	KY511037	KY910793
Sa7	TMR	27 31.005	32 41.425	14	Alcyoniidae	*Sarcophyton*	sp.	KY511038	KY910794
Sa8	TMR	27 31.005	32 41.425	14	Alcyoniidae	*Sarcophyton*	sp.	KY511039	KY910795
Sa9	TMR	27 31.005	32 41.425	14	Alcyoniidae	*Sarcophyton*	sp.	KY511040	KY910796
1	LS	27 52.513	32 36.370	18	Alcyoniidae	*Lobophytum*	sp.	MG583579	-
32	NMR	27 24.818	32 43.647	18	Alcyoniidae	*Lobophytum*	*venustum*	-	MG583533
117	TMR	27.52223	32.68668	11	Alcyoniidae	*Lobophytum*	sp.	-	MG583538
278	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Lobophytum*	*depressum*	-	MG583548
6	LS	27 52.513	32 36.370	18	Alcyoniidae	*Lobophytum*	*depressum*	MG583580	-
11	LS	27 52.350	32 36.354	18	Alcyoniidae	*Lobophytum*	*patulum*	MG583581	-
27	LS	27 52.328	32 36.340	18	Alcyoniidae	*Lobophytum*	sp.	MG583583	-
106	TMR	27.51815	32.68987	18	Alcyoniidae	*Lobophytum*	sp.	-	MG583535
111	TMR	27.51815	32.68987	18	Alcyoniidae	*Lobophytum*	sp.	-	MG583536
276	TMR	27.5271	32.6868	16.8	Alcyoniidae	*Lobophytum*	sp.	-	MG583547
132	KR	26.92825	32.88568	9.5	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583540
144	KR	26.92825	32.88568	9.5	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583542
193	SMR	27.4515	32.7118	20.7	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583543
136	KR	26.92825	32.88568	9.5	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583541

Table 4.2 continued…

Sample	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth (m)**	**Family**	**Genus**	**Species**	***MutS***	***28S rRNA***
15	LS	27 52.350	32 36.354	18	Alcyoniidae	*Lobophytum*	*crassum*	MG583582	-
63	SR	26.92641	32.88772	11	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583534
116	TMR	27.52223	32.68668	11	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583537
125	TMR	27.52223	32.68668	11	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583539
207	NMR	27.4148	32.72662	17.6	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583544
210	NMR	27.4148	32.72662	17.6	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583545
216	NMR	27.4148	32.72662	17.6	Alcyoniidae	*Lobophytum*	*crassum*	-	MG583546
286 (KS)	KR	27.4918	32.6899	37	Ellisellidae	*Junceella*	sp.	MG583631	MG583551
285 (KS)	KR	27.4918	32.6899	37	Ellisellidae	*Verrucella*	sp.	MG583630	MG583549
282 (KS)	TMR	27.5275	32.6920	33	Gorgoniidae	*Leptogorgia*	sp.	MG583640	MG583561
107	TMR	27.51815	32.68987	18	Gorgoniidae	*Rumphella*	sp.	MG583633	MG583554
114	TMR	27.52223	32.68668	11	Gorgoniidae	*Rumphella*	sp.	-	MG583552
150	RR	26.92807	32.8875	14.3	Gorgoniidae	*Rumphella*	sp.	MG583641	MG583562
155	RR	26.92807	32.8875	14.3	Gorgoniidae	*Rumphella*	sp.	-	MG583557
201	NMR	27.4148	32.72662	17.6	Gorgoniidae	*Rumphella*	sp.	MG583637	MG583559
287 (KS)	KR	27.4919	32.6900	37	Nidaliidae	*Chironephthya*	sp.	MG583632	MG583553
126 (KS)	RR	26.99189	32.90918	36	Parasphaerascleridae	*Parasphaerasclera*	*aurea*	MG583642	-
289 (KS)	DBN	27 31.257	32 41.384	40	Parasphaerascleridae	*Parasphaerasclera*	*rotifera*	MG583634	MG583555
290 (KS)	DBN	27 31.257	32 41.384	40	Parasphaerascleridae	*Parasphaerasclera*	*rotifera*	MG583635	MG583556
291 (KS)	DBN	27 31.257	32 41.384	40	Parasphaerascleridae	*Parasphaerasclera*	*rotifera*	MG583636	MG583558
127 (KS)	RR	26.99189	32.90918	36	Plexauridae	*Euplexaura*	sp.	MG583638	MG583560
284 (KS)	KR	27.4918	32.6899	37	Plexauridae	*Euplexaura*	sp.	-	MG583550
281 (KS)	TMR	27.5275	32.6920	33	Plexauridae	*Paraplexaura*	sp.	MG583639	-
283 (KS)	KR	27.4918	32.6899	37	Nephtheidae	*Dendronephthya*	sp.	MG583629	-
71	NMR	27.41491	32.72666	12	Nephtheidae	*Dendronephthya*	sp.	MG583585	MG583525
74	SR	26.92641	32.88772	11	Nephtheidae	*Dendronephthya*	sp.	MG583586	-

Table 4.2 continued…

Sample	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth (m)**	**Family**	**Genus**	**Species**	***MutS***	***28S rRNA***
96	TMR	27.51815	32.68987	18	Nephtheidae	*Dendronephthya*	sp.	MG583588	-
97	TMR	27.51815	32.68987	18	Nephtheidae	*Dendronephthya*	sp.	MG583589	-
100	TMR	27.51815	32.68987	18	Nephtheidae	*Dendronephthya*	sp.	MG583591	-
102	TMR	27.51815	32.68987	18	Nephtheidae	*Dendronephthya*	sp.	MG583592	MG583526
104	TMR	27.51815	32.68987	18	Nephtheidae	*Dendronephthya*	sp.	MG583593	-
105	TMR	27.51815	32.68987	18	Nephtheidae	*Dendronephthya*	sp.	MG583594	-
108	TMR	27.51815	32.68987	18	Nephtheidae	*Dendronephthya*	sp.	MG583595	MG583527
120	TMR	27.52223	32.68668	11	Nephtheidae	*Dendronephthya*	sp.	MG583597	-
122	TMR	27.52223	32.68668	11	Nephtheidae	*Dendronephthya*	sp.	MG583599	-
137	KR	26.92825	32.88568	9.5	Nephtheidae	*Dendronephthya*	sp.	MG583602	-
161	RR	26.92807	32.8875	14.3	Nephtheidae	*Dendronephthya*	sp.	MG583606	-
163	TMR	27.5278	32.68482	14.3	Nephtheidae	*Dendronephthya*	sp.	MG583607	-
166	TMR	27.5278	32.68482	16.9	Nephtheidae	*Dendronephthya*	sp.	MG583608	-
167	TMR	27.5278	32.68482	17.9	Nephtheidae	*Dendronephthya*	sp.	MG583609	-
173	TMR	27.5278	32.68482	17.9	Nephtheidae	*Dendronephthya*	sp.	MG583611	-
185	SMR	27.4515	32.7118	20.7	Nephtheidae	*Dendronephthya*	sp.	MG583616	MG583531
186	SMR	27.4515	32.7118	20.7	Nephtheidae	*Dendronephthya*	sp.	MG583617	-
187	SMR	27.4515	32.7118	20.7	Nephtheidae	*Dendronephthya*	sp.	MG583618	-
231	NMR	27.41497	32.72675	12.8	Nephtheidae	*Dendronephthya*	sp.	MG583622	-
264	TMR	27.5271	32.6868	16.8	Nephtheidae	*Dendronephthya*	sp.	MG583627	-
128 (KS)	RR	26.99189	32.90918	36	Nephtheidae	*Scleronephthya*	sp.	MG583600	MG583528
119	TMR	27.52223	32.68668	11	Nephtheidae	*Stereonephthya*	sp.	MG583596	-
174	TMR	27.5278	32.68482	17.9	Nephtheidae	*Stereonephthya*	sp.	MG583612	MG583529
176	TMR	27.5278	32.68482	17.9	Nephtheidae	*Stereonephthya*	sp.	MG583613	MG583530
180	TMR	27.5278	32.68482	17.9	Nephtheidae	*Stereonephthya*	sp.	MG583614	-
217	NMR	27.4148	32.72662	17.6	Nephtheidae	*Stereonephthya*	sp.	MG583619	MG583532

Table 4.2 continued…

Sample	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth (m)**	**Family**	**Genus**	**Species**	***MutS***	***28S rRNA***
218	NMR	27.4148	32.72662	17.6	Nephtheidae	*Stereonephthya*	sp.	MG583620	-
219	NMR	27.4148	32.72662	17.6	Nephtheidae	*Stereonephthya*	sp.	MG583621	-
251	TMR	27.52105	32.6867	12.8	Nephtheidae	*Stereonephthya*	sp.	MG583623	-
258	TMR	27.5271	32.6868	16.8	Nephtheidae	*Stereonephthya*	sp.	MG583624	-
259	TMR	27.5271	32.6868	16.8	Nephtheidae	*Stereonephthya*	sp.	MG583625	-
260	TMR	27.5271	32.6868	16.8	Nephtheidae	*Stereonephthya*	sp.	MG583626	-
77	LS	27.87455	32.6064	19	Nephtheidae	*Litophyton*	sp.	MG583587	-
99	TMR	27.51815	32.68987	18	Nephtheidae	*Litophyton*	sp.	MG583590	-
121	TMR	27.52223	32.68668	11	Nephtheidae	*Litophyton*	sp.	MG583598	-
130 (KS)	RR	26.99189	32.90918	36	Nephtheidae	*Litophyton*	sp.	MG583601	-
140	KR	26.92825	32.88568	9.5	Nephtheidae	*Litophyton*	sp.	MG583603	-
149	RR	26.92807	32.8875	14.3	Nephtheidae	*Litophyton*	sp.	MG583604	-
158	RR	26.92807	32.8875	14.3	Nephtheidae	*Litophyton*	sp.	MG583605	-
169	TMR	27.5278	32.68482	17.9	Nephtheidae	*Litophyton*	sp.	MG583610	-
184	SMR	27.4515	32.7118	20.7	Nephtheidae	*Litophyton*	sp.	MG583615	-
267	TMR	27.5271	32.6868	16.8	Nephtheidae	*Litophyton*	sp.	MG583628	-
154	RR	26.92807	32.8875	14.3	Xeniidae	*Anthelia*	*glauca*	MG583646	MG583566
228	NMR	27.41497	32.72675	12.8	Xeniidae	*Anthelia*	*glauca*	MG583651	MG583572
232	TMR	27.52105	32.6867	12.8	Xeniidae	*Anthelia*	*glauca*	MG583652	MG583573
241	TMR	27.52105	32.6867	12.8	Xeniidae	*Anthelia*	*glauca*	MG583653	MG583574
265	TMR	27.5271	32.6868	16.8	Xeniidae	*Anthelia*	*glauca*	-	MG583577
272	TMR	27.5271	32.6868	16.8	Xeniidae	*Anthelia*	*glauca*	MG583656	-
206	NMR	27.4148	32.72662	17.6	Xeniidae	*Heteroxenia*	*fuscescens*	MG583649	MG583570
159	RR	26.92807	32.8875	14.3	Xeniidae	*Sansibia*	sp.	MG583647	MG583567
204	NMR	27.4148	32.72662	17.6	Xeniidae	*Sansibia*	sp.	MG583584	-
179	TMR	27.5278	32.68482	17.9	Xeniidae	*Sympodium*	*caeruleum*	-	MG583569

Table 4.2 continued…

Sample	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth (m)**	**Family**	**Genus**	**Species**	***MutS***	***28S rRNA***
242	TMR	27.52105	32.6867	12.8	Xeniidae	*Sympodium*	*caeruleum*	MG583654	MG583575
255	TMR	27.52105	32.6867	16.8	Xeniidae	*Sympodium*	*caeruleum*	MG583655	MG583576
288	TMR	27.52105	32.6867	12	Xeniidae	*Sympodium*	*caeruleum*	MG583657	MG583578
129 (KS)	RR	26.99189	32.90918	36	Xeniidae	*Xenia*	sp.	MG583644	MG583564
152	RR	26.92807	32.8875	14.3	Xeniidae	*Xenia*	sp.	MG583645	MG583565
177	TMR	27.5278	32.68482	17.9	Xeniidae	*Xenia*	sp.	MG583648	MG583568
95	LS	27.87465	32.60798	11	Xeniidae	*Xenia*	sp.	MG583643	MG583563

(TMR) Two Mile Reef; (KR) Kosi Reef; (LS) Leadsman Shoal; (NMR) Nine Mile Reef; (SMR) Seven Mile Reef; (SR) Saxon Reef; (RR) Rabbit Rock; (IR) Isipingo Rocks; (DBN) Durban. Collector (KS) Kerry Sink (SANBI); (RJ) Roy Jackson (UKZN). ”-“ data unavailable.

Prior to fixation, small tissue subsamples (approximately 1cm^3) were excised from the collected specimen sample, placed into individual cryofuge tubes and frozen at -80 °C for subsequent genetic analyses.

4.3.2 *Morphological analyses*

Preliminary identifications of specimens to the genus and species-level (where possible) were carried out according to the protocols and monographs by Fabricius & Alderslade (2001) and Williams (1992a, d) (Table 4.2). Colony morphologies were analysed using a combination of *in situ* photographs and examination under a stereomicroscope (Z730 Stereo Series; Unitron) at 20X power. Diagnostic tissues were carefully excised with a sterile scalpel for slide preparation and subsequent sclerite examination. The excised tissues were dissolved in 12% sodium hypochlorite on a microscope slide to release the sclerites which were subsequently analysed under a compound microscope with a digital camera (Eclipse 80i; Nikon) at 40 and 100X magnification. Species-level identifications were carried out for taxa known to occur in the study region that had clearly defined species boundaries with published descriptions that made them easily discernible without further expertise. However, species-level identifications were not possible for all taxa at the time due to a lack of appropriate taxonomic revisions of the assigned genera (Benayahu et al. 2012).

4.3.3 *Genomic DNA extraction and amplification*

Tissue samples were flash frozen in liquid Nitrogen and ground to a fine powder using a sterilized pestle and mortar prior to genomic isolation. DNA was extracted from the homogenized tissues using a Zymogen ZR Genomic DNA^{TM} Tissue MiniPrep kit. Protocols were carried out according to the manufacturer's instructions and included an extended digestion period of 16 hours. DNA quality and concentration were subsequently measured using a NanoDrop 2000 (Thermo Scientific^{TM}, Wilmington, DE, USA). Fragments of mitochondrial *MutS* and nuclear *28S rRNA* were amplified using a combination of forward and reverse primers described in Table 4.3. Oligonucleotides were designed for specimens that were recently reassigned to a new genus (Beta) that failed amplification with known *MutS* primers. Primers *MutS*$_{KF}$ (5'-ATGAATCAAATACC

TGCGCAAT-3') and *MutS*$_{KR}$ (5'-ACACGGCAAGTTGGTTAATACT-3') were designed based on GenBank sequences of Beta *kashmani* using PRIMER3 (Rozen & Skaletsky

2000). Each PCR reaction consisted of 1 X DreamTaq Green PCR Master Mix (Thermo Scientific^{TM}); 0,18 µM of forward and reverse primer; 10mg/ml BSA; 50-150ng genomic DNA and nuclease-free water to a final volume of 25 µl. Amplification was carried out using a T100^{TM} Thermal cycler (Bio-Rad) according to the protocols specified in Table 4.3.

Table 4.3 Primer combinations and PCR amplification protocols implemented in this study.

Gene region	Primer	Amplicon size (bp)	PCR protocol	Reference
MutS	ND42599F^{1}	735^{1,4}	58°C, 90:90:60	France & Hoover 2002
	ND4L2475F^{2}	990^{2,4}	58°C, 90:90:60	Brugler & France 2008
	AnthoCorMSH^{3}	800^{3,4}	50°C, 30:45:60	Herrera et al. 2010
	MUT3458R^{4}		58°C, 90:90:60	Sánchez et al. 2003
	MutS$_{\mathrm{KF}}^{5}$	710^{5,6}	58°C, 30:30:60	this study
	MutS$_{\mathrm{KR}}^{6}$		58°C, 30:30:60	this study
28S	*28S*-Far^{1}	810^{1,2}	60°C, 30:30:60	McFadden & van Ofwegen 2013
	28S-Rar^{2}		60°C, 30:30:60	McFadden & van Ofwegen 2013
	28S-Rab^{3}	740^{1,3}	60°C, 30:30:60	McFadden & van Ofwegen 2013

PCR initial denaturation step took place for 3 min at 95°C, with a final elongation step of 5 min at 72°C. All protocols were run for 35X cycles. PCR protocol as follows: annealing temperature; seconds for denaturation at 95°C; seconds at annealing temperature; seconds for elongation at 72°C.

The PCR products, along with a negative control and GeneRuler 100bp DNA Ladder (Thermo Scientific^{TM}), were electrophoresed on a 1% agarose gel for 60 min and examined under a Molecular Imager$^{®}$ ChemiDoc^{TM} XRS (Bio-Rad) to confirm successful amplification of the targeted gene regions. The amplicons were subsequently sent to the Central Analytical Facilities unit of Stellenbosch University for purification, quantification and Sanger dideoxy sequencing with an ABI 3730xl DNA analyser (Thermo Scientific^{TM}). Raw sequences were examined and annotated using BLASTN (Altschul et al. 1990) and manually edited in MEGA v 6.06 (Tamura et al.

2013) prior to their submission to GenBank. A list of GenBank accession numbers can be found in Table 4.2.

4.3.4 Phylogenetic analyses

Maximum likelihood and Bayesian phylogenies were constructed using 313 sequences of mitochondrial *MutS* (710bp), 250 sequences of nuclear *28S rRNA* (881bp) and a concatenation of 212 sequences of *MutS+28S* (1626bp) respectively. The datasets included 219 *MutS*, 180 *28S rRNA* and 160 concatenated (*MutS+28S*) sequences from specimens collected in this study. GenBank sequences of *MutS* and *28S rRNA* were incorporated into analyses in order to increase the representation of genera and facilitate specimen identifications from the phylogenetic framework. GenBank sequences were selected for vouchered Alcyonacea taxa that have been recorded in the study region (Appendix B Table 7.5). *Briareum hamrum* ZMTAU CO34187 (Scleraxonia) was used as an outgroup for *MutS* whereas *Cornularia pabloi* USNM 1178390 (Stolonifera) was used to root *28S rRNA* since both taxa belong to subordinal groups that form the base of the octocorallian tree (McFadden & van Ofwegen 2012).

Nuclear data were phased in PHASE v 2.1.1 (Flot 2010) to account for alleles represented by sequences displaying ambiguities. Multiple sequence alignments were carried out for each dataset using the MUSCLE (Edgar 2004) operation in MEGA v 6.06 (Tamura et al. 2013). MEGA v 6.06 (Tamura et al. 2013) was used to calculate pairwise genetic distances (uncorrected *p*) and distance among and within families for all datasets. DnaSP v 5.10.1 (Librado & Rozas 2009) was used to calculate haplotypes for each dataset including haplotype and nucleotide diversities. Gap regions were taken into consideration for datasets displaying numerous polymorphisms and indels. Phased alleles of nuclear data shared the same haplotype per individual and were therefore not indicated in subsequent phylogenetic analyses.

General Time Reversible (GTR) with invariant sites and a gamma distribution (GTR+I+G) was selected as the substitution model for both *MutS* and *28S rRNA* under the Akaike information criterion (AIC) (Akaike 1973) calculated in jModelTest v 2.1.10 (Guindon & Gascuel 2003, Darriba et al. 2012). Phylogenetic analyses were carried out for each dataset (*MutS*; *28S rRNA*; *Mut+28S*) using both maximum likelihood and Bayesian inferences with an applied model of evolution (GTR+I+G). Maximum likelihood analyses were carried out with Randomized Axelerated maximum likelihood RAxML) (Stamatakis 2014) using the web server RAxML Black Box[12] (Stamatakis et al. 2008) and Phylogenetic Maximum Likelihood (PhyML) v 3.0 (Guindon et al. 2010) online server[13] for 2000 bootstraps respectively. Remaining parameters were left at default. Bayesian phylogenetic analyses were carried out in MrBayes v 3.2.6 (Ronquist & Huelsenbeck 2003) simulating four chains for 20,000,000 generations and a burn-in of 10%. Tracer v 1.6 (Rambaut et al. 2018) was used to assess Markov Chain Monte Carlo (MCMC) convergence and verify that effective sample size (ESS) values were greater than 200.

Maximum likelihood and Bayesian outputs displayed corresponding phylogenies that differed only in branch support values. The most likely trees from Maximum likelihood were rooted and edited in FigTree v 1.4 (Rambaut 2012); and presented with mapped bootstrap support values and posterior probabilities.

4.4 Results

4.4.1 Morphological analyses and specimen identifications

In total, the study of 239 collected taxa yielded ~61 putative species assigned to 23 genera within 8 families (Table 4.2) and distributed among three subordinal groups, Alcyoniina, Holaxonia and Calcaxonia (barring Parasphaerascleridae). The collection included 161 colonies of unidentified species that were tentatively assigned to genera (Table 4.2). The genera collected, and their respective number of samples included *Sinularia* (67); *Lobophytum* (21); *Sarcophyton* (44); *Cladiella* (3); Beta (21); *Aldersladum* (5); *Xenia* (4); *Heteroxenia* (1); *Sansibia* (2); *Sympodium* (4); *Anthelia* (6); *Dendronephthya* (23); *Stereonephthya* (11); *Scleronephthya* (1); *Litophyton* (10); *Chironephthya* (1); *Verrucella* (1); *Junceella* (1); *Rumphella* (5); *Leptogorgia* (1); *Parasphaerasclera* (4); *Euplexaura* (2); and *Paraplexaura* (1). The subordinal group, Alcyoniina, included 4 families: Alcyoniidae (Figure 4.14); Nephtheidae (Figure 4.2); Xeniidae (Figure 4.3); and Nidaliidae (Figure 4.6 F). The families Plexauridae (Figure 4.6 A & B) and Gorgoniidae (Figure 4.6 C) represented the Holaxonia subordinal group

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[12] http://www.genome.jp/tools/raxml/

[13] http://www.atgc-montpellier.fr/phyml/

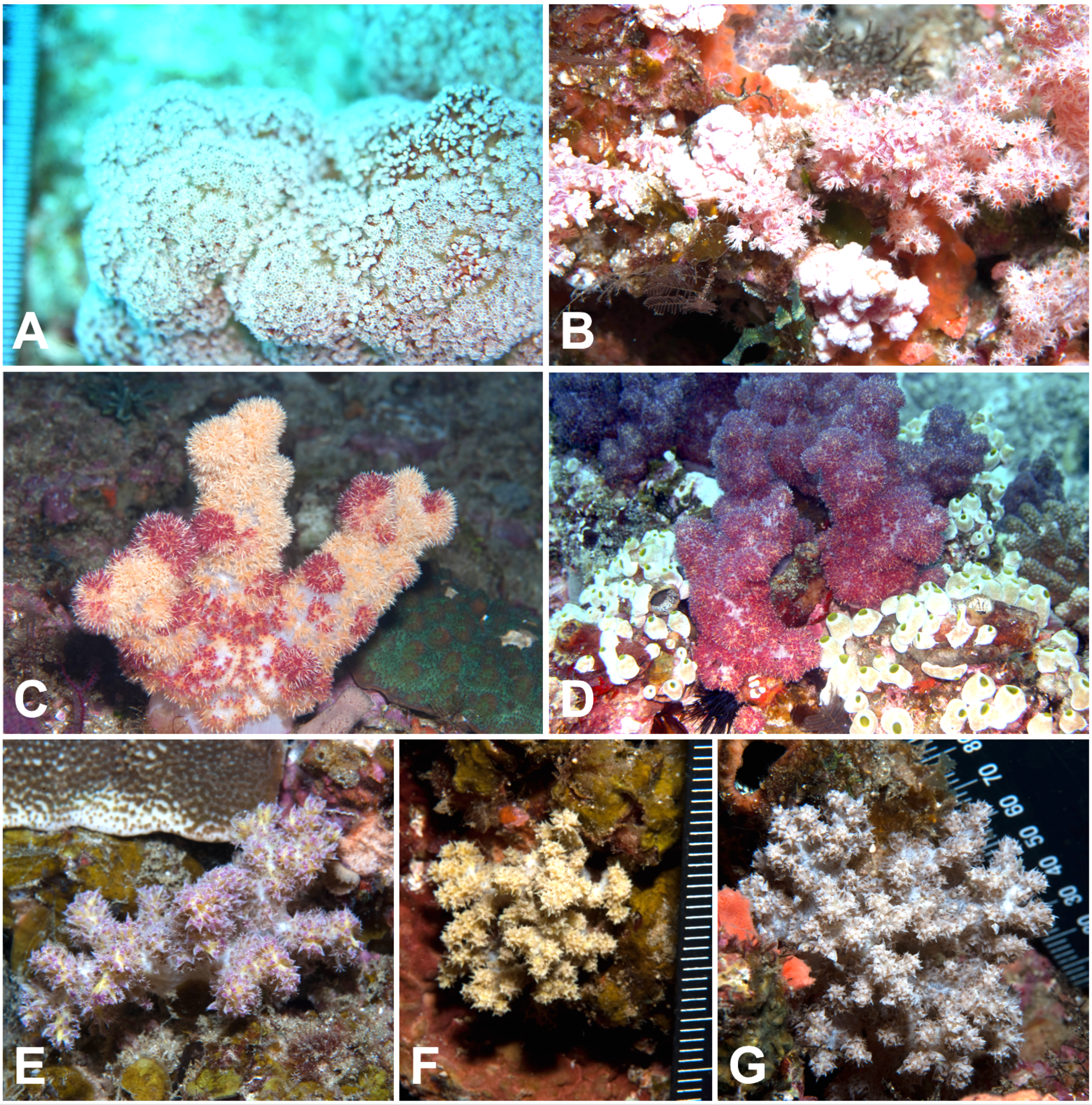


Figure 4.2 Colony photos of Alcyonacea (Family Nephtheidae) from the iSimangaliso Wetland Park. A, C, D: *Dendronephthya* spp.; B: *Scleronephthya* sp.; E-G: *Stereonephthya* spp.

and Ellisellidae was the sole representative of the Calcaxonia group (Figure 4.6 D & E). The monogeneric family Parasphaerascleridae (Figure 4.6 G) does not belong to a subordinal group (McFadden & van Ofwegen 2013a). Several morphotypes were noted throughout the collection. The genus *Dendronephthya* yielded three colour morphs within the collection: 1) yellow and red polyps with a white stalk (Figure 4.2 C); 2) dark pink and orange with a white stalk (Figure 4.2 D; and 3) purple and white polyps with a white stalk. Four colour morphs were observed within the genus *Stereonephthya*: 1) pale purple and yellow with a white stalk (Figure 4.2 E); yellow and white polyps with a white stalk (Figure 4.2 F); cream and brown coloured polyps with a white stalk (Figure 4.2 G); and purple polyps with a white stalk. Two morphotypes were noted for the genus *Xenia* (Figure 4.3 A) which may form two different species recorded from the region and require expert identification. One morphotype is characterised by longer tentacles and fewer rows of pinnules than its counterpart. *Sansibia* yielded an unfamiliar morphotype that was pink in colour. Furthermore, *Sinularia brassica* showed evidence of two morphologically distinct colony forms: 1) digitate and 2) lobate and encrusting.

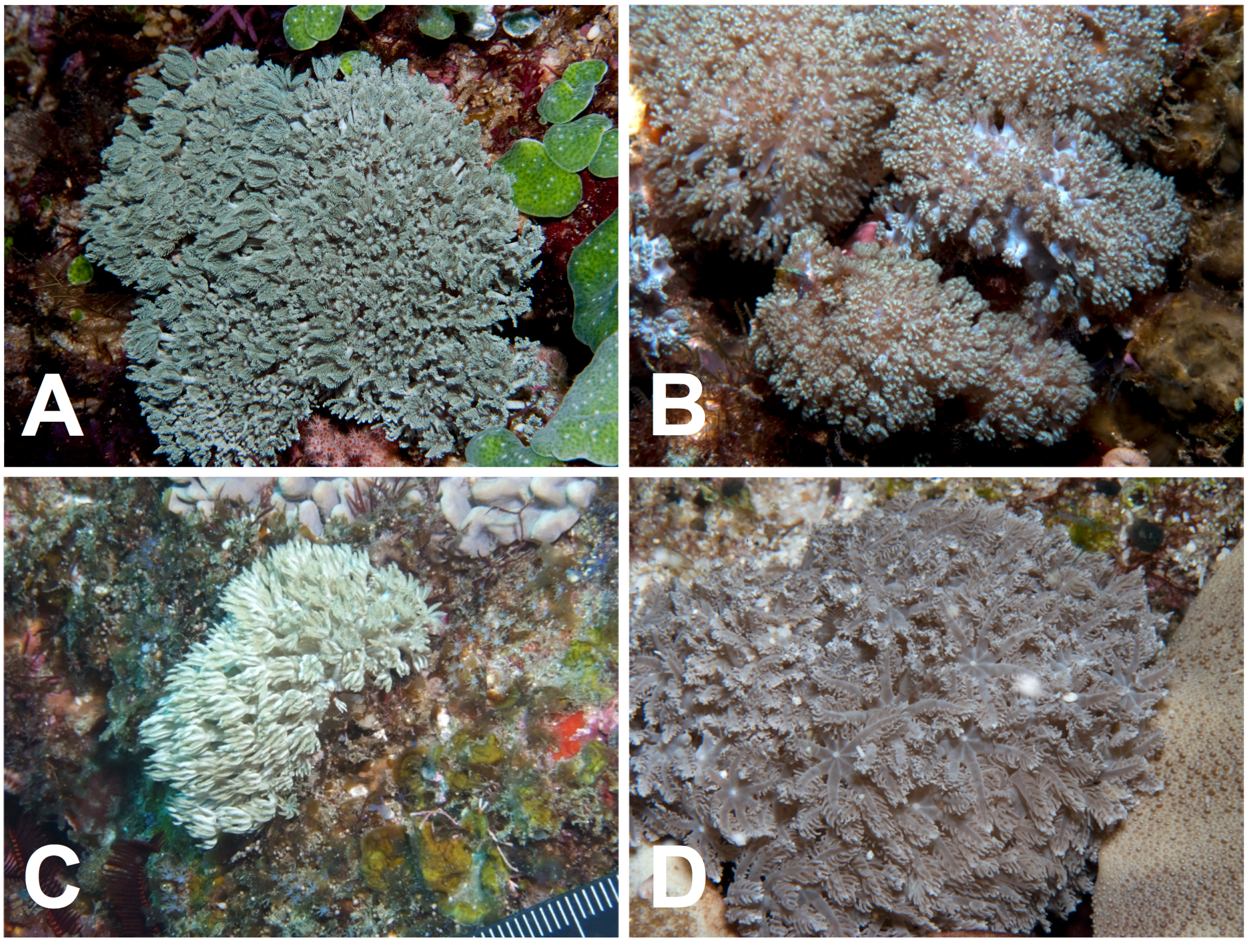


Figure 4.3 Colony photos of Alcyonacea (Family Xeniidae) from the iSimangaliso Wetland Park. A: *Xenia* sp.; B: *Sympodium caeruleum*; C: *Heteroxenia fuscescens*; D: *Anthelia glauca*.

4.4.2 New records of Alcyonacea genera from Northern KwaZulu-Natal, South Africa

The genera *Scleronephthya*, *Paraplexaura*, and *Chironephthya* collected in this study form new zoogeographic records and are reported for the first time in South Africa. Fragments of each specimen were collected by SCUBA from the iSimangaliso Wetland Park from over 30m depth by Dr. KJ Sink from the South African National Biodiversity Institute (SANBI) (Table 4.2). Specimens were stored in 70% ethanol for subsequent taxonomic analyses. Identifications to the genus-level were carried out according to the generic monograph by Fabricius & Alderslade (2001) and confirmed by a taxonomic expert, Dr Phil Alderslade. Species-level identifications were not possible at the time due to a lack of appropriate taxonomic revisions of the assigned genera (Benayahu et al. 2012).

4.4.2.1 New record of the genus *Scleronephthya* Studer, 1887; Tiger Reef, KwaZulu-Natal, South Africa

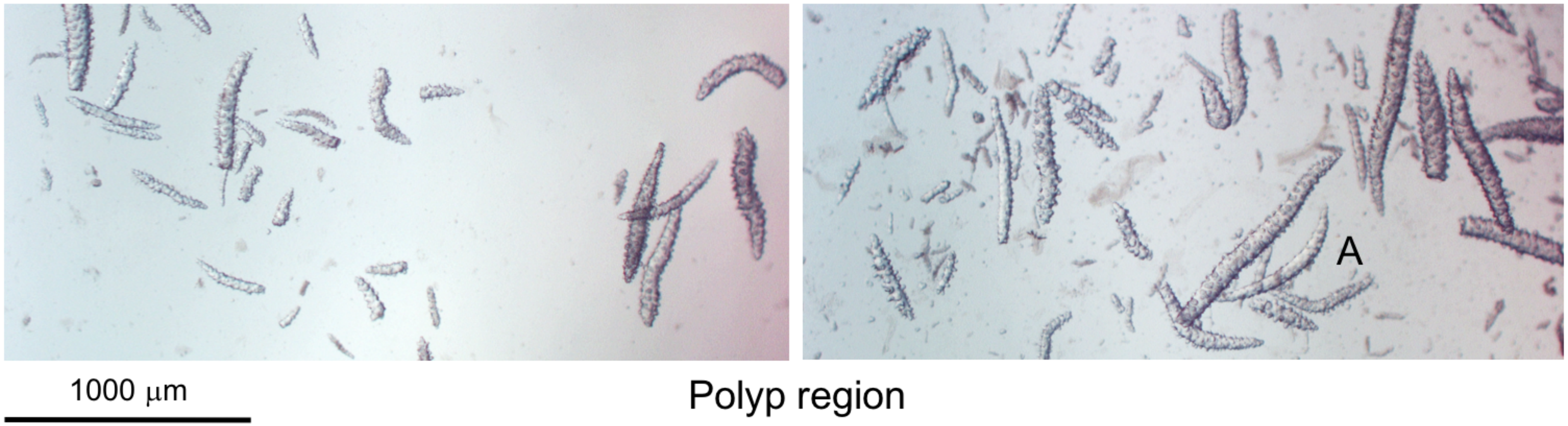
Subordinal group Alcyoniina
Family Nephtheidae Gray, 1862
Genus *Scleronephthya* Studer, 1887
Scleronephthya sp.

Material

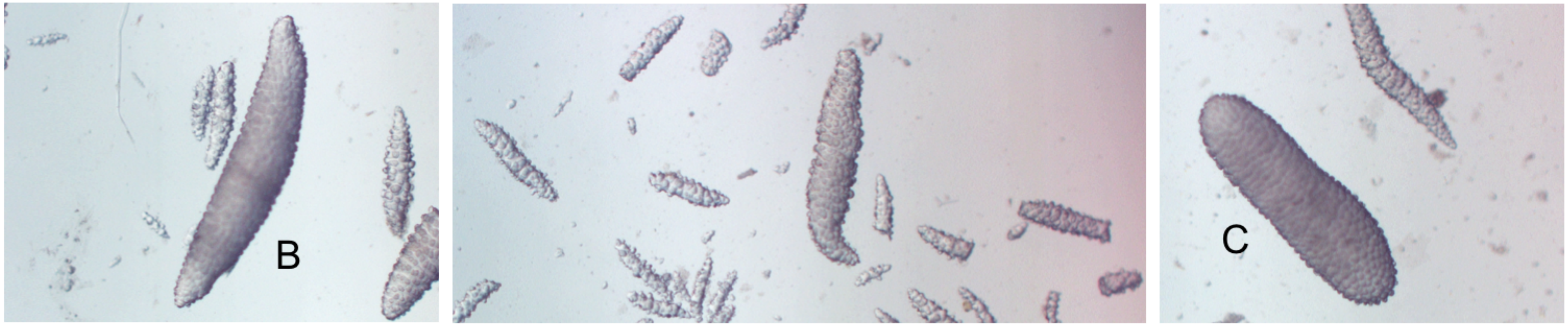
The colony was recorded and collected from Tiger Reef, Kosi Bay, iSimangaliso Wetland Park, KwaZulu-Natal, South Africa on the 24th April 2015 at Latitude -26.99188 and Longitude 32.90918 from a depth of 36m at a water temperature of 26°C (Table 4.2.)

Description

The colony is monomorphic, relatively small (<8cm), and with an arborescent appearance. The colony colour is pale pink (*in situ*) with red polyp mouths and is highly contractile (Figure 4.2 B). The colony forms a creamy white colour in alcohol. A closer examination of the sclerites and their relative distribution throughout the colony reveal typical characteristics of the genus (Figure 4.4). Point sclerites are clearly distinguishable in the polyp heads. The sclerites are colourless and the polyp region



Polyp region



Branch surface Base surface Base interior

Figure 4.4 Microscopic view of *Scleronephthya* sp. sclerites (40X). The polyp is mainly distinguished by sticks and spindles of varied size, some of which are curved (A). The remaining tissues have large spindles and cylinders with numerous rounded warts, a typical feature of the genus (B, C). Scale represents 1000 µm.

predominantly contains sticks and spindles of varied size (from 0.1mm to over 0.2mm) with some marginally club-like forms. A few spindles are large and curved, which is indicative of the pseudo-collaret formed when the polyp is contracted (Figure 4.4 A). Sclerites commonly found in the branch and base surface are conspicuously warty spindles (Figure 4.4 B) and the interior sclerites are cylindrical (Figure 4.4 C). All sclerites have numerous tall and rounded warts which are key diagnostic features of this genus (Fabricius & Alderslade 2001).

4.4.2.2 New record of the genus *Paraplexaura* Kuekenthal, 1909; Roonies Reef, Sodwana Bay, KwaZulu-Natal, South Africa

Subordinal group Holaxonia
Family Plexauridae Gray, 1859
Genus *Paraplexaura* Kuekenthal, 1909
Paraplexaura sp.

Material

The colony was recorded and collected from Roonies Reef, iSimangaliso Wetland Park, KwaZulu-Natal, South Africa on May 1st, 2015 at Latitude -27.5275 and Longitude 32.6920 from a depth of 33m at a water temperature of 26°C (Table 4.2).

Description

The specimen is an orange-red colour (*in situ*) and approximately 10cm height. The colony is bush-like in appearance though sparsely branched. The polyps are monomorphic and completely retractile with inconspicuous calyces. This genus belongs to the distinct subordinal group Holaxonia as it contains an internal axis made from gorgonin with a central, hollow, cross-chambered core and loculi (spaces filled with amorphous or fine crystalline calcium carbonate) (Fabricius & Alderslade 2001).

Examination of the sclerites and their relative distribution throughout the colony reveals typical characteristics of the genus (Figure 4.5). The sclerites are varied in form and orange in colour. The calyces contain thorn scales (Figure 4.5 A) and thick thorn scales (Figure 4.5 B). Sclerites are sparsely found in the polyp region and relatively small in the form of sticks and rods (from less than 0.1mm to over 0.2mm).

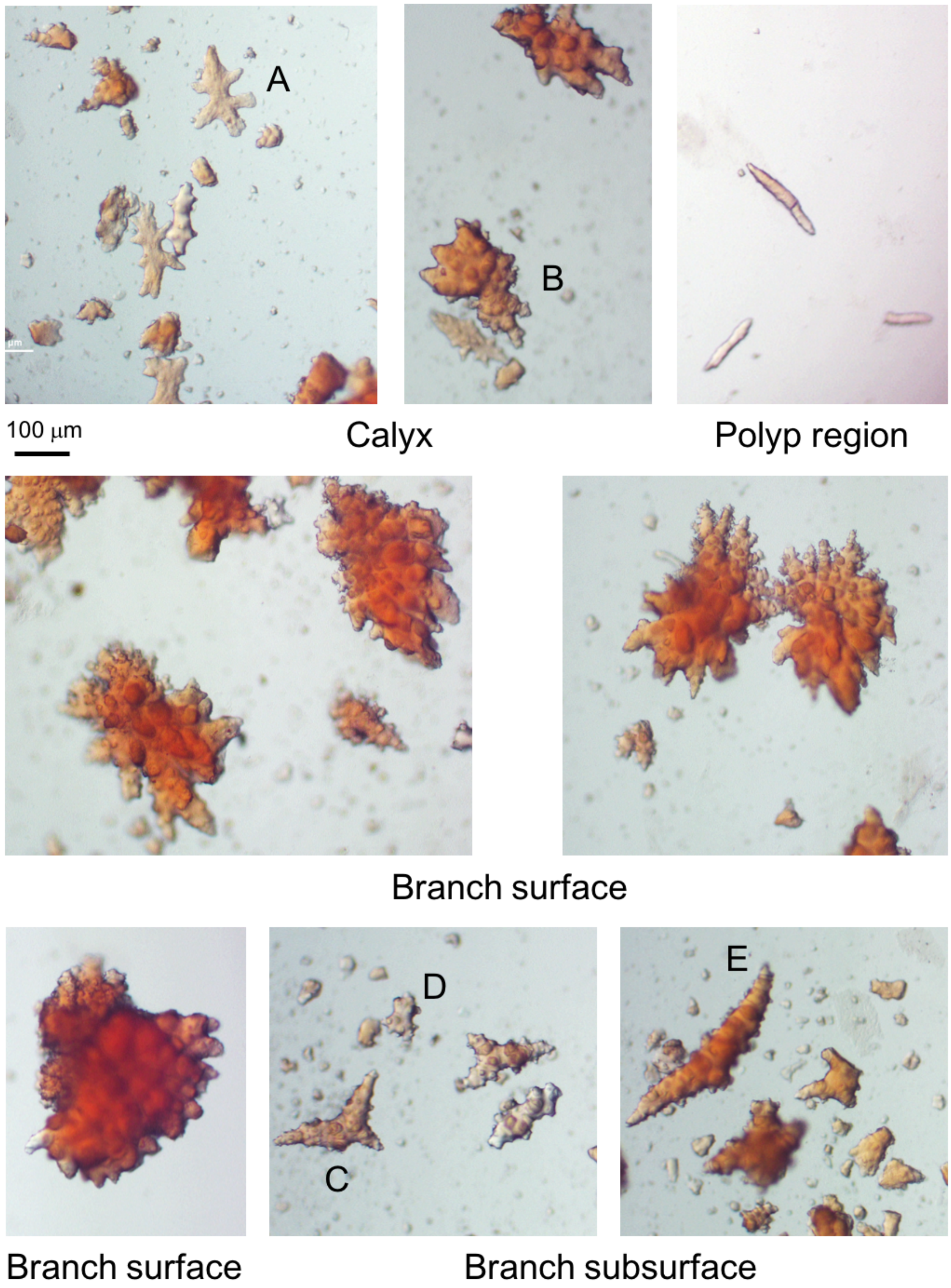


Figure 4.5 Microscopic view of *Paraplexaura* sp. colony sclerites (100X). Scale represents 100 μm. Sclerites from the calyx includes thorn scales (A) and thick thorn scales (B). The polyp region consists of sticks and rods; the branch surface and subsurface contains branched spindles (C), capstans (D) and warty spindles (E).

The branch surface contains large and varied unilateral, spiny and foliate spheroids with densely tuberculate bases which is a major diagnostic feature of the genus (Fabricius & Alderslade 2001). The subsurface of the branch contains branched warty spindles (Figure 4.5 C), capstans (Figure 4.5 D) and regular spindles (Figure 4.5 E). Species identification is unlikely without a thorough revision of the genus *Paraplexaura* (Samimi-Namin & van Ofwegen 2012, Williams & Gosliner 2014).

4.4.2.3 New record of the genus *Chironephthya* Gray, 1869; Gotham Reef, KwaZulu-Natal, South Africa

Subordinal group Alcyoniina
Family Nidaliidae
Genus *Chironephthya* Gray, 1869
Chironephthya sp.

Material

The colony was recorded and collected from Gotham Reef, near Rabbit Rock, iSimangaliso Wetland Park, KwaZulu-Natal, South Africa on May 1st, 2015 at Latitude -27.4919 and Longitude 32.6900 from a depth of 37m at a water temperature of 26°C.

Colony description and sclerites

The colony is monomorphic, highly branched and bush-like in appearance with prominent calyces. The colony is pale in colour (*in situ*), with a progressive orange colouring at the terminals of the branches (Figure 4.6 F). The extended polyps are white with pink-purple coloured tentacles and is retractile. The calyx sclerites are shaped in a split "Rabbit Ear-like" manner which is a distinguishing feature of this genus (Bayer 1981, Fabricius & Alderslade 2001). The point and collaret sclerites of the polyp are bright pink spindles, those of the collaret are curved (Figure 4.7). Sclerites from the colony surface are large, elongated and complex warty spindles of a brown colour (Figure 4.7). The interior of the colony contains short, narrow spindles and rods.

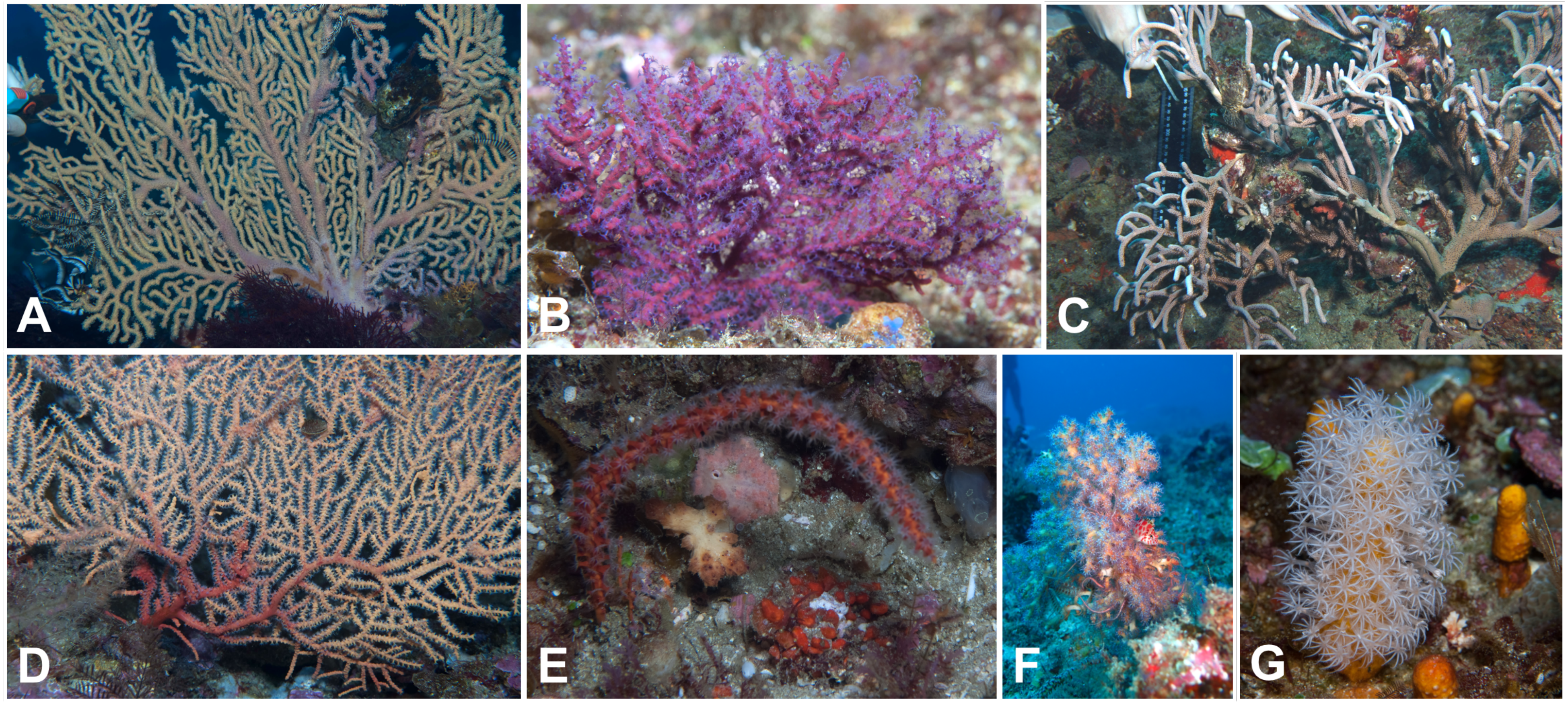


Figure 4.6 Colony photos of Alcyonacea from the iSimangaliso Wetland Park. Holaxonia subordinal group comprised of families Plexauridae (A: *Euplexaura* sp.) and Gorgoniidae (B: *Leptogorgia* sp., C: *Rumphella* sp.). Calcaxonia subordinal group: Ellisellidae (D: *Verrucella* sp., E: *Junceella* sp.). Alcyoniina family Nidaliidae (F: *Chironephthya* sp.). The family Parasphaerascleriidae (G: *Parasphaerasclera* sp.).

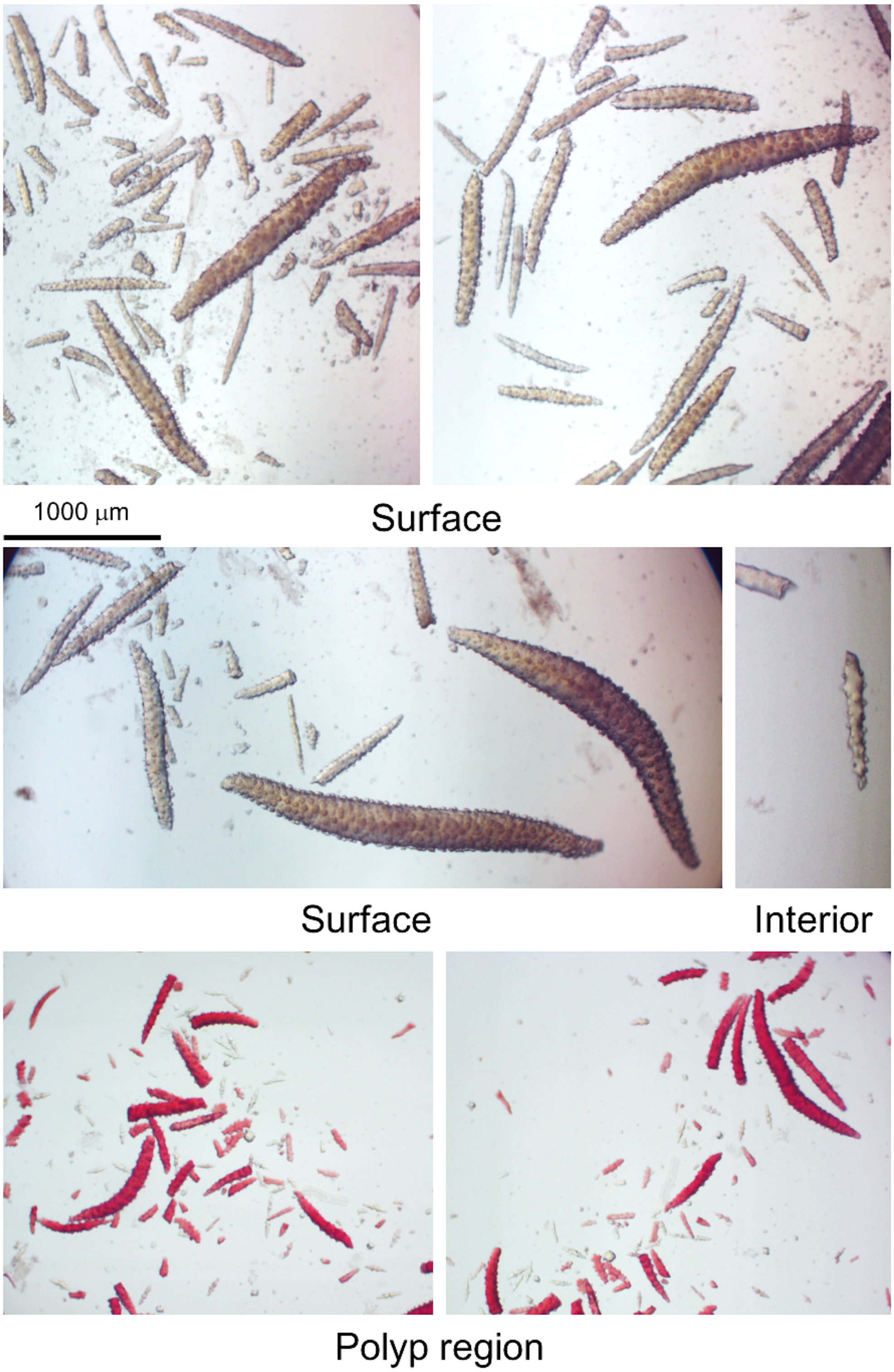


Figure 4.7 Microscopic view of *Chironephthya* sp. sclerites (40X). Scale represents 1000 µm. The surface is distinguished by large, elongated spindles. The interior typically has narrow spindles and rods and the polyp has spindles in a collaret and point configuration.

4.4.3 Molecular identifications and phylogenetic analyses

Inferences from the phylogenies of *MutS* (Figure 4.8) *28S rRNA* (Figure 4.9) and concatenated *MutS+28S* (Figure 4.10), in combination with morphological data and photographs, yielded ~61 species from the collection. Seventy-eight specimens were identified to 19 known species whereas the remaining 161 specimens were assigned to ~42 putative species (Table 4.2). Several specimens in the collection comprised 9 of the 42 species that require expert identification and form existing records i.e. *X. crassa*, *X. garciae*, and *X. kuekenthali*. These species are not clearly phylogenetically separated (Figures 4.8, 4.9 and 4.10) and therefore cannot be delineated to species-level. The remainder of the unidentified specimens were assigned to ~33 putative species which are considered new records that are previously not known to occur in the iSWP or may possibly represent new taxa that require taxonomic description, either of which cannot be ascertained without further taxonomic investigation (Table 4.2). The total number of putative species per genera included *Sinularia* (22); *Lobophytum* (5); *Sarcophyton* (4); *Cladiella* (1); Beta (3); *Aldersladum* (1); *Xenia* (2); *Heteroxenia* (1); *Sansibia* (2); *Sympodium* (1); *Anthelia* (1); *Dendronephthya* (3); *Stereonephthya* (4); *Scleronephthya* (1); *Litophyton* (1); *Chironephthya* (1); *Verrucella* (1); *Junceella* (1); *Rumphella* (1); *Leptogorgia* (1); *Parasphaerasclera* (2); *Euplexaura* (1); and *Paraplexaura* (1) (Table 4.2).

Analyses of the *MutS* (Figure 4.8)*, 28S rRNA* (Figure 4.9) and concatenated *MutS+28S* (Figure 4.10) loci recovered phylogenies that were mostly congruent with one another. The major phylogenies (Figures 4.8, 4.9 and 4.10) exhibited collapsed clades for several Alcyoniina families that were represented as separate subtrees (Figures 4.11, 4.12 and 4.13) in order to facilitate readability. The evolutionary relationships demonstrated among genera, families and subordinal groups across the gene regions were largely consistent with previous studies (McFadden, France, et al. 2006, McFadden & van Ofwegen 2012, 2013a, Cairns & Wirshing 2015, Quattrini et al. 2017). Polyphyletic clusters of the families Alcyoniidae and Gorgoniidae were found throughout all major phylogenies (Figures 4.8, 4.9 and 4.10) and resulted in a mixed clade of Holaxonia-Alcyoniina (HA) subordinal groups that corresponded to previous reports by McFadden, France, et al. (2006). Calcaxonia was recovered as a monophyletic subordinal group that fell outside of the HA clade for all phylogenies. The family Parasphaerascleridae also fell outside of the HA clade, consistent with the

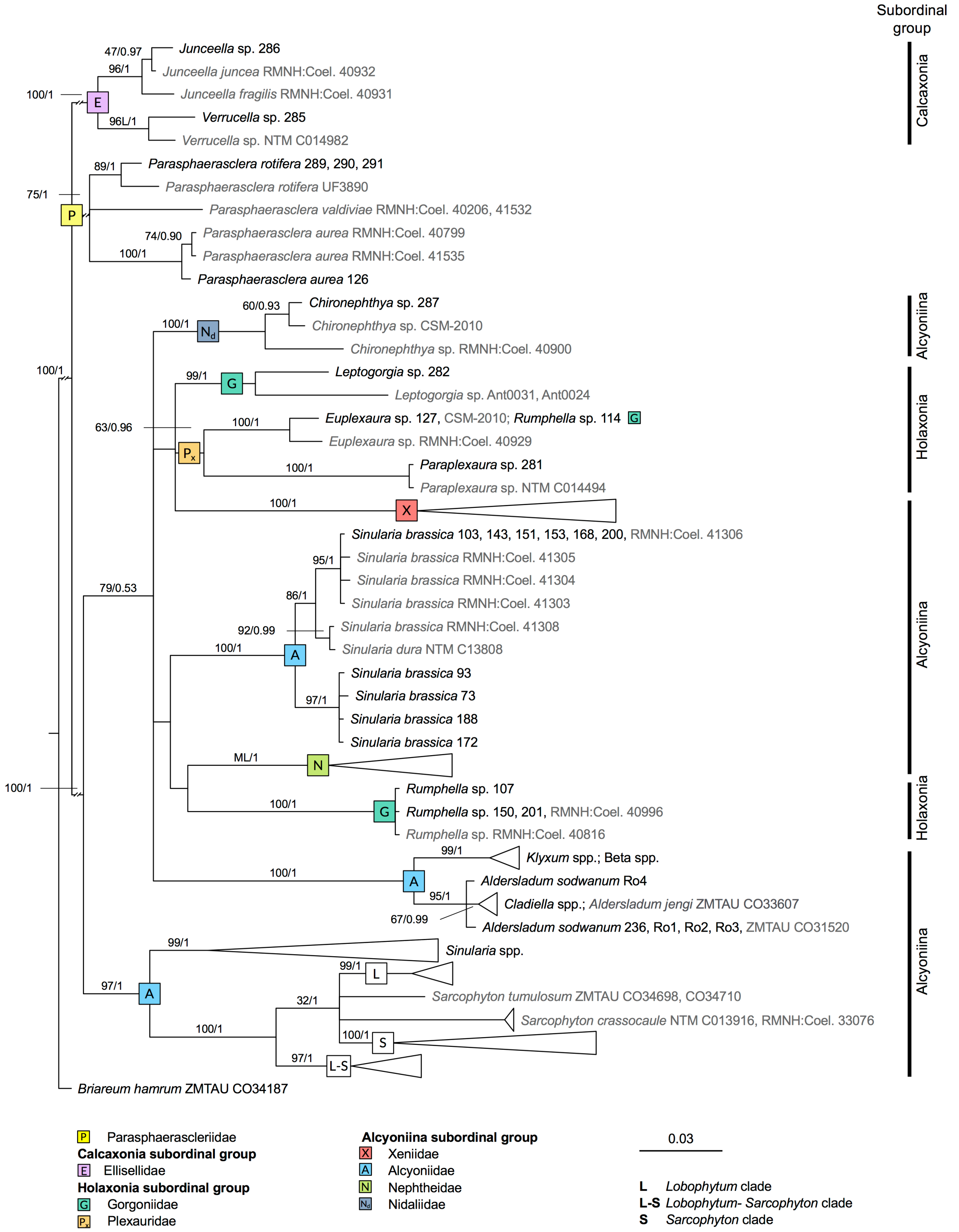


Figure 4.8 Maximum likelihood tree of Alcyonacea based on bacterial MutS homologue (*MutS*) sequence data. Octocoral families are colour-coded and their respective subordinal groups indicated by vertical bars. Larger clades were collapsed to facilitate readability. *Sarcophyton* and *Lobophytum* clade labels correspond to clades discussed in McFadden, Alderslade, et al. (2006). Branch length scales are given; branch labels indicate bootstrap support values and posterior probabilities respectively. GenBank sequences are denoted in grey.

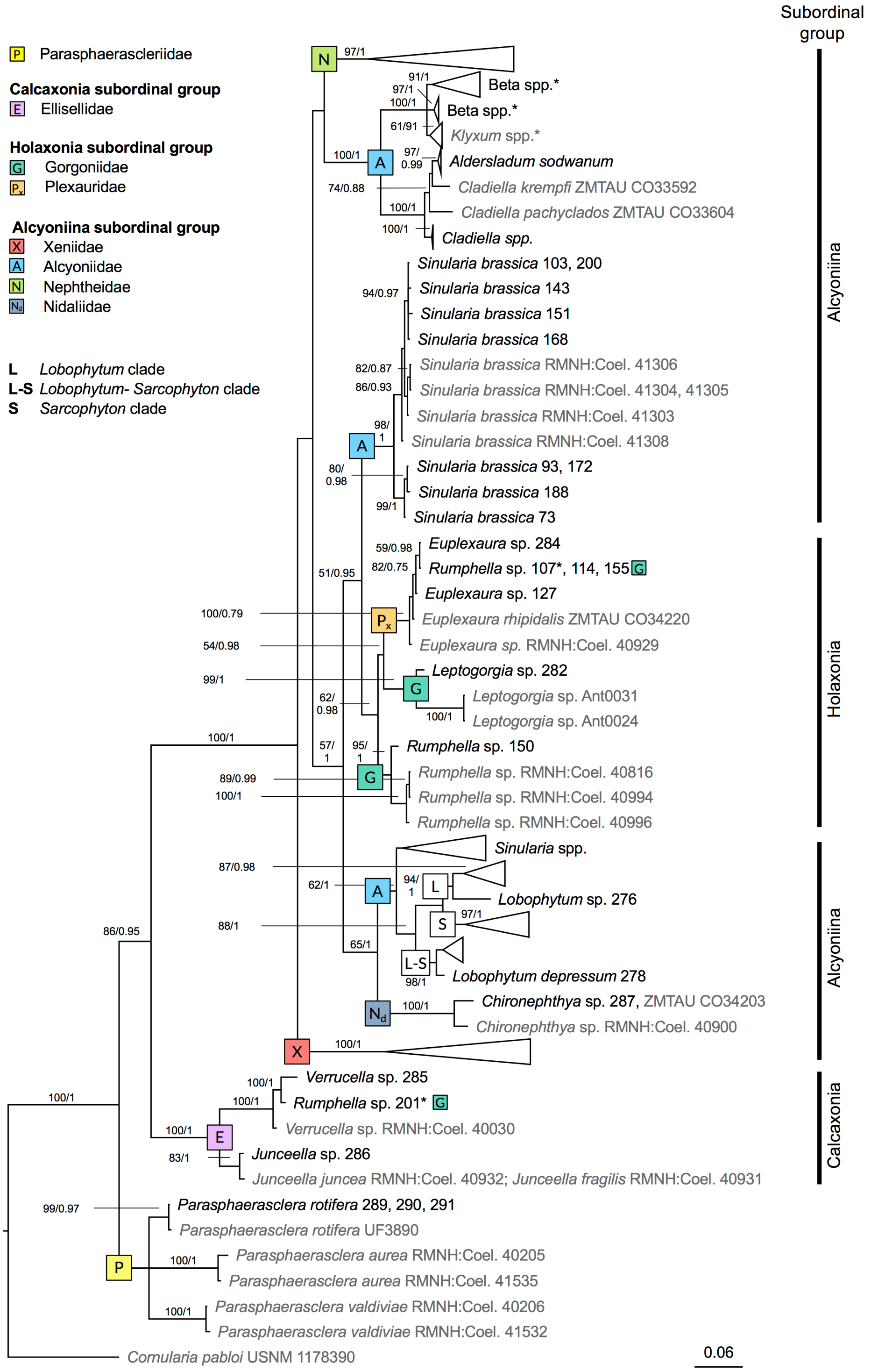


Figure 4.9 Maximum likelihood tree of Alcyonacea based on nuclear 28S ribosomal RNA (*28S rRNA*). Octocoral families are colour-coded and their respective subordinal groups indicated by vertical bars. Large clades were collapsed to improve intelligibility. *Sarcophyton* and *Lobophytum* clade labels correspond to clades discussed in McFadden, Alderslade, et al. (2006). Branch length scales are given; branch labels indicate bootstrap support values and posterior probabilities respectively. GenBank sequences are denoted in grey. Asterisks indicate taxa or clades inconsistent with the *MutS* phylogeny.

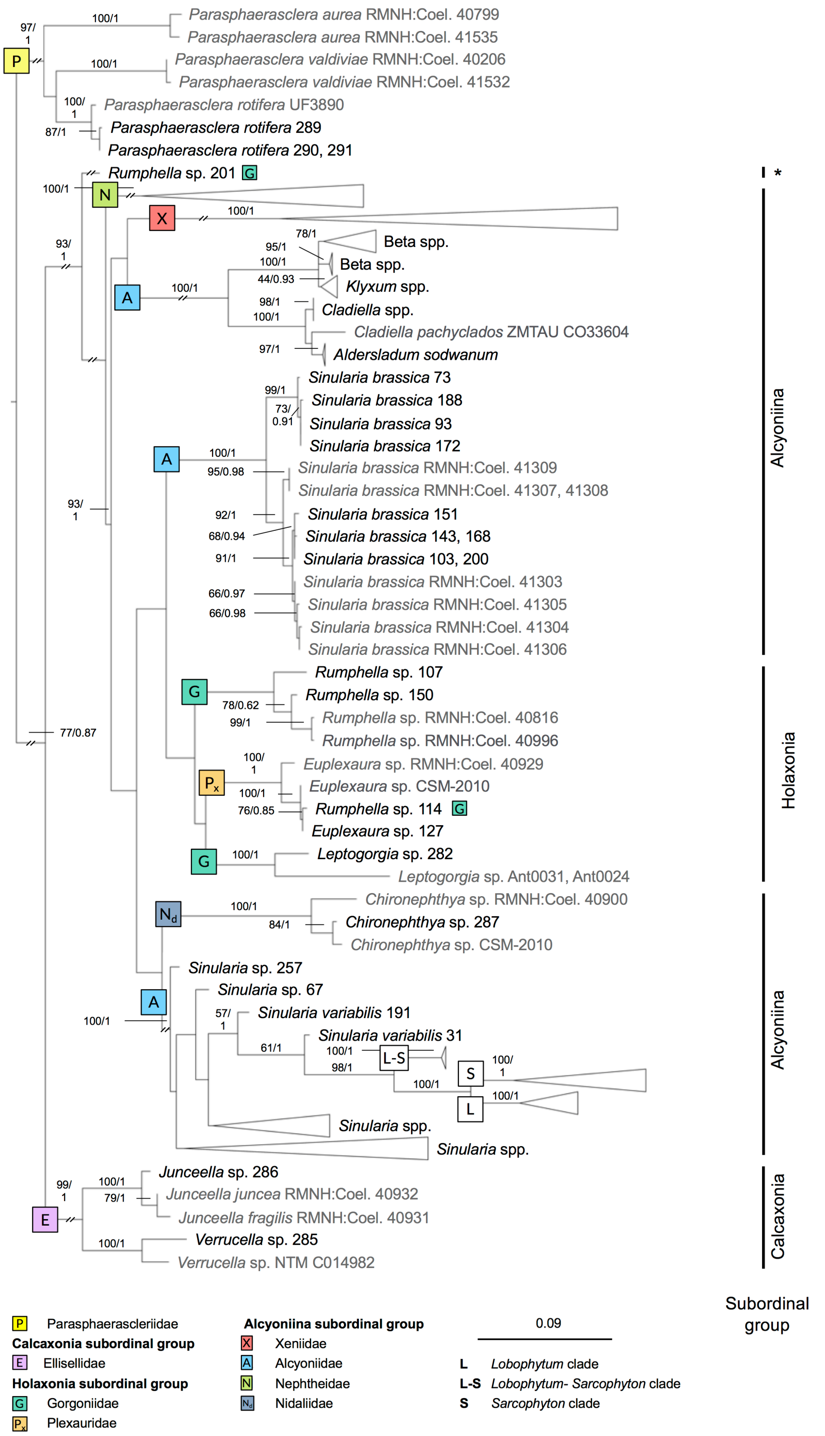


Figure 4.10 Maximum likelihood tree of Alcyonacea based on concatenated bacterial MutS homologue (*MutS*) sequence data and nuclear 28S ribosomal RNA (*28S rRNA*). Octocoral families are colour-coded and their respective subordinal groups indicated by vertical bars. Major clades were collapsed to improve intelligibility. *Sarcophyton* and *Lobophytum* clade labels correspond to clades discussed in McFadden, Alderslade, et al. (2006). Branch length scales are given; branch labels indicate bootstrap support values and posterior probabilities respectively. GenBank sequences are denoted in grey. The asterisk indicates the Holaxonia group.

fact that it has no designated subordinal group (McFadden & van Ofwegen 2013a). The remaining families were recovered as monophyletic clades across all phylogenies which was consistent with previous studies of these loci within these taxa (McFadden, France, et al. 2006, Benayahu et al. 2012, McFadden & van Ofwegen 2013a, b, Haverkort-Yeh et al. 2013, McFadden, Reynolds, et al. 2014, Cairns & Wirshing 2015). However, inferences made regarding the monophyly of these families or genera were limited by taxon sampling, as the phylogeny only includes taxa sampled from the study region and not all species or genera belonging to a family.

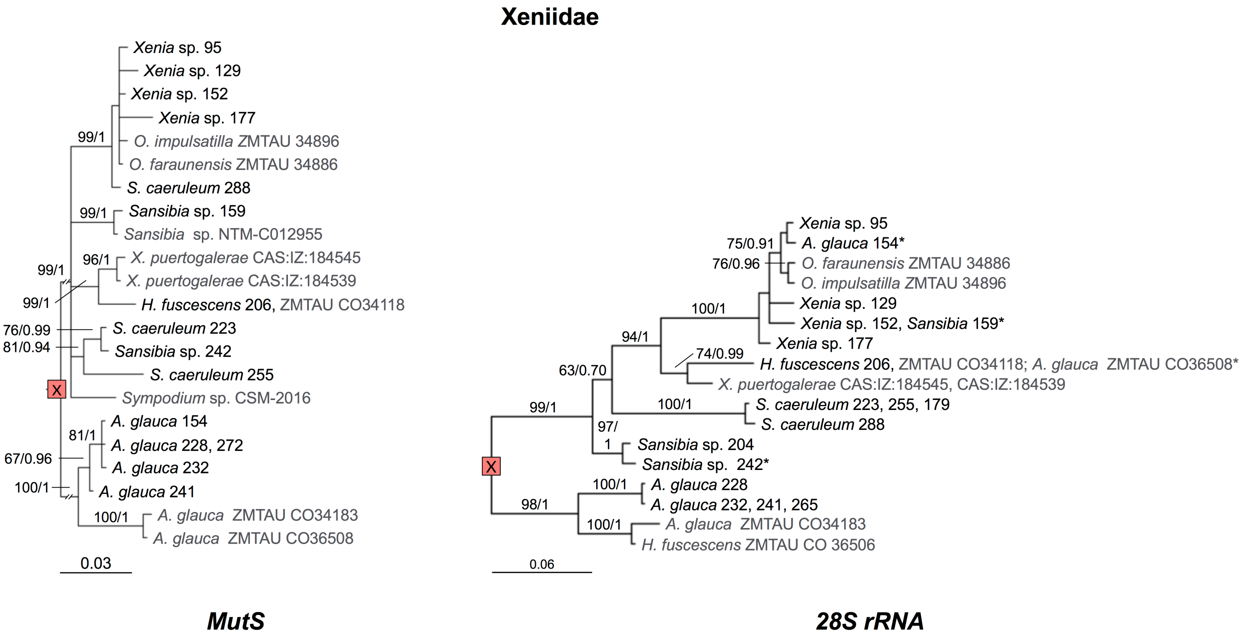
Parasphaerascleriidae, a monogeneric family comprised of the genus *Parasphaerasclera*, revealed significant species delineation across all gene regions (Figures 4.8, 4.9 and 4.10) as per previous reports by McFadden & van Ofwegen (2013a). However, sequences from South African samples of *P. rotifera* and *P. aurea* did not share haplotypes with their respective GenBank conspecifics. The genera *Junceella* and *Verrucella*, both representatives of shallow water Calcaxonia, were both monophyletic in the major phylogenies (Figures 4.8, 4.9 and 4.10). Species inferences were challenging with few available representatives of *Verrucella* and a lack of species assignments. However, *MutS* and *MutS+28S* (Figures 4.8 and 4.10) genetically differentiated between species of *Junceella* whereas *J. juncea* and *J. fragilis* exhibited a shared haplotype in the *28S rRNA* phylogeny (Figure 4.9).

The families Plexauridae and Gorgoniidae of the Holaxonia subordinal group, were recovered amongst Alcyoniina across all datasets (Figures 4.8, 4.9 and 4.10). *Paraplexaura*, *Euplexaura* and *Leptogorgia* formed well-supported monophyletic clades. *Rumphella* was polyphyletic within all phylogenies comparable to studies by McFadden, France, et al. (2006) and Mcfadden & van Ofwegen (2017). Species of *Rumphella* were distributed among the genus *Euplexaura* and outside of the clade of taxa that form the bulk of the Holaxonia group. Additionally, *Rumphella* was recovered in the Calcaxonia clade for *28S rRNA* (Figure 4.9).

Most of the taxa collected in this study were members of the Alcyoniina group, which was the most widespread group in the iSWP. Species of *Chironephthya* (Nidaliidae) were recovered in a monophyletic clade (Figures 4.8, 4.9 and 4.10) in which an unidentified South African taxon shared a haplotype with an unidentified GenBank sequence for the *28S rRNA* gene region (Figure 4.9). Alcyoniidae was the largest and most prevalent family in all phylogenies. Two distinct clades of closely related alcyoniid genera consisting of (1) *Aldersladum* and *Cladiella*, and (2) Beta and *Klyxum*, were recovered across the major phylogenies (Figures 4.8, 4.9 and 4.10). *Klyxum* sequences were sourced from Genbank as there is no record of these taxa from South Africa. The respective genera were all monophyletic for gene regions *28S rRNA* (Figure 4.9) and *MutS+28S* (Figure 4.10) whereas little generic resolution was provided by mitochondrial *MutS* for Beta and *Klyxum*. Both genera displayed paraphyly (Figure 4.8) and species of *Klyxum* shared haplotypes with Beta specimens (see Chapter 2 for more details).

Sinularia (with the exception of *S. brassica*), *Lobophytum* and *Sarcophyton* are closely related genera and were consistently recovered on the same alcyoniid clade, comparable to previous studies by Benayahu et al. (2012) and McFadden & van Ofwegen (2013a). The genus *Sinularia* was polyphyletic given that all sequences of *Sinularia brassica* formed a separate, well-supported monophyletic clade outside of the remaining *Sinularia* taxa (Figures 4.8, 4.9 and 4.10). The species, *S. brassica*, was separated by an average genetic distance of 9.3% (uncorrected *p*) from the remaining congeners across all loci (Appendix C Table 7.10, 7.11 and 7.12). Furthermore, two morphotypes of *S. brassica* from within the collection formed two distinct clades that were genetically differentiated, suggestive of two separate species. *S. brassica* specimens 103, 143, 151, 153, 168 and 200 were recovered in the same clade across all phylogenies and exhibited digitate growth forms whereas the remaining *S. brassica* specimens 73, 93, 172 and 188 that displayed lobate, encrusting growth forms were recovered in a separate clade (Figures 4.8, 4.9 and 4.10). The remaining *Sinularia* species were recovered in subclades that were congruent with the systematic clades defined in McFadden et al. (2009) which were consequently collapsed throughout the major phylogenies for convenience (see Chapter 3 for in depth analyses and species inferences). *Sinularia* specimens collected in this study yielded 6 known species: *S. brassica*, *S. variabilis*, *S. heterospiculata*, *S. notanda* and *S. abrupta*, and a new zoogeographic record for *S. grandilobata*. The remaining specimens were assigned to 16 putative species that either form new records that require expert identification or new taxa that require taxonomic description.

Xeniidae was recovered as a well-supported monophyletic family throughout all phylogenies and consistent with previous studies (McFadden, Reynolds, et al. 2014). *Anthelia* was among two of the monophyletic genera recovered in the xeniid *MutS* phylogeny (Figure 4.11).



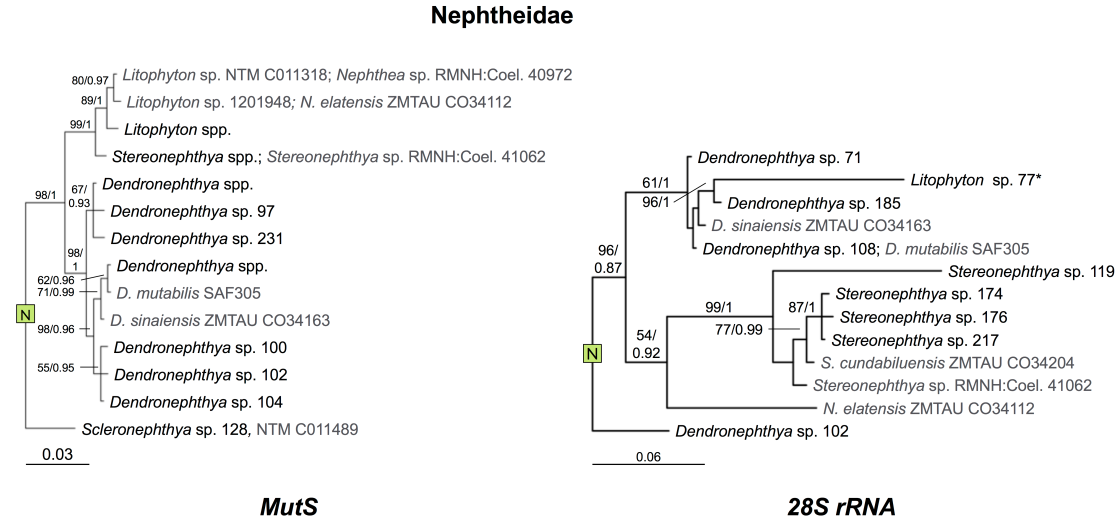


Figure 4.11 Maximum likelihood trees of bacterial MutS homologue (*MutS*) and 28S ribosomal RNA (*28S rRNA*) of the Alcyoniina families Xeniidae and Nephtheidae. GenBank sequences are represented in grey, branch length scales are given; branch labels indicate bootstrap support values and posterior probabilities respectively.

However, mitochondrial and nuclear discord was evident in that *Anthelia* exhibited polyphyly in the *28S rRNA* phylogeny (Figure 4.11) and *MutS+28S* (Figure 4.13) where sequences of *A. glauca* from South Africa (*A. glauca* 154) and from GenBank were distributed amongst the genera *Xenia* and *Heteroxenia* respectively. Furthermore, a GenBank sequence of *A. glauca* shared a haplotype with the GenBank sequence for *Heteroxenia fuscescens* in *28S rRNA* (Figure 4.11). *Xenia* was recovered as a polyphyletic genus in that several species were distributed amongst the genera *Ovabunda* and *Heteroxenia* for all gene regions, like that of previous studies by Janes & Mary (2012), Haverkort-Yeh et al. (2013), Stemmer et al. (2013) and McFadden, Reynolds, et al. (2014). *Xenia* morphotypes were not distinguished by phylogenetic differences. *Heteroxenia* was monophyletic for *MutS* (Figure 4.11), which is likely an artefact of the few sequences included in mitochondrial analyses. *Heteroxenia* was polyphyletic throughout *28S rRNA* (Figure 4.11) and *MutS+28S* (Figure 4.13), which is consistent with previous studies (Janes et al. 2014, McFadden, Reynolds, et al. 2014). *Sympodium caeruleum* was paraphyletic for *MutS* (Figure 4.11) and monophyletic for *28S rRNA* (Figure 4.11) and *MutS+28S* (Figure 4.13). *S. caeruleum* 288 was consistently differentiated from other conspecifics throughout the phylogenies. *Sansibia* sp. 242, a pink coloured morphotype, did not share haplotypes with other *Sansibia* sequences and displayed polyphyly in both the *MutS* and *MutS+28S* phylogenies (Figure 4.11 and 4.13). The remaining two *MutS* sequences of *Sansibia* sp. were recovered in a well-supported clade in which the South African sequence was distinct from the GenBank sequence. However, *Sansibia* sp. 159 appeared to be in an unresolved relationship with *Xenia* in the nuclear and concatenated phylogenies and was consequently polyphyletic (Figure 4.11 and 4.13). Furthermore, the sequence shared a haplotype with *Xenia* sp. 152 for *28S rRNA*.

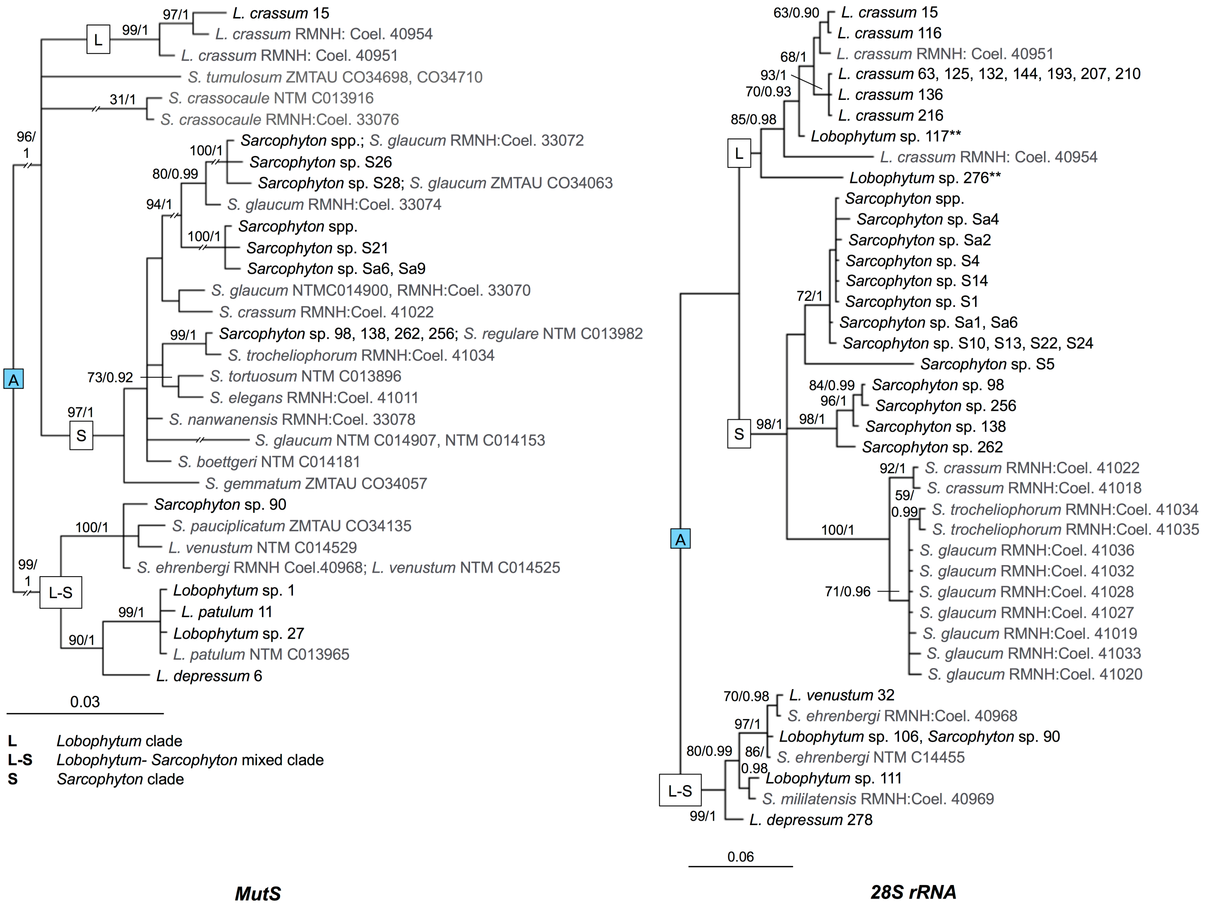
Phylogenies of the family Nephtheidae can be found in Figure 4.11 and Figure 4.13 for all gene regions. Similar to previous studies by McFadden et al. (2011) and Haverkort-Yeh et al. (2013), genera within these phylogenies are generally monophyletic barring *Nephthea* and *Litophyton*. *Scleronephthya* was monophyletic for *MutS* (Figure 4.11) and formed an outer clade to a clade consisting of more closely related genera that included *Dendronephthya*, *Litophyton*, *Nephthea* and *Stereonephthya*, consistent with previous reports for this gene region (McFadden et al. 2011). No *28S rRNA* sequences were available for *Scleronephthya*. *Litophyton* and *Nephthea* were nested in the same clade with shared haplotypes between the two genera and were paraphyletic for *MutS*. However, this was not the case for the nuclear and concatenated phylogenies, wherein few sequence representatives were available and *Litophyton* was nested in *Dendronephthya*. *Stereonephthya* was monophyletic for all phylogenies (Figure 4.11 and 4.13) and its morphotypes were not distinguished by phylogenetic differences. *Dendronephthya* was monophyletic in the *MutS* and concatenated phylogeny and paraphyletic in the nuclear phylogeny (Figure 4.11, Figure 4.13). Morphotypes of *Dendronephthya* were not genetically distinct from one another.

The genera *Lobophytum* and *Sarcophyton* were recovered in three well defined paraphyletic clades across all gene regions (Figure 4.12 and 4.13) that paralleled those discussed in McFadden, Alderslade, et al. (2006). Species recovered in either of these clades exhibited the typical morphological features of their respective genera (McFadden, Alderslade, et al. 2006). A third clade (L-S), exhibited a combination of nominal species of *Lobophytum* and *Sarcophyton* with intermediate morphological generic features as defined in McFadden, Alderslade, et al. (2006). *S. tumulosum* and *S. crassocaule* fell outside of the three sub clades (Figure 4.12 *MutS*) as was previously noted by McFadden, Alderslade, et al. (2006) and Benayahu et al. (2012).

All sequences of *Lobophytum crassum* (Figure 4.14 A) were recovered in the *Lobophytum* (L) clade for all datasets in which *L. crassum* is the only species previously recorded from the iSWP to feature in this clade (Table 4.1). None of the *L. crassum* sequences shared haplotypes in the *MutS* or *MutS+28S* phylogenies (Figure 4.12 and Figure 4.13), however, several taxa from South Africa shared haplotypes in the nuclear phylogeny, *28S rRNA* (Figure 4.12). In addition to *L. crassum*, two unidentified species of *Lobophytum* were recovered in the L clade for *28S rRNA* (Figure 4.12) whereas no sequences were obtained for their *MutS* counterparts. *Lobophytum* sp. 117 and 276, assumed to be conspecifics that form a new record of *Lobophytum* in South Africa (Figure 4.14 B, C). These paraphyletic taxa were distributed among species of *L. crassum* in the L clade for *28S rRNA*.

Molecular phylogenies did not exhibit species delineation within the *Sarcophyton* (S) clade nor the mixed L-S clade for the *MutS* subtree (Figure 4.12). *MutS* sequences of the South African *Sarcophyton* specimens were distributed amongst various

Sarcophyton sequences taken from GenBank (Figure 4.12). Unidentified specimens of *Sarcophyton* sp. S1-S29 and Sa1-Sa9 from South Africa are considered to be a mixture of either *S. glaucum*, *S. trocheliophorum* or *S. crassum* and constitute species that form previous records from this region and that fall within the S clade (Table 4.1). The nuclear and concatenated phylogenies (Figures 4.12 and 4.13) provided greater taxonomic resolution in the S clade wherein GenBank sequences of *S. glaucum*, *S. trocheliophorum* and *S. crassum* were recovered in distinct clades and were also separated from South African specimens. Unlike *MutS*, *28S rRNA* and *MutS+28S* subtrees did not exhibit shared haplotypes between sequences from GenBank and South African taxa.



MutS ***28S rRNA***

Figure 4.12 Maximum likelihood trees of bacterial MutS homologue (*MutS*) and 28S ribosomal RNA (*28S rRNA*) for alcyoniid genera *Lobophytum* and *Sarcophyton*. GenBank sequences are represented in grey. *Sarcophyton* and *Lobophytum* clade labels correspond to clades discussed in McFadden, Alderslade, et al. (2006). Branch length scales are given; branch labels indicate bootstrap support values and posterior probabilities respectively.

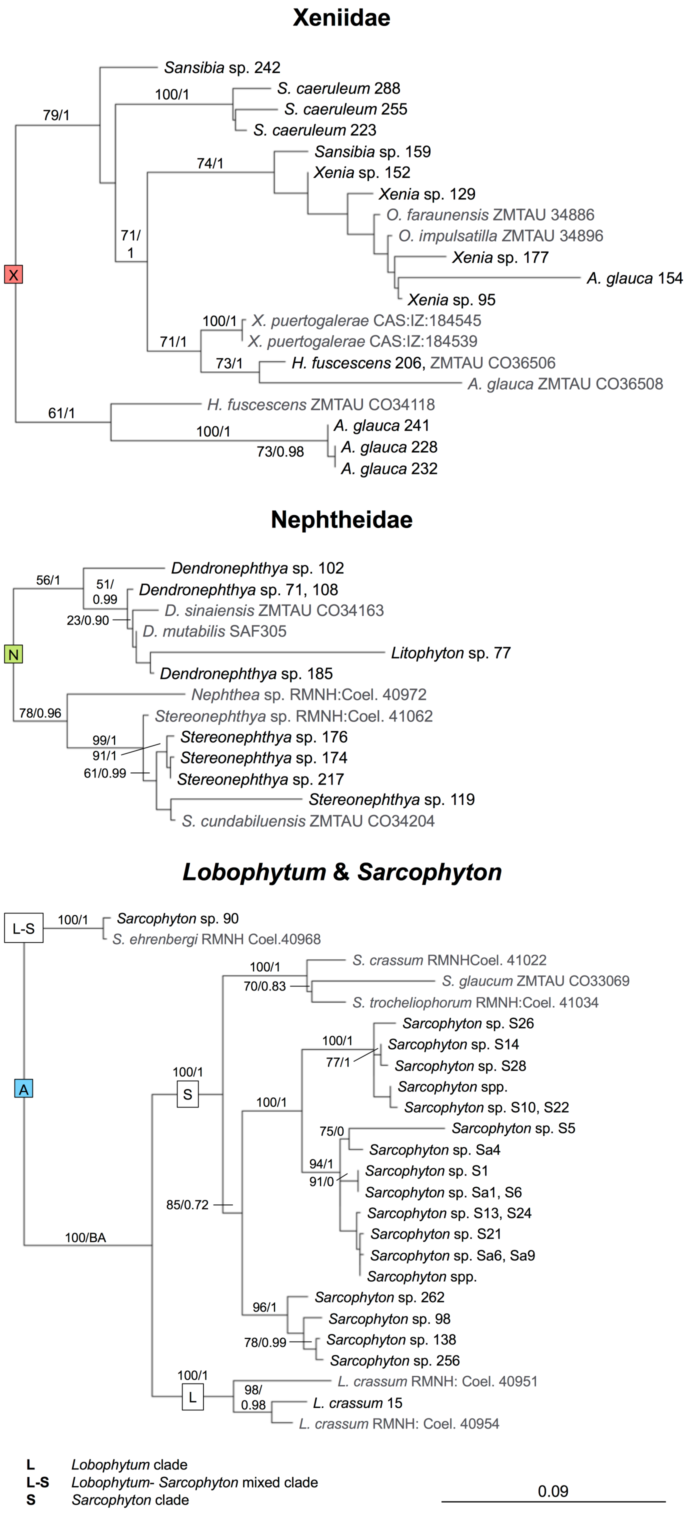


Figure 4.13 Maximum likelihood trees of bacterial MutS homologue (*MutS*) and 28S ribosomal RNA (*28S rRNA*) for alcyoniid genera *Lobophytum* and *Sarcophyton*. GenBank sequences are represented in grey. *Sarcophyton* and *Lobophytum* clade labels correspond to clades discussed in McFadden, Alderslade, et al. (2006). Branch length scales are given, branch labels indicate bootstrap support values and posterior probabilities respectively.

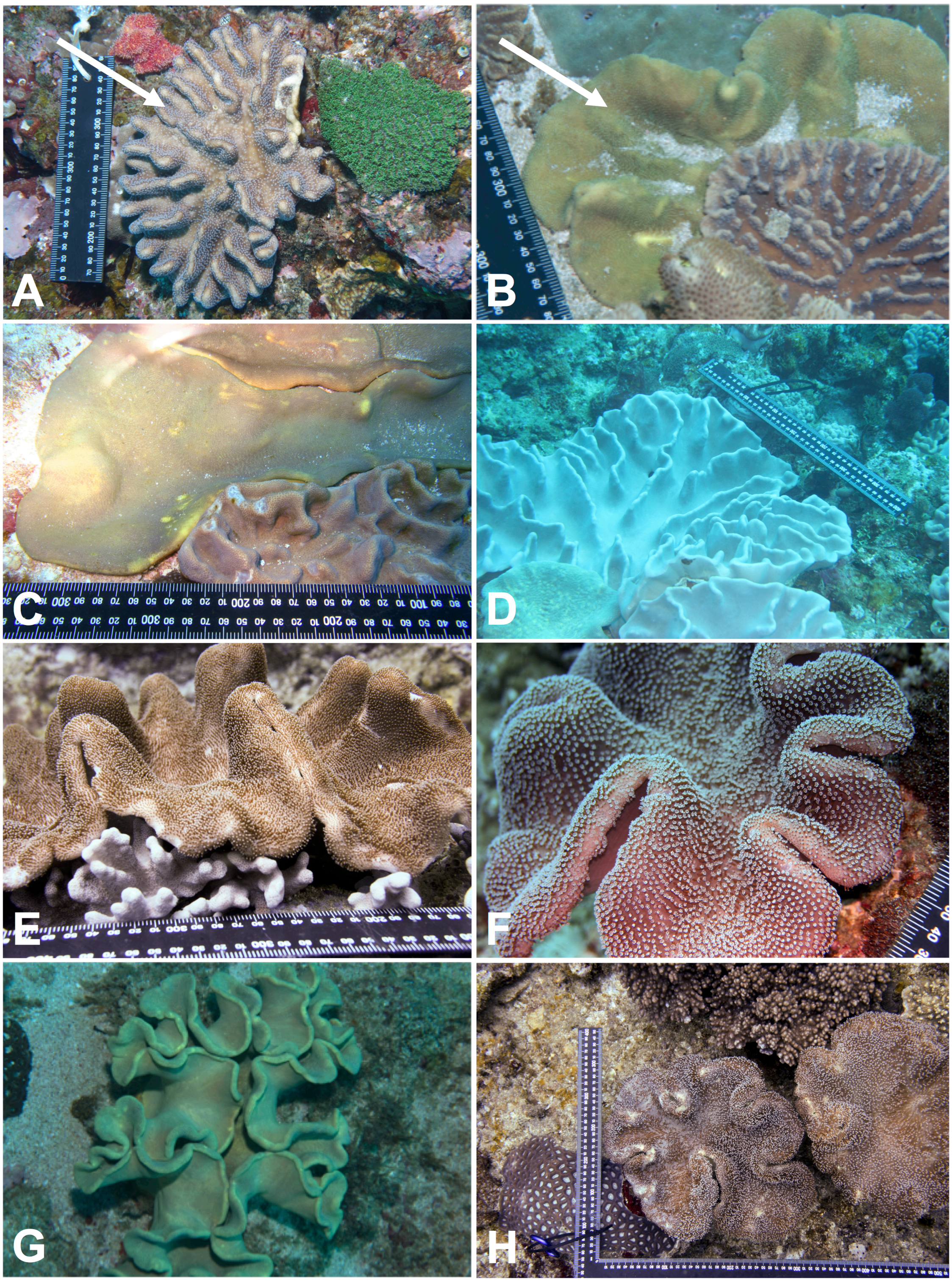


Figure 4.14 Colony photos of *Sarcophyton* and *Lobophytum* from iSimangaliso Wetland Park. A: *Lobophytum crassum* (L clade); B-C: *Lobophytum* sp. (L clade). D: *Lobophytum* sp. (L-S clade), E: *Lobophytum* sp. (L-S clade); F-H: *Sarcophyton* spp. (S clade).

Throughout all datasets (Figures 4.12 and 4.13), *Sarcophyton* sp. 98, 138, 256 and 262 formed a separate subclade (with significant support) from the remaining South African taxa that more typically resembled colonies of *S. glaucum*, *S. trocheliophorum* and *S. crassum* (Figure 4.14 H). Unlike other species of *Sarcophyton* that are typically brown and yellow in colour, *Sarcophyton* sp. 98, 138 and 256 exhibited colonies that were brown and purple in colour and are likely conspecific (Figure 4.14 F). *Sarcophyton* sp. 262, however, was bright yellow in colour with highly folded margins and a short stalk (Figure 4.14 G). It is possible that these taxa form two new records of *Sarcophyton* for the iSWP.

The *Lobophytum*-*Sarcophyton* mixed clade (L-S) exhibited a combination of *Lobophytum* and *Sarcophyton* taxa with intermediate morphologies (Figures 4.12 and 4.13). The gene regions did not delimit species and revealed haplotypes that were shared between the two genera throughout *MutS* and *28S rRNA* phylogenies (Figure 4.12). Four species of *Lobophytum* previously recorded from South Africa: *L. patulum, L. venustum*, *L. latilobatum* and *L. depressum*, fell within the L-S clade for all datasets. *Sarcophyton* sp. 90 was consistently recovered in the mixed L-S clade whereby, based on its position in the clade and colony features, it could be either one of the two species that occur in the region, namely *S. ehrenbergi* or *S. flexuosum*, though further taxonomic investigation would be required to ascertain this.

4.4.4 Genetic divergence and comparison of molecular markers

The number of base substitutions per site averaging over all sequence pairs within families are shown for each barcode region in Table 4.4. The average genetic distances (uncorrected *p*) for all gene regions within families were greatest for Alcyoniidae (7.7%) followed by Xeniidae and Gorgoniidae (5.8%), Parasphaerascleridae (3.8%), Nephtheidae (3.2%) Ellisellidae (3%), Plexauridae (1.7%), and Nidaliidae (1.4%). The little variation found within the latter three families is an artefact of the few records of representative taxa from this region. Uncorrected pairwise sequence divergence between families for each marker can be found in Table 4.5. Interfamily distances ranged from 6.4% to 18,0% for *MutS*, 5.6% to 18.1% for *28S* and 6.4% to 17.2% for *MutS*+*28S*. On average, the most distant families were Ellisellidae and Xeniidae whereas the closest families were Gorgoniidae and Plexauridae.

Table 4.4 Estimates of evolutionary divergence (uncorrected *p*) over sequence pairs within families for bacterial MutS homologue (*MutS*), 28S ribosomal RNA (*28S rRNA*) and *MutS+28S* gene regions.

Family	***MutS***	***28S rRNA***	***MutS+28S***
Alcyoniidae	0,087	0,066	0,078
Xeniidae	0,049	0,066	0,059
Nephtheidae	0,012	0,048	0,036
Nidaliidae	0,019	0,00	0,012
Ellisellidae	0,019	0,039	0,031
Gorgoniidae	0,049	0,063	0,062
Parasphaerascleridae	0,03	0,044	0,039
Plexauridae	0,044	0	0,006

Uncorrected pairwise genetic distances between genera for each marker can be found in Tables 7.10- 7.12 (Appendix C). Intergeneric distances ranged from 0.0% to 18.8% for *MutS*, 0.9% to 19.9% for *28S* and 0.8% to 19.6% for *MutS+28S*. On average, the most distant genera were *Verrucella* and *Xenia* whereas the closest related genera were Beta and *Klyxum*.

Table 4.5 Estimates of evolutionary divergence (uncorrected *p*) over sequence pairs between families for bacterial MutS homologue (*MutS*), 28S ribosomal RNA (*28S rRNA*) and *MutS+28S* gene regions.

Family	**Alcyoniidae**	**Xeniidae**	**Nephtheidae**	**Nidaliidae**	**Ellisellidae**	**Gorgoniidae**	**Parasphaerascleridae**
MutS							
Xeniidae	0,147						
Nephtheidae	0,117	0,128					
Nidaliidae	0,106	0,112	0,075				
Ellisellidae	0,156	0,180	0,154	0,147			

Table 4.5 continued…

Family	**Alcyoniidae**	**Xeniidae**	**Nephtheidae**	**Nidaliidae**	**Ellisellidae**	**Gorgoniidae**	**Parasphaerascleridae**
Gorgoniidae	0,111	0,110	0,080	0,070	0,148		
Parasphaerascleridae	0,119	0,141	0,102	0,103	0,110	0,101	
Plexauridae	0,106	0,115	0,079	0,071	0,143	0,064	0,091
			28S rRNA				
Xeniidae	0,115						
Nephtheidae	0,088	0,127					
Nidaliidae	0,081	0,113	0,094				
Ellisellidae	0,150	0,174	0,143	0,142			
Gorgoniidae	0,095	0,123	0,093	0,075	0,145		
Parasphaerascleridae	0,133	0,172	0,127	0,137	0,107	0,141	
Plexauridae	0,087	0,116	0,090	0,071	0,169	0,048	0,149
			MutS + 28S				
Xeniidae	0,130						
Nephtheidae	0,101	0,130					
Nidaliidae	0,091	0,116	0,086				
Ellisellidae	0,153	0,181	0,147	0,142			
Gorgoniidae	0,102	0,121	0,092	0,075	0,144		
Parasphaerascleridae	0,130	0,165	0,121	0,125	0,108	0,127	
Plexauridae	0,092	0,117	0,087	0,070	0,157	0,056	0,130

The mean uncorrected pairwise sequence divergence was highest for *MutS* (10.4%) compared to *28S rRNA* (9,2%) and 10,1% for *MutS+28S* (Table 4.5). Similar to the results of Table 4.4 and 4.5, the discrepancies between these diversity estimates are likely an artefact of the number of representative sequences available for the various gene regions used in this study. Likewise, the number of haplotypes, haplotype diversity and nucleotide diversity were greatest for *MutS* (Table 4.6). However, *MutS+28S* exhibited the greatest number of parsimony-informative sites (430) compared with *28S rRNA* (252) and *MutS* (116) (Table 4.6).

Table 4.6 Diversity estimates and mean evolutionary distance for genetic barcodes used in this study.

Barcode	bp	n	H	Hd	π	S	S_p	D
MutS	735	312	96	0.975	0.10415	127	116	0.104
28S rRNA	881	250	84	0.957	0.07714	813	252	0.092
MutS+28S	1626	212	92	0.974	0.08375	430	328	0.101

bp= number of base pairs. n= number of sequences. H= number of haplotypes; Hd= haplotype diversity; π= nucleotide diversity; S= number of variable sites; S_p= number of parsimony-informative sites; D= estimates of average evolutionary divergence.

4.5 Discussion

4.5.1 Taxonomic and molecular identifications

The Alcyonacea specimens collected in this study yielded 61 putative species within 22 genera and 8 families using a combination of molecular approaches, traditional taxonomy and *in situ* photographs. Four taxa from this collection constitute new zoogeographic records for the iSWP. This is the first survey that employs molecular data for this diverse group of Alcyonacea from this understudied region. By comparison, previous taxonomic surveys of this area by Williams (1989a, b) provided estimates of 26 species within 15 genera and 9 families and a subsequent study by Benayahu 1993 and Benayahu & Schleyer (1996) documented 37 species within 10 genera and 4 families. These earlier surveys were performed using considerable taxonomic expertise and relied solely on morphological characters to separate species.

Traditional species-level assignments are complex and time consuming, therefore molecular markers were tested for resolution to the genus-level in this diverse group of taxa. In this regard, the molecular approach used in this survey resulted in increased diversity estimates with the use of limited taxonomic expertise. This initial approach allowed for rapid screening of identifications using mitochondrial and nuclear loci *MutS* and *28S rRNA* respectively. These genetic markers distinguished most genera and clades therein which consequently reduced species identities to fewer candidates, similar to that of previous studies by Benayahu et al. (2012) and McFadden, Brown, et al. (2014). Furthermore, genetic data for this collection revealed cryptic species and potential hybrids. Molecular systematics cannot replace traditional taxonomic approaches in octocorals as species level assignments continue to be complex and problematic. However, molecular approaches facilitate the identification process and elucidate more appropriate diagnostic characters (McFadden, Alderslade, et al. 2006, McFadden et al. 2009, 2017, Halàsz et al. 2014).

Species were poorly resolved throughout the phylogenies (Figures 4.8- 4.13) as is often the case in molecular studies of octocorals (Wirshing et al. 2005, McFadden, France, et al. 2006, McFadden et al. 2011, Brockman & McFadden 2012, Quattrini et al. 2014). Molecular inferences provided species-level identifications for 31% of the collected alcyonacean specimens in this study without further need for morphological differentiation. Species inferences were restricted due to the limited availability of reference sequences with species-level identifications and a narrow range of representative taxa, which is an ongoing challenge in Alcyonacea studies (McFadden, Brown, et al. 2014). Little phylogenetic information was available for inferences of species identifications for several genera from the iSWP in this study (i.e. *Rumphella* and Nephtheidae). All remaining taxa were identified to the generic level wherein 79% of the putative species (n=33) recovered comprise either new records or new species within the iSWP and require taxonomic expertise to resolve. New zoogeographic records for the genera *Chironephthya*, *Scleronephthya* and *Paraplexaura* were reported for the iSWP region. The generic assignments for these new records were verified in the molecular phylogenies recovered across all loci (Figures 4.8- 4.10). However, these taxa require taxonomic identification to the species-level.

4.5.2 Phylogenetic analyses

The major phylogenies and subtrees recovered in this study reiterate previous reports for these taxa across these gene regions wherein the families Alcyoniidae and Gorgoniidae were mostly polyphyletic, which is typical of the Alcyoniina-Holaxonia mixed clade and characteristic of octocoral phylogenies (Berntson et al. 2001, McFadden, France, et al. 2006, Daly et al. 2007, McFadden et al. 2010, Zapata et al. 2015). Similar to previous studies, Calcaxonia was recovered as a monophyletic clade that was distinct from other subordinal groups (Berntson et al. 2001, McFadden, France, et al. 2006, McFadden et al. 2010, Pante et al. 2013) as was also the case for Parasphaerascleridae (McFadden & van Ofwegen 2013a). The polyphyletic genera *Leptogorgia* (Poliseno et al. 2017), *Paraplexaura* and *Chironephthya* (McFadden, Brown, et al. 2014) were recovered as monophyletic clades as an artefact of limited taxon sampling and few representative sequences that were available from GenBank.

Similar to other studies, mitochondrial *MutS* and nuclear *28S rRNA* molecular markers identified specimens to the genus and clade level in most cases but could not discriminate among species within some clades of closely related taxa. Species were poorly resolved and it is generally accepted that many of these taxa will not necessarily reflect a phylogenetic association with their assigned taxonomic groups (Wirshing et al. 2005, McFadden, France, et al. 2006, Benayahu et al. 2012, McFadden & van Ofwegen 2012, 2013a, Haverkort-Yeh et al. 2013, Quattrini et al. 2014, Cairns & Wirshing 2015). Morphological assignments that were not always supported by genetics across the gene regions included genera within the families Alcyoniidae, Xeniidae, Nephtheidae and Gorgoniidae respectively i.e. *Sinularia*, *Xenia*, *Anthelia*, *Sansibia*, *Heteroxenia*, *Litophyton*, *Nephthea* and *Rumphella* species respectively.

S. brassica formed a highly supported polyphyletic clade that was significantly distinct from all remaining congeners. Although it is acknowledged that *S. brassica* forms a 'sister clade to the remaining congeners (McFadden et al. 2009, Benayahu, McFadden, & Shoham 2017, Benayahu, McFadden, Shoham, et al. 2017), this is the first case of polyphyly reported for this genus as it is the most taxonomically diverse phylogeny in which *S. brassica* has been contextualized to date. Similar to the observations made by McFadden et al. (2009), it was noted that *S. brassica* was differentiated from other *Sinularia* species by an average genetic distance of 8.4% (uncorrected *p*), comparable to the intergeneric distances displayed by other Alcyoniidae i.e. *Sarcophyton* and *Lobophytum* 5.8% (uncorrected *p*) (Appendix C Table 7.10). Further analyses are required in order to ascertain whether the species, *S. brassica*, warrants generic status and merits re-assignment to a separate genus. Additional sequences of *S. brassica* specimens from widespread locations are needed in order to comprehensively investigate their phylogenetic placement amongst members of the Alcyoniina group. Accordingly, a thorough taxonomic revision of the genus would be required as *S. brassica* is the type species on which the genus description is based (Verseveldt 1980), which would subsequently result in the reassignment of all remaining congeners of *Sinularia* to another genus (McFadden et al. 2009). Diagnostic characters for the *Sinularia* genus would require re-examination if they are found to be inconclusive, which may be due to homoplasy (convergent evolution), incomplete lineage assortment or ancient hybridization-introgression events (Ament-Velásquez et al. 2016). Furthermore, two morphotypes of *S. brassica* were recovered in two supported clades that displayed pairwise differences equivalent to interspecific distances (see Chapter 3). These taxa indicate two separate species and warrant further taxonomic attention (McFadden et al. 2009, Benayahu et al. 2012) that may influence final species estimates for the iSWP (See Chapter 3). Furthermore, a new record of *S. grandilobata* was discovered amongst the taxa collected in this study (see Chapter 3).

The Alcyoniidae phylogenies for the genera *Cladiella*, Beta, *Aldersladum* and *Sinularia* were recovered using sequences from Chapters 2 and 3 respectively. The incorporation of these previous studies served to provide phylogenetic insight in a larger context with other Alcyoniidae and families for the specimens in this study. Nuclear and concatenated phylogenies recovered *Cladiella*, Beta and *Klyxum* as monophyletic genera and *Aldersladum sodwanum* as a monophyletic species.

Lobophytum and *Sarcophyton* (Alcyoniidae) are closely related alcyoniids for which three paraphyletic clades were recovered, consistent with those described by McFadden, Alderslade, et al. (2006). Similar to the phylogenetic classification system of *Sinularia* (McFadden et al. 2009), species identifications for *Lobophytum* and *Sarcophyton* can be narrowed to a few candidate species that share the same clade designation discussed in McFadden, Alderslade, et al. (2006). All three clade topologies were congruent to those of previous studies (McFadden, Alderslade, et al. 2006, Aratake et al. 2012). Specimens of *L. depressum* were recovered in the mixed L-S clade. This is the first record of *L. depressum* in phylogenetic analyses and it was consequently not assigned to a taxonomic clade until now. The paraphyletic mixed clade of the two genera (L-S), containing intermediary morphological forms, warrant further investigation into their evolutionary relationships and generic boundaries in order to uncover valid diagnostic characters for taxonomic assignments as their taxonomy is notoriously difficult (McFadden, Alderslade, et al. 2006). A new record of *Lobophytum* from the L clade was recorded and requires identification to the species-level. Two putative species of *Sarcophyton* were morphologically and phylogenetically distinct from other congenerics in the S clade and may form new records that warrant taxonomic investigation.

Among the five xeniid genera collected in this study only *Anthelia* was monophyletic for the mitochondrial locus. *Heteroxenia* and *Xenia* were polyphyletic for both loci, similar to previous reports for these taxa (McFadden, France, et al. 2006, Janes & Mary 2012, Stemmer et al. 2013, Janes et al. 2014, McFadden, Reynolds, et al. 2014). Furthermore, mitochondrial and nuclear DNA markers exhibited some inconsistencies between taxa which was also the case for other Alcyonacea studies that utilized the same loci (McFadden & van Ofwegen 2012, Haverkort-Yeh et al. 2013, Cairns & Wirshing 2015). *Sansibia* was polyphyletic for both loci as opposed to previous reports of paraphyly for these taxa that were likely an artefact of the few samples used in previous analyses (McFadden, France, et al. 2006, Janes & Mary 2012, McFadden, Brown, et al. 2014). Inconsistencies for the same taxa across mitochondrial and nuclear loci may be a result of incomplete lineage assortment or introgressive hybridisation between groups (McFadden & Hutchinson 2004, McFadden et al. 2010) and has previously been reported for certain xeniid taxa (McFadden et al. 2017). *Anthelia* was monophyletic for *MutS* but displayed polyphyly for *28S rRNA* as sequences of taxa from both this study and from GenBank belonged to disparate clades. This may have resulted due to either incomplete lineage assortment, hybridisation events or possibly misidentification but it is challenging to ascertain which of these may be the case. The pink colour morph of *Sansibia* was phylogenetically distinguished from other congenerics and may constitute a new record that requires identification. In addition, *Sympodium caeruleum* 288 might indicate a cryptic species that also warrants further taxonomic attention.

McFadden, France, et al. 2006 noted the discordance between morphological assignments of several Nephtheidae genera and their phylogenetic histories. Members of Nephtheidae lack sufficient taxonomic description (van Ofwegen & Groenenberg 2007) and closely related genera *Litophyton*, *Nephthea*, *Dendronephthya* and *Stereonephthya* form complexes. Most species of *Stereonephthya* and

Dendronephthya were recovered in distinct clades (McFadden et al. 2011, Haverkort-Yeh et al. 2013) as was the case in this study. However, paraphyletic polytomies of *Litophyton* and *Nephthea* in this study corroborate those of other studies (Lee & Song 2000, Song Jun & Lee Young 2000, McFadden, France, et al. 2006) and further support the recommendation that these two genera be synonymized (van Ofwegen & Groenenberg 2007). Furthermore, mitochondrial and nuclear DNA markers exhibited inconsistencies between taxa where *Litophyton* was recovered in a paraphyletic clade along with *Nephthea* for *MutS* but was nested in the *Dendronephthya* clade for *28S rRNA.* Though fewer sequences were included in the nuclear phylogeny, it is difficult to ascertain whether these disparities are due to lack of sequences or whether they are attributed to hybridisation events or incomplete lineage assortment. There were numerous examples of distinct colour morphs of *Stereonephthya* and *Dendronephthya* within clades that nonetheless shared identical sequences with dissimilar morphs or showed no genetic differentiation, an indication of morphological polymorphism within these taxa (Haverkort-Yeh et al. 2013). The molecular markers used in this study and those used in past studies of nephtheids (Lee & Song 2000, Song Jun & Lee Young 2000, McFadden, France, et al. 2006, van Ofwegen & Groenenberg 2007, Haverkort-Yeh et al. 2013) lack the variation to delineate species boundaries let alone species complexes.

Few sequences for taxa from South Africa exhibited shared haplotypes with their respective congeners and conspecifics from various geographic regions. This is not surprising as Bilewitch et al. (2010, 2014) and Poliseno et al. (2017) noted inconsistencies at the paraphyletic and even polyphyletic level between conspecifics from different geographic regions. Conspecifics from distant geographic ranges, with different haplotypes, may have formed separate populations, part of a species complex or undergone speciation in cases of temporal and spatial isolation (Fabricius & Alderslade 2001). Mcfadden & van Ofwegen (2017) attributed high levels of alcyonacean endemism in the Agulhas region of South Africa to isolation, though little has been researched with regards to the iSWP region. Phylogenetic studies of extensively sampled regions and species representatives would be required to ascertain whether this is the case for the taxa in this study.

The inclusion of a nuclear marker in addition to a mitochondrial marker for studies of these diverse taxa provides insight into evolutionary events as their gene regions typically exhibit different mutation rates (Reijnen et al. 2014, McFadden, Reynolds, et al. 2014). Comparisons of the taxonomic resolution for the loci *MutS* and *28S rRNA* were limited due to a lack of corresponding sequences over the gene regions. However, the nuclear marker yielded more parsimony informative sites than the mitochondrial region even with significantly fewer sequences of *28S rRNA*. DNA barcoding studies of alcyoniid taxa in previous studies demonstrated the greatest resolution for the multilocus barcode, *MutS+28S,* of the markers tested (Haverkort-Yeh et al. 2013, Halász et al. 2014). Application of this barcode across more diverse taxa recovered comparable estimates of putative species in conjunction with character analysis (McFadden, Brown, et al. 2014, McFadden, Reynolds, et al. 2014).

Mitochondrial and nuclear discordance between phylogenies may demonstrate cases of hybridization-introgression or incomplete lineage assortment (as a result of a recent speciation event) as has previously been reported in Alcyonacea (McFadden & van Ofwegen 2013a, Cairns & Wirshing 2015, Ament-Velásquez et al. 2016). Hybridization events are not uncommon amongst alcyonacean corals in which broadcasting and brooding soft corals have both exhibited hybridization (McFadden 1999, McFadden & Hutchinson 2004, Slattery et al. 2008, van Ofwegen et al. 2013, McFadden et al. 2017). The high latitudinal reefs of the iSWP form the lowest distribution limits of many Alcyonacea in the Indian Ocean (Schleyer & Celliers 2003a); it has been observed that corals on such marginal reefs share an overlap in distribution and composition with other taxa, thereby increasing the likelihood of hybridization events (Beger et al. 2014). Interspecific gene flow between corals increases their adaptive potential and subsequent resilience in subtropical conditions (van Oppen & Gates 2006, Richards et al. 2008). However, molecular markers are needed that are be able to delimit species in the presence of incomplete lineage sorting and hybridization events.

The updated species list for this area, that includes the results of this work and recent literature, has resulted in a significant increase from previous reports of 37 species to 81 species, 11 to 47 genera and 4 to 17 families, including 4 new records. Although the revised species estimates for the iSWP were considerable, estimates will continue to evolve with the rates of discovery and taxonomic descriptions. The samples collected in this study are estimated to yield an additional 33 species once the specimens have been identified. However, the need for taxonomic revisions of genera are required before unknown species can be correctly assigned an identification (McFadden, Brown, et al. 2014) and considering the dwindling octocoral taxonomic expertise (Fabricius & Alderslade 2001, Neigel et al. 2007, Griffiths et al. 2010, Radulovici et al. 2010), it may take some time before a final estimate of the total number of species can be made for this region.

4.6 Conclusion

This is the first genetic study for this diverse group of Alcyonacea from South Africa. This study serves as an example of the utility of DNA markers to provide systematic and evolutionary insight into the biodiversity of these ecologically important yet complex communities at high latitudinal reefs. Rapid identification assignments to the genus-level (and species-level in certain taxa) can be inferred using phylogenetic approaches based on the multilocus barcode *MutS+28S*. The use of these gene regions in concatenation show great potential for evolutionary and biodiversity studies of Alcyonacea from this region. However, additional sequence representatives for a wider range of taxa with species-level identifications would significantly increase the taxonomic utility of these barcodes and assist the identification process. The updated biodiversity estimates for this region are useful for conservation-related decisions, especially in under-sampled areas in which high endemism levels are anticipated. Revised biodiversity estimates provide taxonomic insight into conservation targets for the iSWP, where published assessments forecast a decline in soft coral cover. This research will ameliorate some of the future challenges in identifications of soft corals in this area.

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CHAPTER 5: A CHECKLIST FOR SOFT CORAL (ALCYONACEA) IN SOUTH AFRICA (PAPER 4)

5.1 Abstract

Records of species estimates of Alcyonacea (Octocorallia) from South Africa are dispersed, outdated and neglected. The constant flux in taxonomic revisions, nomenclature and the increased discoveries of new taxa are additional factors that render existing national species lists incomplete and outdated. A compilation of 51 published papers that contained valid taxonomic records of Alcyonacea in South Africa were reviewed. Species records were cross-referenced, consolidated and compiled throughout the literature in order to establish an updated national species list for Alcyonacea in South Africa. The species list reflected current biodiversity estimates, taxonomic identifications and included the basis of records for the region. This updated reference list provides the basis for assessing species diversity, highlighting areas of endemism, protected species, invasive species and conducting spatial and temporal comparative studies, all of which can be utilized for management decisions in conservation. Approximately 226 species within 89 genera and distributed among 25 families of Alcyonacea were reported. This was almost twice the last published species estimates of 130 taxa nationwide. Seventy-five of these species were endemic (32%) and hailed mainly from the Agulhas bioregion of South Africa. Furthermore, new records were reported for the genera *Scleronephthya, Paraplexaura*, and *Chironephthya* and the species, *Sinularia grandilobata* from the Delagoa bioregion, an understudied region of interest that forms the lowest distribution limit for key taxa of Alcyonacea. However, a significant number of species remain undescribed throughout South Africa that potentially form new records or new species that require taxonomic description.

Keywords: *biodiversity, soft corals, South Africa, taxonomy*

5.2 Introduction

Octocorallia are a large, taxonomically diverse subclass of Anthozoa (Phylum Cnidaria) that consists of three orders: Alcyonacea (soft corals and gorgonians), Helioporacea (blue corals), and Pennatulacea (sea pens) (Williams 1993, Fabricius &

Alderslade 2001). Unlike Scleractinia, Octocorallia species have polyps with eight tentacles containing one or more rows of pinnules down each side (Branch et al. 2008, King & Fraser 2014). Alcyonacea are further subdivided into six subordinal groups based on their skeletal morphology and colony structure. Gorgonians (sea fans and sea whips) are organized into the subordinal groups Scleraxonia, Holaxonia and Calcaxonia, based on the structure of internal axial support comprised of gorgonin and/or calcareous matter whereas the remaining groups of Alcyoniina, Protoalcyonaria, and Stolonifera lack internal axes (Grasshoff 1999). Holaxonia and Calcaxonia are considered to be the only two distinct subordinal groups delineated by morphological apomorphies (Grasshoff 1999, Daly et al. 2007). The Scleraxonia group have a central supporting structure consisting of fused or cemented sclerites or closely packed sclerites reinforced with a matrix of gorgonin (Fabricius & Alderslade 2001, Daly et al. 2007, McFadden et al. 2010).

The rate of discovery and subsequent taxonomic descriptions of marine taxa has increased rapidly in the last decade with advancements in technology, *in situ* studies and molecular systematics (Costello et al. 2013). The current accepted classification of Alcyonacea is based on similarities of morphological features, especially those pertaining to the distribution and forms of tiny spicules of calcium carbonate in the colony tissues known as sclerites (Bayer 1981, Fabricius & Alderslade 2001). Daly et al. (2007) reported 305 genera and 2739 identified species of Alcyonacea worldwide which Appeltans et al. (2012) later updated to 2951 species. It is predicted that a further 1600 Alcyonacea taxa remain unidentified whereas an estimated 435-577 Scleractinia remain undescribed (Appeltans et al. 2012). At present, approximately 37 families; 349 genera and 3377 valid species of Alcyonacea have been described (Ratnasingham & Hebert 2007, Horton et al. 2017).

The taxonomy of corals is notoriously confounding because of antiquated, ambiguous species descriptions based on poorly preserved, improperly curated type specimens from the 18th and 19th century that cannot be verified by modern day taxonomists (Williams 1992a, Veron 2013). Alcyonacea have received little attention compared to their Scleractinian counterparts as their classification is in constant dispute due to their complex taxonomy and ambiguous species boundaries (Bayer 2001, Riegl 2003, Samimi Namin & van Ofwegen 2010, McFadden et al. 2010, 2011). Comprehensive field guides equivalent to those of Veron's 'Corals of the World' do not exist for Alcyonacea (Veron & Stafford-Smith 2000, Fabricius & Alderslade 2001). Soft coral taxonomy remains understudied, poorly understood and according to experts- in need of a complete revision (Bayer 2001, McFadden et al. 2006, Daly et al. 2007).

National species lists and biodiversity estimates of Alcyonacea from South Africa were published over two decades ago. Taxonomic and biogeographic studies of Alcyonacea and Pennatulacea in South Africa were carried out by an octocoral taxonomic expert in the late eighties and early nineties with identifications based on morphological characters and sclerite forms (Williams 1990a, 1992a, Williams 1992b). This work revised and expanded upon the pioneering work of J. S. Thompson who initially compiled taxonomic records of South African Alcyonacea in 1917 and 1921 (Thomson 1917, 1921, Williams 1992a, b). However, relatively few of Thompson's identifications could be verified due to the damaged state of the specimens on which the records were based and lacked sufficient material for examination. Furthermore, many of these taxa were misidentified by Thompson due to misinterpretations of morphological characters based on antiquated descriptions (Williams 1992a). Identifications of additional taxa were challenging due to lost type specimens and insufficient species descriptions that lacked sclerite descriptions and made identifications an impossible task e.g. *Alcyonium membranaceum*, *Melithaea rosea*, *Alcyonium novarae* and *Metalcyonium lanceatum* (Thomson 1917, 1921, Williams 1992a, b). Undertakings of this nature were further challenged by a lack of access to appropriate literature where many original descriptions are either lost, confined to unavailable hard copies or require translation.

The discovery of formerly neglected morphological characters of taxonomic value and the addition of molecular phylogenies have resulted in a surge of revisions and modified classifications in the last two decades (McFadden et al. 2006, 2009, Williams & Lundsten 2009, Radulovici et al. 2010, Benayahu & McFadden 2011, McFadden & van Ofwegen 2013, Reijnen et al. 2014). A recent revision of endemic Alcyoniina taxa in the Agulhas bioregion of South Africa resulted in the description of three new families, four new genera and four new species (Mcfadden & van Ofwegen 2017). Notwithstanding this, there have been relatively few new published species from South Africa in the last two decades despite increased soft coral collections from this region (van Ofwegen & Schleyer 1997, Williams & Lindo 1997, Gibbons et al. 1999, Williams 2000b, 2003, Williams & Starmer 2000, Williams & Little 2001, McFadden & van Ofwegen 2012a, b). Gibbons et al. (1999) noted the dwindling taxonomic expertise in South Africa for marine taxa including octocorals. There is insufficient taxonomic expertise for this important group at a global level and specimens cannot be identified and described at the rate of their discovery (Appeltans et al. 2012). Consequently, biodiversity estimates and species lists for South African Alcyonacea are outdated and warrant revision (Williams 2000b, 2003, Reijnen et al. 2014).

Williams (2000b) reported 130 valid species of Alcyonacea in South Africa and over 50% endemism for Octocorallia (including Pennatulacea) (Riegl et al. 1995, Gibbons et al. 1999, Griffiths et al. 2010). Many of these identifications were based on taxa that were sampled from the much-studied Agulhas bioregion which comprises the western and southern coast of South Africa (Williams et al. 1992, Mcfadden & van Ofwegen 2017). Coral communities are found at high latitudes on the east coast of South Africa in northern KwaZulu-Natal (Riegl et al. 1995, Muthiga et al. 2008, Schleyer et al. 2008). These reefs support the southernmost coral assemblages and sublittoral reefs in East Africa and fall within a world heritage site, the iSWP, situated in the Delagoa Bioregion (Benayahu et al. 2003, Schleyer & Celliers 2003). Their faunal component is dominated by Alcyonacea instead of Scleractinia (Williams 1993). The Alcyoniidae genera *Sinularia*, *Sarcophyton* and *Lobophytum* constitute the top three soft coral genera that together constitute nearly two thirds of the reef biota (Williams 1989a, 1993, Riegl et al. 1995, Benayahu et al. 2003). Of the few taxonomic studies of soft corals from the iSWP region, approximately 37 confirmed species within 11 genera and distributed among 4 families have been recorded (Benayahu 1993, Williams 1993, Benayahu & Schleyer 1996).

This review serves to consolidate all previously published taxonomic records of Alcyonacea in South Africa with updated taxonomic nomenclature in order to provide a national reference base that reflects more precise biodiversity estimates. In addition, it also highlights taxa from the iSWP, an understudied region of great significance for these data deficient taxa and includes new records as a result of this research. The biogeography of these taxa is beyond the scope of this review and has been sufficiently covered by Williams et al. (1992). Presented here is an updated and curated species list for soft corals of the order Alcyonacea in South Africa that can be utilized in biodiversity studies and conservation management decisions.

5.3 Method and materials

Taxonomic records were sourced from peer-reviewed journal papers and published expedition reports found either online or as physical copies. Eligible records were obtained from 51 literature sources wherein species identifications were carried by present day taxonomic experts and zoogeographic records were provided. Additionally, these species were checked for valid taxonomic nomenclature and updated using the World Register of Marine Species (Costello et al. 2013, Horton et al. 2017). Historic and ambiguous records, that could neither be verified nor eliminated by taxonomists, were included in the national list with an indication of the validity of their record. In addition to species records sourced from the literature, four new zoogeographic records of corals that were discovered during this research (see Chapter 4, section 4.4.2) were included in the national species list for Alcyonacea. The biogeography and distribution of Alcyonacea taxa in South Africa was beyond the scope of the work.

5.4 Results

5.4.1 Alcyonacea recorded from South Africa

Species accounts were cross-referenced and consolidated throughout the taxonomic literature and combined with records from previous chapters in this thesis in order to establish an updated national species list for Alcyonacea in South Africa. Species of Alcyonacea recorded from South Africa are presented in Table 5.1 along with their respective taxonomic authority and basis of record. Approximately 226 species within 89 genera distributed among 25 families were recorded in total. Seventy-five of these species are reportedly endemic (32%), with 16 endemic genera (18%) and 4 endemic families (16%). Within the iSWP, 81 species belonging to 47 genera within 17 families were reported (including 12 endemic species). Thirty of the species listed in Table 5.1 require taxonomic description by an octocoral expert.

Table 5.1 Octocorals of the order Alcyonacea Lamouroux, 1812 recorded from South Africa. Highlighted rows indicate taxa recorded from the iSimangaliso Wetlands Park World Heritage Site, Northern KwaZulu-Natal.

Family	**Genus**	**Scientific name**	**Authority**	**Basis of record**
Family	**Genus**	**Scientific name**	**Authority**	**Basis of record**
Acanthogorgiidae	*Acanthogorgia*	*Acanthogorgia armata* *	Verrill, 1878	Williams 1992a
		Acanthogorgia ramosissima *	Wright & Studer, 1889	Williams 1992a
		Acanthogorgia sp.	Gray, 1857	Williams 1992b
	Anthogorgia	*Anthogorgia* sp.	Verrill, 1868	Williams 1992b
	Muricella	*Muricella ramosa*	Thomson & Henderson, 1905	Williams 1992a
Acrophytidae (e)	*Acrophytum* (e)	*Acrophytum claviger* (e)	Hickson, 1900	Williams 1992a
	Lampophyton (e)	*Lampophyton planiceps* (e)	(Williams, 1986)	Williams 1986
		Lampophyton spinatum (e)	McFadden & van Ofwegen, 2017	McFadden & van Ofwegen 2017
	Pieterfaurea (e)	*Pieterfaurea equicalceola* (e)	Williams, 2000	Williams 2000a
		Pieterfaurea khoisaniana (e)	(Williams, 1988)	Williams 1992a
		Pieterfaurea lampas (e)	Williams, 2000	Williams 2000c
		Pieterfaurea sinuosa (e)	Williams, 2000	Williams 2000c
		Pieterfaurea unilobata (e)	(Thomson, 1921)	Williams 1992a
Alcyoniidae	*Alcyonium*	*Alcyonium burmedju* **	Sampaio, Stokvis & van Ofwegen, 2016	Williams 1992a
		Alcyonium compactofestucum (e)	Verseveldt & van Ofwegen, 1992	Verseveldt & van Ofwegen 1992
		Alcyonium dolium (e)	McFadden & van Ofwegen, 2017	McFadden & van Ofwegen 2017
		Alcyonium elegans (e)	(Kuekenthal, 1902)	Williams 1992a
		Alcyonium faura (e)	Thomson, 1910	Williams 1992a
		Alcyonium foliatum (e)	Thomson, 1921	Williams 1992a

Table 5.1 continued…

Family	Genus	Scientific name	Authority	Basis of record
		Alcyonium glomeratum ✢	(Hassall, 1843)	Thomson 1921
		Alcyonium patagonicum	(May, 1899)	Thomson 1921
		Alcyonium reptans *	Kuekenthal, 1906	Thomson 1921
		Alcyonium roseum *	(Tixier-Durivault, 1954)	Williams 1992a
		Alcyonium rotiferum	Thomson, 1910	Thomson 1921
		Alcyonium sollasi **	Wright & Studer, 1889	Thomson 1921
		Alcyonium sp.	Linnaeus, 1758	Williams 1992a
	Aldersladum	*Aldersladum sodwanum* (e)	(Benayahu, 1993)	Benayahu 1993
	Anthomastus	*Anthomastus giganteus* (e)	Tixier-Durivault, 1954	Williams 1992a
		Anthomastus grandiflorus	Verrill, 1878	Thomson 1921
		Anthomastus granulosus	Kuekenthal, 1910	Thomson 1921
		Anthomastus hicksoni	Bock, 1938	Molodtsova 2013
		Anthomastus sp.	Verrill, 1878	Williams et al. 1992c
	Bellonella	*Bellonella clavata* **	(Pfeffer, 1889)	Verseveldt & Bayer 1988
		Bellonella rubra	Brundin, 1896	Thomson 1921
		Bellonella sp.	Gray, 1862	Verseveldt & Bayer 1988
		Bellonella unilobata	(Thomson, 1921)	Thomson 1921
	Beta	Beta *australis*	(Benayahu & Schleyer, 1996)	Benayahu & Schleyer 1996
		Beta *kashmani* (e)	(Benayahu & Schleyer, 1996)	Benayahu & Schleyer 1996
		Beta sp.	(Benayahu & Schleyer, 1996)	Alderslade & Etsebeth (in prep)
	Cladiella	*Cladiella* spp.	Gray, 1869	Alderslade & Etsebeth (in prep)
	Eleutherobia	*Eleutherobia studeri* (e)	(Thomson, 1910)	Williams 1992a
		Eleutherobia variabile (e)	(Thomson, 1921)	Williams 1992a
		Eleutherobia vinadigitaria (e)	Williams & Little, 2001	Williams & Little 2001

Table 5.1 continued…

Family	Genus	Scientific name	Authority	Basis of record
	Lanthanocephalus (e)	*Lanthanocephalus clandestinus* (e)	Williams & Starmer, 2000	Williams & Starmer 2000
	Lobophytum	*Lobophytum crassum*	von Marenzeller, 1886	Williams 1992a
		Lobophytum depressum	Tixier-Durivault, 1966	Benayahu 1993
		Lobophytum latilobatum	Verseveldt, 1971	Benayahu 1993
		Lobophytum patulum	Tixier-Durivault, 1956	Benayahu 1993
		Lobophytum sarcophytoides *	Moser, 1919	Thomson 1921
		Lobophytum spp.	von Marenzeller, 1886	Williams 1992a
		Lobophytum venustum	Tixier-Durivault, 1957	Benayahu 1993
	Malacacanthus (e)	*Malacacanthus capensis* (e)	(Hickson, 1900)	Williams 1987b
	Paraminabea	*Paraminabea cosmarioides* (e)	(Williams, 1992)	Williams 1992c
		Paraminabea kosiensis (e)	(Williams, 1992)	Williams 1992c
	Sarcophyton	*Sarcophyton crassum*	Tixier-Durivault, 1946	Benayahu 1993
		Sarcophyton ehrenbergi	(von Marenzeller, 1886)	Benayahu 1993
		Sarcophyton flexuosum	Tixier-Durivault, 1966	Benayahu 1993
		Sarcophyton glaucum	(Quoy & Gaimard, 1833)	Benayahu 1993
		Sarcophyton infundibuliforme	Tixier-Durivault, 1958	Benayahu 1993
		Sarcophyton spp.	Lesson, 1834	Williams 1992a
		Sarcophyton trocheliophorum	von Marenzeller, 1886	Williams 1992a
	Sinularia	*Sinularia abrubta*	Tixier-Durivault, 1970	Benayahu 1993
		Sinularia brassica	May, 1898	Benayahu 1993
		Sinularia erecta	Tixier-Durivault, 1945	Benayahu 1993
		Sinularia firma	Tixier-Durivault, 1970	Benayahu 1993
		Sinularia gardineri	(Pratt, 1903)	Benayahu 1993
		Sinularia grandilobata (n)	Verseveldt, 1980	Etsebeth (in prep)

Table 5.1 continued…

Family	**Genus**	**Scientific name**	**Authority**	**Basis of record**
		Sinularia gravis	Tixier-Durivault, 1970	Benayahu 1993
		Sinularia heterospiculata	Verseveldt, 1970	Benayahu 1993
		Sinularia hirta	(Pratt, 1903)	Benayahu 1993
		Sinularia muralis	(May, 1899)	Benayahu 1993
		Sinularia notanda	Tixier-Durivault, 1966	Benayahu 1993
		Sinularia querciformis	(Pratt, 1903)	Benayahu 1993
		Sinularia schleyeri (e)	Benayahu, 1993	Benayahu 1993
		Sinularia spp.	May, 1898	Williams 1992a
		Sinularia triangula	Tixier-Durivault, 1970	Benayahu 1993
		Sinularia variabilis	Tixier-Durivault, 1945	Benayahu 1993
	Verseveldtia (e)	*Verseveldtia bucciniforme* (e)	Williams, 1990	Williams 1992a
		Verseveldtia sp. (e)	Williams, 1990	Williams 1990a
		Verseveldtia trochiforme (e)	(Hickson, 1900)	Williams 1992a
Anthothelidae	*Williamsium*	*Williamsium parviflora* (e)	(Thomson, 1917)	Williams 1992b
Arulidae	*Arula*	*Arula petunia* (e)	McFadden & van Ofwegen, 2012	McFadden & Van Ofwegen 2012a
Chrysogorgiidae	*Chrysogorgia*	*Chrysogorgia* sp.	Duchassaing & Michelotti, 1864	Williams 1992b
	Helicogorgia	*Helicogorgia capensis* (e)	(Simpson, 1910)	Williams 1992d
		Helicogorgia flagellata (e)	(Simpson, 1910)	Williams 1992d
		Helicogorgia ramifera	Williams, 1992	Williams 1992d
		Helicogorgia sp.	Verrill, 1883	Williams 1992d
		Helicogorgia spiralis (e)	(Hickson, 1904)	Williams 1992d
		Helicogorgia squamifera	(Kuekenthal, 1919)	Williams 1992d
	Radicipes	*Radicipes* sp.	Stearns, 1883	Williams 1992b
	Trichogorgia	*Trichogorgia capensis* (e)	(Hickson, 1904)	Williams et al. 1992

Table 5.1 continued…

Family	Genus	Scientific name	Authority	Basis of record
		Trichogorgia flexilis (e)	Hickson, 1904	Williams 1992b
		Trichogorgia sp.	Hickson, 1904	Williams 1992b
	Xenogorgia	*Xenogorgia sciurus* * (e)	Bayer & Muzik, 1976	Williams 1992b
		Xenogorgia sp.	Bayer & Muzik, 1976	Williams 1992b
Clavulariidae	*Bathytelesto*	*Bathytelesto tubuliporoides* (e)	Williams, 1989	Williams 1989c
	Carijoa	*Carijoa* sp.	F. Mueller, 1867	McFadden & van Ofwegen 2012b
	Clavularia	*Clavularia capensis* *	(Studer, 1879)	Williams 1992a
		Clavularia cylindrica **	Wright & Studer, 1889	Williams 1992a
		Clavularia diademata	Broch, 1939	Williams 1992a
		Clavularia elongata **	Wright & Studer, 1889	Williams 1992a
		Clavularia parva	Tixier-Durivault, 1964	Williams 1992a
		Clavularia sp.	Blainville, 1830	Williams 1989b
	Denhartogia (e)	*Denhartogia hartogi* (e)	Ocaña & van Ofwegen, 2003	Ocaña & Ofwegen 2003
	Inconstantia (e)	*Inconstantia exigua* (e)	McFadden & van Ofwegen, 2012	McFadden & van Ofwegen 2012b
		Inconstantia pannucea (e)	McFadden & van Ofwegen, 2012	McFadden & van Ofwegen 2012b
		Inconstantia procera (e)	McFadden & van Ofwegen, 2012	McFadden & van Ofwegen 2012b
	Scleranthelia	*Scleranthelia thomsoni*	Williams, 1987	Williams 1987a
	Scyphopodium	*Scyphopodium ingolfi*	(Madsen, 1944)	Williams 1989c
		Scyphopodium sp.	Bayer, 1981	Williams 1989c
	Stereotelesto	*Stereotelesto corallina*	Duchassaing, 1870	Williams 1989c
	Telesto	*Telesto arborea* *	Wright & Studer, 1889	McFadden & van Ofwegen 2012b
		Telesto sp.	Lamouroux, 1812	Thomson 1921
		Telesto trichostemma *	(Dana, 1846)	McFadden & van Ofwegen 2012b
	Telestula	*Telestula* sp.	Madsen, 1944	McFadden & van Ofwegen 2012b

Table 5.1 continued…

Family	**Genus**	**Scientific name**	**Authority**	**Basis of record**
Coelogorgiidae	*Coelogorgia*	*Coelogorgia palmosa*	Milne Edwards & Haime, 1857	Williams 1992a
Cornulariidae (e)	*Cornularia* (e)	*Cornularia pabloi* (e)	McFadden & van Ofwegen, 2012	McFadden & van Ofwegen 2012b
Corymbophytidae (e)	*Corymbophyton* (e)	*Corymbophyton bruuni* (e)	(Bayer, 1995)	Bayer 1995
Ellisellidae	*Ellisella*	*Ellisella flagellum* ✢	(Johnson, 1863)	Thomson 1917
		Ellisella sp. **	Gray, 1858	Williams 1992b
	Junceella	*Junceella* sp.	Valenciennes, 1855	Williams 1992b
	Verrucella	*Verrucella bicolor*	Nutting, 1908	Thomson 1917
Gorgoniidae	*Eunicella*	*Eunicella alba*	(Esper, 1766)	Williams 1987b
		Eunicella albicans (e)	(Koelliker, 1865)	Williams 1992b
		Eunicella papillosa (e)	(Esper, 1797)	Williams 1992b
		Eunicella tricoronata (e)	Velimirov, 1971	Williams 1992b
	Leptogorgia	*Leptogorgia barnardi* (e)	Stiasny, 1940	Williams 1992b
		Leptogorgia bayeri	Williams & Lindo, 1997	Williams & Lindo 1997
		Leptogorgia capensis	(Hickson, 1900)	Williams 1992b
		Leptogorgia flammea	(Ellis & Solander, 1786)	Williams 1992b
		Leptogorgia gilchristi (e)	(Hickson, 1904)	Williams 1992b
		Leptogorgia lütkeni **	(Wright & Studer, 1889)	Williams & Lindo 1997
		Leptogorgia palma (e)	(Pallas, 1766)	Williams 1992b
		Leptogorgia pusilla *	Kuekenthal, 1919	Thomson 1917
		Leptogorgia rigida **	Verrill, 1864	Thomson 1917
		Leptogorgia sp.	Milne Edwards, 1857	Williams 1992b
		Leptogorgia tenuissima *	Kuekenthal, 1919	Thomson 1917
	Rumphella	*Rumphella aggregata* *	(Nutting, 1910)	Williams et al. 2014
		Rumphella sp.	Bayer, 1955	Williams 1992b

Table 5.1 continued…

Family	Genus	Scientific name	Authority	Basis of record
Isididae	*Acanella*	*Acanella* sp.	Gray, 1870	Williams 1992b
	Chathamisis	*Chathamisis ramosa* (e)	(Hickson, 1904)	Williams 1992b
	Keratoisis	*Keratoisis* sp.	Wright, 1869	Williams 1992b
Keroeididae	*Ideogorgia*	*Ideogorgia capensis* (e)	(Simpson, 1910)	Williams 1992b
Leptophytidae (e)	*Circularius* (e)	*Circularius wilsoni* (e)	(Thomson, 1921)	McFadden & van Ofwegen 2017
	Leptophyton (e)	*Leptophyton benayahui* (e)	van Ofwegen & Schleyer, 1997	van Ofwegen & Schleyer 1997
		Leptophyton fustis (e)	McFadden & van Ofwegen, 2017	McFadden & van Ofwegen 2017
	Porphyrophyton (e)	*Porphyrophyton distinctum* (e)	(Williams, 1988)	McFadden & van Ofwegen 2017
	Tenerodus (e)	*Tenerodus fallax* (e)	(Luettschwager, 1922)	McFadden & van Ofwegen 2017
		Tenerodus pollex (e)	McFadden & van Ofwegen, 2017	McFadden & van Ofwegen 2017
		Tenerodus verseveldti (e)	(Bayer, 1995)	Bayer 1995
Melithaeidae	*Melithaea*	*Melithaea africana*	(Kuekenthal, 1908)	Reijnen et al. 2014
		Melithaea capensis *	(Studer, 1878)	Williams 1992b
		Melithaea coccinea *	(Ellis & Solander, 1786)	Williams 1992b
		Melithaea dichotoma	(Linnaeus, 1758)	Matsumoto & van Ofwegen 2015
		Melithaea furcata **	(Thomson, 1916)	Williams 1992b
		Melithaea rubra	(Esper, 1789)	Williams 1992b
		Melithaea singularis **	(Thomson, 1916)	Thomson 1917; van Ofwegen
		Melithaea sp.	Milne Edwards, 1857	Reijnen et al. 2014
		Melithaea trilineata **	(Thomson, 1917)	Thomson 1917
		Melithaea valdiviae	(Kuekenthal, 1908)	Reijnen et al. 2014
		Melithaea wrighti *	Reijnen et al., 2013	Williams 1992b
Nephtheidae	*Capnella*	*Capnella* sp.	Gray, 1869	Williams 1992a
		Capnella susanae (e)	Williams, 1988	Williams 1992a

Table 5.1 continued…

Family	Genus	Scientific name	Authority	Basis of record
	Eunephthya	*Eunephthya thyrsoidea* (e)	Verrill, 1869	Williams 1992a
	Dendronephthya	*Dendronephthya inhacaensis* *	Verseveldt, 1960	Williams 1992a
		Dendronephthya mirabilis * (e)	(Tixier-Durivault & Prevorsek, 1962)	Williams 1992a
		Dendronephthya mutabilis *	Tixier-Durivault & Prevor, 1962	Williams 1992a
		Dendronephthya spp.	Kuekenthal, 1905	Williams 1992a
		Dendronephthya vervoorti	Verseveldt & van Ofwegen, 1991	Verseveldt & van Ofwegen 1991
	Drifa	*Drifa* sp.	Danielssen, 1886	Williams 2000b
	Eunephthya (e)	*Eunephthya celata* (e)	McFadden & van Ofwegen, 2012	McFadden & Van Ofwegen 2012b
		Eunephthya ericius (e)	McFadden & van Ofwegen, 2012	McFadden & van Ofwegen 2012a
		Eunephthya granulata (e)	McFadden & van Ofwegen, 2012	McFadden & van Ofwegen 2012a
		Eunephthya shirleyae (e)	McFadden & van Ofwegen, 2012	McFadden & van Ofwegen 2012a
		Eunephthya susanae (e)	(Williams, 1988)	McFadden & van Ofwegen 2012a
		Eunephthya thyrsoidea (e)	Verrill, 1869	McFadden & van Ofwegen 2012a
	Gersemia	*Gersemia liltvedi* (e)	(Verseveldt & Williams, 1988)	Verseveldt & Williams 1998
	Litophyton	*Litophyton* sp.	Forskål, 1775	Williams 1992a
	Scleronephthya	*Scleronephthya* sp. (n)	Studer, 1887	Etsebeth (in prep)
	Stereonephthya	*Stereonephthya* spp.	Kuekenthal, 1905	Riegl et al. 1995
Nidaliidae	*Chironephthya*	*Chironepthya* sp. (n)	Studer, 1887	Etsebeth (in prep)
	Siphonogorgia	*Siphonogorgia* sp.	Koelliker, 1874	Williams 1992a
Paralcyoniidae	*Dimorphophyton* (e)	*Dimorphophyton mutabiliforme* (e)	(Williams, 1988)	Williams 1988
Parasphaerascleridae	*Parasphaerasclera*	*Parasphaerasclera aurea* (e)	(Benayahu & Schleyer, 1995)	Benayahu & Schleyer 1995
		Parasphaerasclera morifera	(Tixier-Durivault, 1954)	Williams 1992a
		Parasphaerasclera rotifera (e)	(Thomson, 1910)	Verseveldt & Bayer 1988
		Parasphaerasclera valdiviae (e)	(Kuekenthal, 1906)	Williams 1992a

Table 5.1 continued…

Family	Genus	Scientific name	Authority	Basis of record
Plexauridae	*Acanthomuricea*	*Acanthomuricea pulchra* (e)	(Thomson, 1911)	Williams 1992b
	Astromuricea	*Astromuricea fusca* (e)	(Thomson, 1911)	Williams 1992b
		Astromuricea sp.	Germanos, 1895	Williams 1992b
	Echinomuricea	*Echinomuricea* sp.	Verrill, 1869	Williams 1992b
	Euplexaura	*Euplexaura capensis*	Verrill, 1870	Williams 1992b
		Euplexaura sp.	Verrill, 1869	Williams 1992b
	Menella	*Menella* sp.	Gray, 1870	Williams et al. 1992
	Paraplexaura	*Paraplexaura* sp. (n)	Kuekenthal, 1909	Etsebeth (in prep)
Primnoidae	*Callogorgia*	*Callogorgia* sp.	Gray, 1858	Williams 1992b
	Calyptrophora	*Calyptrophora* sp.	Gray, 1866	Williams 1992b
	Narella	*Narella gilchristi*	(Thomson, 1911)	Williams 1992b
		Narella sp.	Gray, 1870	Williams 1992b
	Primnoeides	*Primnoeides* sp.	Studer & Wright, 1887	Williams 1992b
	Primnoella	*Primnoella* sp.	Gray, 1858	Williams 1992b
	Thouarella	*Thouarella brucei*	(Thomson & Ritchie, 1906)	Taylor et al. 2013
		Thouarella clavata	Kuekenthal, 1908	Taylor et al. 2013
		Thouarella hicksoni (e)	Thomson, 1911	Williams 1992b
		Thouarella sp.	Gray, 1870	Williams 1992b
Spongiodermidae	*Diodogorgia*	*Diodogorgia capensis* (e)	(Thomson, 1911)	Williams 1992b
	Homophyton	*Homophyton verrucosum* (e)	(Moebius, 1861)	Williams 1992b
Tubiporidae	*Tubipora*	*Tubipora* sp. (nt)	Linnaeus, 1758	Benayahu 1993
Xeniidae	*Anthelia*	*Anthelia glauca*	Lamarck, 1816	Williams 1992a
		Anthelia profunda *	Tixier-Durivault, 1964	Williams 1992a
		Anthelia sp.	Lamarck, 1816	Williams 1992a

Table 5.1 continued…

Family	Genus	Scientific name	Authority	Basis of record
	Cespitularia	*Cespitularia coerula* *	May, 1898	Williams 1992a, c
	Heteroxenia	*Heteroxenia elisabethae*	Koelliker, 1874	Williams 1992a
		Heteroxenia fuscescens	(Ehrenberg, 1834)	Benayahu 1993
		Heteroxenia membranacea *	Schenk, 1896	Williams 1992a
		Heteroxenia rigida *	(May, 1899)	Williams 1992a
	Sansibia	*Sansibia flava*	(May, 1899)	Williams 1992a
	Sympodium	*Sympodium caeruleum*	Ehrenberg, 1834	Benayahu 1993
	Xenia	*Xenia crassa*	Schenk, 1896	Benayahu 1993
		Xenia dayi * (e)	Tixier-Durivault, 1959	Williams et al. 1992
		Xenia florida *	(Lesson, 1826)	Williams 1992a
		Xenia garciae	Bourne, 1894	Benayahu 1993
		Xenia kükenthali	Roxas, 1933	Benayahu 1993
		Xenia sp.	Lamarck, 1816	Williams 1992a
		Xenia umbellata *	Lamarck, 1816	Williams 1992a
		Xenia viridis *	Schenk, 1896	Williams 1992a

Authority names in brackets indicate species that were originally in different genera when first described (in accordance with the International Code of Zoological Nomenclature, ICZN),

(n) New zoogeographical record,

(e) Endemic species,

(nt) 'Near threatened' status. The IUCN Red List of Threatened Species^{TM},

(nn) Two nominal species of *Cladiella* are assigned to a new genus and the name Beta has been selected as a placeholder for that genus while the full taxonomic description is being prepared for publication,

* Based on historical literature records only with no current material available for verification by present-day taxonomists,

** Specimen material is available though the species is not presently considered identifiable,

✢ Specimen materials were too damaged or incomplete for valid identification by present-day taxonomists.

New records for genera *Chironephthya*, *Paraplexaura*, *Scleronephthya* and the species *Sinularia grandilobata* are reported in South Africa for the first time. (See Chapter 4 p. 109; Chapter 3 p. 47). Tentative identifications of several species are indicated in Table 5.1 with an explicative of their taxonomic status: material for two of the specimens were too damaged for valid identification; 26 species records were with that did not have any material available for examination were based on historical literature records; and twelve records were available for species that are not currently considered identifiable by present-day taxonomists. Only one species, *Tubipora musica*, appears on the IUCN Red list of Threatened species (Table 5.1). This stoloniferous taxon is one of only seven alcyonacean species assessments available and was determined as 'near threatened' in 2008 though it's status is in need of an update (Obura et al. 2008).

A summary of the families and genera within their designated subordinal groups is provided in Figure 5.1. Alcyoniidae exhibited the greatest diversity of all the families

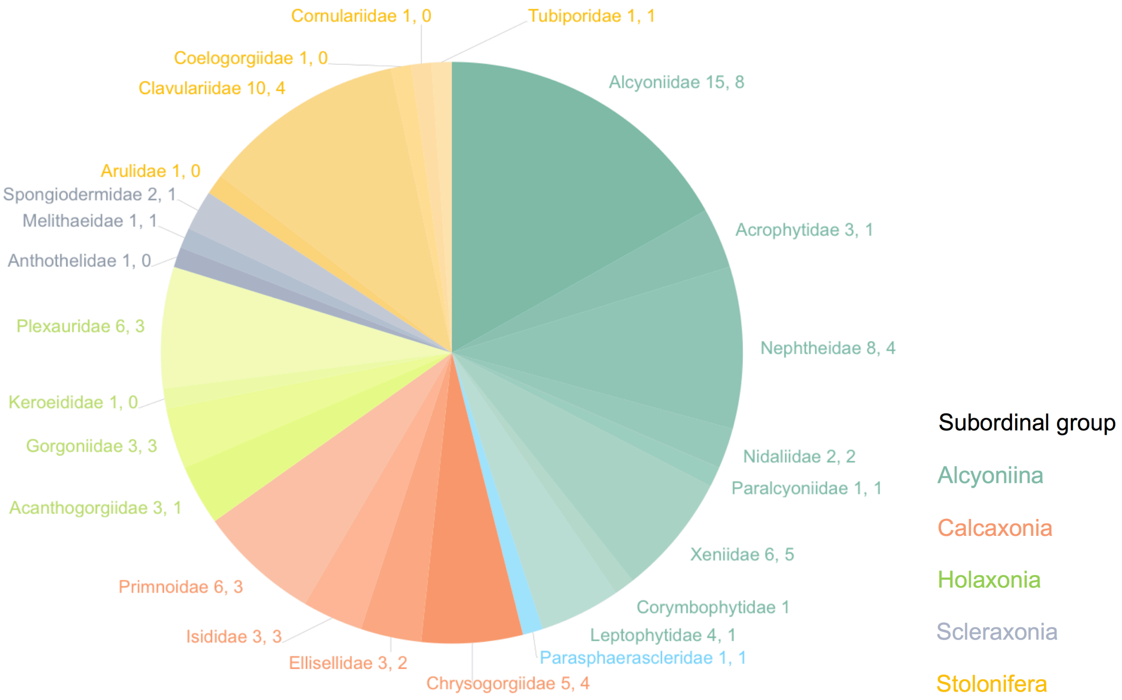

Figure 5.1 Families of Alcyonacea and their respective genera from South Africa and the iSimangaliso Wetland Park. Families are colour-coded according to their subordinal groups barring Parasphaerascleridae, which lies outside of these groups.

with the highest number of genera in South Africa (15) and in the iSWP (8). Alcyoniina was the dominant subordinal group and constituted 45% of the taxa followed by Calcaxonia (19%), Stolonifera (16%), Holaxonia (15%), and, Scleraxonia (5%). No records exist for taxa belonging to the Protoalcyonaria group in South Africa. Within the iSWP, 44% of the taxa constituted the Alcyoniina group followed by Calcaxonia (26%), Holaxonia (15%) Stolonifera (11%) and Scleraxonia (4%).

5.5 Discussion

This work provides a comprehensive national checklist for valid species of Alcyonacea since the last recorded estimates over two decades ago. Taxonomic estimates have been updated to include three newly recorded genera, *Scleronephthya*, *Paraplexaura* and *Chironephthya* and the first record of *Sinularia grandilobata* from this region. The total recorded estimate of Alcyonacea species has almost doubled since the last report by Williams (2000b, a) from 130 to 226 species as a result of this literature synthesis and additional taxonomic work.

Almost a third of Alcyonacea species recorded in South Africa are endemic (32%), however, the majority of taxonomic studies are concentrated in the Agulhas Bioregion which consequently accounts for most of the reported endemism (Williams 2000b, Williams & Starmer 2000, Williams & Little 2001, McFadden & van Ofwegen 2012a, 2013, Mcfadden & van Ofwegen 2017). This ecoregion spans the Cape Peninsula (Atlantic Ocean) to southern Kwa-Zulu Natal (Indian Ocean) and ranges from temperate to sub-tropical conditions respectively (Griffiths et al. 2010). Recent taxonomic revisions of taxa from the Agulhas Bioregion have increased the number of recognized endemic species to 40, where 13 of the recorded 18 genera are endemic to the region (Mcfadden & van Ofwegen 2017). Four endemic families have been described from the Agulhas Bioregion that include Acrophytidae, Cornulariidae, Corymbophytidae and Leptophytidae (McFadden & van Ofwegen 2012a, Mcfadden & van Ofwegen 2017).

Northern KwaZulu-Natal falls within the Delagoa Bioregion, an ecoregion which is characterized by a transition from tropical to subtropical conditions and is maintained by the warm Agulhas current (Riegl et al. 1995, Porter et al. 2013). Due to the unique nature of these reefs, high endemism and biodiversity is forecast (Benayahu & Schleyer 1996, Gibbons et al. 1999, Schleyer 1999, Schleyer & Celliers 2003, Schleyer et al. 2008) though these reefs remain less studied than the Agulhas Bioregion. Species estimates for the iSWP, as a result of this work and collated taxonomic records (Williams 1989b, 1992b, Benayahu 1993, van Ofwegen & Schleyer 1997, Benayahu et al. 2003, Schleyer & Celliers 2003), increased from 37 to 81 species, 11 to 47 genera and 4 to 17 families. Despite anticipated high levels of endemism, relatively few endemic taxa have been recorded in the iSWP (16%) (Benayahu 1993, Schleyer & Celliers 2003). These limited records are an artefact of the lack of research focus in this region. Furthermore, a third of the species recorded for this region require species-level identifications and/or descriptions that require taxonomic expertise. These unidentified taxa may potentially form new records or new species which would consequently increase endemism estimates.

5.6 Conclusion

This updated reference list provides the basis for studies of species diversity in South Africa, levels of endemism, protected species, invasive species and monitoring studies, all of which can be utilized in management decisions. However, these estimates are only as accurate as their taxonomic identifications- many specimens remain undescribed or in need of revision. Species diversity is dynamic and demands continuous attention. The Alcyonacea in South Africa require significant taxonomic consideration and we are only recently starting to realize the extent of soft coral biodiversity in this region and simultaneously compete with the effects of climate change and mounting anthropogenic forces.

5.7 Acknowledgements

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5.8 References

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CHAPTER 6: CONCLUSIVE DISCUSSION

The biodiversity of shallow water soft corals in South Africa was investigated using multilocus DNA barcodes. Identifications were carried out for taxonomically difficult alcyonacean genera that dominate shallow reefs in north eastern South Africa. The first chapter of this thesis introduced the importance of octocorals and the need for approaches that combine traditional morphological and molecular taxonomic approaches. Chapter two highlighted the use of three genetic markers (*COI+MutS+28S*) in determining taxonomic boundaries in closely related alcyoniid genera; *Aldersladum*, *Cladiella* and *Klyxum*. Molecular phylogenies corroborated the reassignment of morphologically aberrant members of *Cladiella* to a new genus. The proposed new genus is characterised by an absence of platelet sclerites in the polyp region and the presence of double heads with complex warts in the coenenchyme.

Chapter three was an investigation into the species diversity of *Sinularia* in the iSimangaliso Wetland Park (iSWP) using multiple loci. Phylogenetic and species delimitation methods provided generally congruent estimates of species richness and facilitated species-level identifications for two thirds of the collected specimens. A new zoogeographic record for *Sinularia grandilobata* was discovered. Twenty of the collected specimens require additional expertise for taxonomic identification, these unidentified specimens were assigned amongst 16 putative species that constitute new records or undescribed species. Hybridization and incomplete lineage assortment are evolutionary factors contributing to the complex taxonomy of these local octocoral communities. *Sinularia brassica* showed evidence of a species complex with two highly supported monophyletic clades and two separate clusters of possible cryptic species that exhibited two morphologically distinct colony forms.

Chapter four focused on the biodiversity of Alcyonacea of the iSWP using molecular systematics. This study presents the first ever phylogenetic analysis of the Alcyonacea community from this marine reserve. Sixty putative species were inferred from phylogenies, divergence estimates, colony photos and basic morphology for specimens collected in this study. Molecular evidence was presented for proposed reassignments of non-*S. brassica* congeners of *Sinularia* to a new genus due to large scale paraphyly. *Sinularia*, *Lobophytum* and *Sarcophyton* comprise the three major Alcyonacea genera in the study region that exhibit taxonomic complexity; additional putative species were recovered that require description. These three genera require further genetic and systematic attention to elucidate their diversity and ecology. New zoogeographic records were reported for the genera *Chironephthya*; *Scleronephthya* and *Paraplexaura*. The result of this work, in combination with previous records, revised biodiversity estimates for the iSWP from 37 to 81 species (with 12% endemism), 11 to 47 genera and 4 to 17 families.

Chapter 5 consisted of a comprehensive review of the Alcyonacea in South Africa. Approximately 226 species within 89 genera, among 25 families of Alcyonacea were reported, of which 75 species were endemic (32%). This national estimate has nearly doubled since the last published estimate of 130 species and includes new records from the results of Chapters 2 to 4.

Species-level assignments in octocorals are generally challenging and therefore carried out by specialist taxonomists. Due to the current state of octocoral taxonomy and the lack of expertise available, identifications of species is very time-consuming and may take months or up to a year to reliably identify specimens in extensive collections. Species designations for all the taxa in this thesis would increase the accuracy of current biodiversity estimates of Alcyonacea for South Africa (especially for the iSWP). Furthermore, extensive species assignations would increase the genetic impact of this work: evolutionary processes may be further elucidated, and genetic markers may be more aptly assessed for their barcoding potential in this diverse group.

This thesis culminated in a revised and updated national species list for South African Alcyonacea. It has provided taxonomic and evolutionary insight into the biodiversity of soft corals in the Delagoa bioregion, provided critical evidence to establish at least one new genus, provided new species records and helped resolve several problematic species assignations.

APPENDIX A: COLLECTION DATA FOR SPECIMENS FOR EACH CHAPTER

Data for specimens collected and utilized in this research are presented for chapters 2-4.

Table 7.1 Collection data for taxa under study for Chapter 2

Sample ID	**Scientific name**	**Date**	**Site**	**Location**	**Latitude (S)**	**Longitude (E)**	**Depth (m)**	**Method**	**Collector**
Ro1	*Aldersladum sodwanum*	06-06-2015	Isipingo Rocks	Durban	-30.00455	30.942	1	Snorkel	Roy Jackson
Ro2	*Aldersladum sodwanum*	06-06-2015	Isipingo Rocks	Durban	-30.00455	30.942	1	Snorkel	Roy Jackson
Ro3	*Aldersladum sodwanum*	06-06-2015	Isipingo Rocks	Durban	-30.00455	30.942	1	Snorkel	Roy Jackson
Ro4	*Aldersladum sodwanum*	06-06-2015	Isipingo Rocks	Durban	-30.00455	30.942	1	Snorkel	Roy Jackson
Ri1	*Cladiella* sp.	06-06-2015	Isipingo Rocks	Durban	-30.00455	30.942	1	Snorkel	Roy Jackson
Ri2	*Cladiella* sp.	06-06-2015	Isipingo Rocks	Durban	-30.00455	30.942	1	Snorkel	Roy Jackson
Ri3	*Cladiella* sp.	06-06-2015	Isipingo Rocks	Durban	-30.00455	30.942	1	Snorkel	Roy Jackson
61	Beta sp.	04-12-2014	Kosi Bay, Saxon Reef	iSWP	-26,92641	32,88771	11	Scuba	Kerry Etsebeth
79	Beta sp.	04-12-2014	Leadsman Shoal	iSWP	-27,87455	32,60641	19	Scuba	Kerry Etsebeth
84	Beta sp.	04-12-2014	Leadsman Shoal	iSWP	-27,87465	32,60798	11	Scuba	Kerry Etsebeth
112	Beta sp.	04-12-2014	Sodwana Bay, Two Mile reef	iSWP	-27,51815	32,68986	18	Scuba	Kerry Etsebeth
113	Beta sp.	04-12-2014	Sodwana Bay, Two Mile reef	iSWP	-27,52223	32,68668	11	Scuba	Kerry Etsebeth
134	Beta sp.	27-04-2015	Kosi Bay, Tridacna	iSWP	-26,92825	32,88568	9,5	Scuba	Kerry Etsebeth

Table 7.1 continued…

Sample ID	Scientific name	Date	Site	Location	Latitude (S)	Longitude (E)	Depth (m)	Method	Collector
135	Beta sp.	27-04-2015	Kosi Bay, Tridacna	iSWP	-26,92825	32,88568	9,5	Scuba	Kerry Etsebeth
142	Beta sp.	27-04-2015	Kosi Bay, Tridacna	iSWP	-26,92825	32,88568	9,5	Scuba	Kerry Etsebeth
157	Beta sp.	27-04-2015	Kosi Bay, Gorgonin	iSWP	-26,92806	32,88753	14,3	Scuba	Kerry Etsebeth
160	Beta sp.	27-04-2015	Kosi Bay, Gorgonin	iSWP	-26,92806	32,88753	14,3	Scuba	Kerry Etsebeth
165	Beta sp.	27-04-2015	Anton’s Reef	iSWP	-27,52783	32,68481	15,9	Scuba	Kerry Etsebeth
192	Beta sp.	28-04-2015	Seven Mile Reef	iSWP	-27,4515	32,7118	20,7	Scuba	Kerry Etsebeth
194	Beta sp.	28-04-2015	Seven Mile Reef	iSWP	-27,4515	32,7118	17,6	Scuba	Kerry Etsebeth
195	Beta sp.	28-04-2015	Seven Mile Reef	iSWP	-27,4515	32,7118	17,6	Scuba	Kerry Etsebeth
198	Beta sp.	28-04-2015	Nine Mile Reef	iSWP	-27,4148	32,72661	17,6	Scuba	Kerry Etsebeth
209	Beta sp.	28-04-2015	Nine Mile Reef	iSWP	-27,4148	32,72661	17,6	Scuba	Kerry Etsebeth
215	Beta sp.	28-04-2015	Nine Mile Reef	iSWP	-27,4148	32,72661	17,6	Scuba	Kerry Etsebeth
220	Beta *kashmani*	28-04-2015	Nine Mile Reef	iSWP	-27,4148	32,72661	16,8	Scuba	Kerry Etsebeth
221	Beta sp.	28-04-2015	Nine Mile Reef	iSWP	-27,4148	32,72661	16,8	Scuba	Kerry Etsebeth
236	*Aldersladum sodwanum*	28-04-2015	Nine Mile Reef	iSWP	-27,4148	32,72661	16,8	Scuba	Kerry Etsebeth
248	Beta sp.	29-04-2015	Sodwana Bay, Two Mile reef	iSWP	-27,52105	32,6867	12,8	Scuba	Kerry Etsebeth
252	Beta sp.	29-04-2015	Sodwana Bay, Two Mile reef	iSWP	-27,52105	32,6867	12,8	Scuba	Kerry Etsebeth

Table 7.2 Collection data for taxa under study for Chapter 3

Sample	Scientific name	Date	Site	Latitude (S)	Longitude (E)	Depth max (m)	Collector
2	*Sinularia* sp.	15-05-2014	Leadsman Shoal	-27 52.513	32 36.370	18	Kerry Etsebeth
4	*Sinularia* sp.	15-05-2014	Leadsman Shoal	-27 52.513	32 36.370	18	Kerry Etsebeth
5	*Sinularia* sp.	15-05-2014	Leadsman Shoal	-27 52.513	32 36.370	18	Kerry Etsebeth
7	*S. abrupta*	15-05-2014	Leadsman Shoal	-27 52.513	32 36.370	18	Kerry Etsebeth
8	*S. heterospiculata*	15-05-2014	Leadsman Shoal	-27 52.513	32 36.370	18	Kerry Etsebeth
9	*Sinularia* sp.	15-05-2014	Leadsman Shoal	-27 52.513	32 36.370	18	Kerry Etsebeth
10	*Sinularia* sp.	15-05-2014	Leadsman Shoal	-27 52.513	32 36.370	18	Kerry Etsebeth
12	*S. abrupta*	15-05-2014	Leadsman Shoal	-27 52.350	32 36.354	18	Kerry Etsebeth
17	*Sinularia* sp.	15-05-2014	Leadsman Shoal	-27 52.350	32 36.354	18	Kerry Etsebeth
25	*S. abrupta*	15-05-2014	Leadsman Shoal	-27 52.328	32 36.340	18	Kerry Etsebeth
31	*S. variabilis*	19-05-2014	Nine Mile Reef	-27 24.818	32 43,647	18	Kerry Etsebeth
36	*Sinularia* sp.	19-05-2014	Nine Mile Reef	-27 24.818	32 43,647	18	Kerry Etsebeth
50	*S. abrupta*	19-05-2014	Two Mile Reef	-27 31.257	32 41.384	18	Kerry Etsebeth
55	*Sinularia* sp.	19-05-2014	Two Mile Reef	-27 31.257	32 41.384	18	Kerry Etsebeth
56	*Sinularia* sp.	19-05-2014	Two Mile Reef	-27 31.257	32 41.384	18	Kerry Etsebeth
57	*S. notanda*	19-05-2014	Two Mile Reef	-27 31.257	32 41.384	18	Kerry Etsebeth
60	*Sinularia* sp.	4-12-2014	Kosi Bay, Saxon Reef	-26.92641	32.88771	11	Kerry Etsebeth

Table 7.2 continued…

Sample	Scientific name	Date	Site	Latitude (S)	Longitude (E)	Depth max (m)	Collector
66	*S. heterospiculata*	4-12-2014	Kosi Bay, Saxon Reef	-26.92642	32.88772	11	Kerry Etsebeth
67	*Sinularia* sp.	4-12-2014	Kosi Bay, Saxon Reef	-26.92643	32.88773	11	Kerry Etsebeth
73	*S. brassica*	4-12-2014	Nine Mile Reef	-27.41491	32.72666	12	Kerry Etsebeth
75	*Sinularia* sp.	5-12-2014	Leadsman Shoal	-27.87455	32.60641	19	Kerry Etsebeth
80	*S. heterospiculata*	5-12-2014	Leadsman Shoal	-27.87455	32.60643	19	Kerry Etsebeth
85	*Sinularia* sp.	5-12-2014	Leadsman Shoal	-27.87465	32.6079	11	Kerry Etsebeth
93	*S. brassica*	5-12-2014	Leadsman Shoal	-27.87465	32.6079	11	Kerry Etsebeth
103	*S. brassica*	6-12-2014	Two Mile Reef	-27.51815	32.68986	18	Kerry Etsebeth
131	*S. abrupta*	27-04-2015	Tridacna Reef	-26.92825	32.8856	9,5	Kerry Etsebeth
139	*S. erecta*	27-04-2015	Tridacna Reef	-26.92825	32.8856	9,5	Kerry Etsebeth
141	*S. heterospiculata*	27-04-2015	Tridacna Reef	-26.92825	32.8856	9,5	Kerry Etsebeth
143	*S. brassica*	27-04-2015	Tridacna Reef	-26.92825	32.8856	9,5	Kerry Etsebeth
146	*S. heterospiculata*	27-04-2015	Tridacna Reef	-26.92825	32.8856	14,3	Kerry Etsebeth
147	*Sinularia* sp.	27-04-2015	Gorgonin Reef	-26.92806	32.8875	14,3	Kerry Etsebeth
148	*S. heterospiculata*	27-04-2015	Gorgonin Reef	-26.92806	32.8875	14,3	Kerry Etsebeth
151	*S. brassica*	27-04-2015	Gorgonin Reef	-26.92806	32.8875	14,3	Kerry Etsebeth
153	*S. brassica*	27-04-2015	Gorgonin Reef	-26.92806	32.8875	14,3	Kerry Etsebeth
164	*S. heterospiculata*	27-04-2015	Anton’s Reef	-27.5278	32.6848	14,3	Kerry Etsebeth
168	*S. brassica*	27-04-2015	Anton’s Reef	-27.5278	32.6848	17,9	Kerry Etsebeth

Table 7.2 continued…

Sample	**Scientific name**	**Date**	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth max (m)**	**Collector**
172	*S. brassica*	27-04-2015	Anton’s Reef	-27.5278	32.6848	17,9	Kerry Etsebeth
183	*S. heterospiculata*	28-04-2015	Seven Mile Reef	-27.4515	32.7118	20,7	Kerry Etsebeth
188	*S. brassica*	28-04-2015	Seven Mile Reef	-27.4515	32.7118	20,7	Kerry Etsebeth
189	*S. heterospiculata*	28-04-2015	Seven Mile Reef	-27.4515	32.7118	20,7	Kerry Etsebeth
191	*S. variabilis*	28-04-2015	Seven Mile Reef	-27.4515	32.7118	20,7	Kerry Etsebeth
199	*S. erecta*	28-04-2015	Nine Mile Reef	-27.4148	32.72661	17,6	Kerry Etsebeth
200	*S. brassica*	28-04-2015	Nine Mile Reef	-27.4148	32.72661	17,6	Kerry Etsebeth
214	*S. heterospiculata*	28-04-2015	Nine Mile Reef	-27.4148	32.72661	17,6	Kerry Etsebeth
224	*Sinularia* sp.	28-04-2015	Nine Mile Reef	-27.41496	32.72675	16,8	Kerry Etsebeth
225	*S. heterospiculata*	28-04-2015	Nine Mile Reef	-27.41496	32.72675	16,8	Kerry Etsebeth
226	*Sinularia* sp.	28-04-2015	Nine Mile Reef	-27.41496	32.72675	16,8	Kerry Etsebeth
227	*S. notanda*	29-04-2015	Nine Mile Reef	-27.41496	32.72675	12,8	Kerry Etsebeth
234	*S. abrupta*	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth
235	*S. notanda*	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth
237	*S. variabilis*	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth
239	*Sinularia* sp.	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth
240	*Sinularia* sp.	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth
243	*S. abrupta*	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth
245	*Sinularia* sp.	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth

Table 7.2 continued…

Sample	**Scientific name**	**Date**	**Site**	**Latitude (S)**	**Longitude (E)**	**Depth max (m)**	**Collector**
246	*Sinularia* sp.	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth
247	*S. heterospiculata*	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth
249	*Sinularia* sp.	29-04-2015	Four Buoy	-27.52105	32.6867	12,8	Kerry Etsebeth
257	*Sinularia* sp.	29-04-2015	Four Buoy	-27.52105	32.6867	16,8	Kerry Etsebeth
263	*Sinularia* sp.	29-04-2015	Sponges/Chain	-27.5271	32.6868	16,8	Kerry Etsebeth
268	*S. grandilobata*	29-04-2015	Sponges/Chain	-27.5271	32.6868	16,8	Kerry Etsebeth
269	*S. notanda*	29-04-2015	Sponges/Chain	-27.5271	32.6868	16,8	Kerry Etsebeth
271	*Sinularia* sp.	29-04-2015	Sponges/Chain	-27.5271	32.6868	16,8	Kerry Etsebeth
274	*S. variabilis*	29-04-2015	Sponges/Chain	-27.5271	32.6868	16,8	Kerry Etsebeth
275	*S. variabilis*	29-04-2015	Sponges/Chain	-27.5271	32.6868	16,8	Kerry Etsebeth
279	*Sinularia* sp.	29-04-2017	Sponges/Chain	-27.5271	32.6868	16,8	Kerry Etsebeth
280	*S. erecta*	29-04-2018	Sponges/Chain	-27.5271	32.6868	16,8	Kerry Etsebeth

APPENDIX B: GENBANK SEQUENCES SOURCED FROM PREVIOUS STUDIES

Voucher numbers and GenBank accession numbers for sequences from previously published studies are presented for each chapter.

Table 7.3 GenBank sequences for voucher specimens from other published authors used in phylogenetic analysis for Chapter 2

Genus	Species	Voucher number	*COI*	*MutS*	*28S rRNA*
Alderseladum	*sodwanum*	ZMTAU CO31520	-	JX991193.1	JX991213.1
Cladiella	*australis*	ZMTAU CO33613	JX991221	-	JX991202.1
Cladiella	*australis*	ZMTAU CO33622	-	-	JX991203.1
Cladiella	*bottai*	ZMTAU CO34648	JX991223	-	JX991204.1
Cladiella	*kashmani*	ZMTAU CO32334	-	JX991195.1	JX991215.1
Cladiella	*kashmani*	ZMTAU CO32482	-	JX991196.1	-
Cladiella	*kashmani*	ZMTAU CO32246	-	JX991194.1	JX991214.1
Cladiella	*krempfi*	ZMTAU CO33592	JX991224	-	JX991205.1
Cladiella	*pachyclados*	ZMTAU CO33604	-	JX991146.1	JX991206.1
Cladiella	*pachyclados*	ZMTAU CO35507	-	JX991197.1	JX991216.1
Cladiella	*pachyclados*	ZMTAU CO34129	GU355976.1	GU356011.1	-
Cladiella	*pachyclados*	ZMTAU CO34130	GU355977.1	-	-
Cladiella	*pachyclados*	ZMTAU CO35508	-	JX991198.1	-
Cladiella	sp.	QM:G334170	-	HG970087.1	HG970073.1
Cladiella	sp.	NTM C012095	-	DQ302807.1	-
Cladiella	*sphaerophora*	ZMTAU CO34132	-	GQ342471.1	JX203653.1
Cladiella	*tuberculoides*	ZMTAU CO34686	-	-	JX991208.1
Cladiella	*tuberculoides*	ZMTAU CO34642	-	JX991147.1	JX991207.1
Cladiella	*tuberculoides*	ZMTAU CO34686	JX991227	JX991148.1	-
Cladiella	*tuberosa*	ZMTAU CO34669	JX991228	-	JX991209.1
Klyxum	*adii*	ZMTAU:CO32636	JX991242.1	JX991199.1	JX991217.1
Klyxum	*utinomii*	ZMTAU:CO34127	GQ342392.1	GQ342476.1	JX203654.1
Klyxum	*utinomii*	ZMTAU:CO34639	JX991232.1	JX991151.1	JX991212.1

NTM=Museum and Art Gallery of the Northern Territory; QM = Queensland Museum; ZMTAU = Zoological Museum, Tel Aviv University. “-“ no sequences available.

Table 7.4 GenBank sequences for voucher specimens from other published authors used in phylogenetic analyses for Chapter 3.

***Sci*entific name**	**Voucher number**	***MutS***	***28S rRNA***	**Clade**
S. brassica	RMNH:Coel. 41306	KF915730.1	KF915494.1	1
S. brassica	RMNH:Coel. 41309	KF915729.1	KF915493.1	1
S. brassica	RMNH:Coel. 41305	KF915728.1	KF915492.1	1
S. brassica	RMNH:Coel. 41304	KF915727.1	KF915491.1	1
S. brassica	RMNH:Coel. 41308	KF915726.1	KF915490.1	1
S. brassica	RMNH:Coel. 41303	KF915725.1	KF915489.1	1
S. brassica	RMNH:Coel. 41307	KF915724.1	KF915488.1	1
S. dura	NTM C13808	FJ621402.1	-	1
S. grandilobata	RMNH:Coel. 41313	KF915745.1	-	2
S. grandilobata	RMNH:Coel. 41312	KF915744.1	-	2
S. loyai	ZMTAU CO4154	FJ621442.1	-	2
S. flaccida	RMNH:Coel. 38731	FJ621408.1	-	2
S. fungoides	RMNH:Coel. 41314	KF915739.1	KF915502.1	3
S. querciformis	ZMTAU CO34096	FJ621469.1	JX203652.1	4A
S. flexibilis	RMNH:Coel. 41315	KF915737.1	KF915500.1	4A
S. variabilis	NTM C14164	FJ621486.1	-	4A
S. variabilis	NTM C14134	FJ621485.1	-	4A
S. cruciata	ZMTAU CO34152	FJ621395.1	-	4B
S. heterospiculata	RMNH:Coel. 41320	KF915747.1	KF915508.1	4B
S. heterospiculata	NTM C14003	FJ621426.1	-	4B
S. heterospiculata	NTM C13968	FJ621425.1	-	4B
S. ultima	RMNH:Coel. 41324	KF915763.1	KF915523.1	4B
S. sublimis	RMNH:Coel. 41323	KF915760.1	-	4B
S. humilis	RMNH:Coel. 41318	KF915752.1	KF915514.1	4B
S. sobolifera	RMNH:Coel. 38748	FJ621479.1	-	4B
S. levi	ZMTAU CO34138	FJ621466.1	-	4B
S. numerosa	NTM C13790	FJ621453.1	-	4B
S. notanda	NTM C14555	FJ621452.1	-	4B
S. curvata	RMNH:Coel. 38436	FJ621396.1	-	4B
S. gravis	RMNH:Coel. 41328	KF915746.1	-	4C
S. gravis	RMNH:Coel. 38736	FJ621422.1	-	4C
S. conferta	NTM C13972	FJ621389.1	-	4D
S. ornate	ZMTAU CO34651	JX991176.1	-	4D
S. polydactyla	ZMTAU CO34142	FJ621387.1	-	4D
S. polydactyla	ZMTAU CO34140	FJ621386.1	-	4D
S. polydactyla	ZMTAU CO34150	FJ621388.1	-	4D
Sinularia sp.	RMNH:Coel. 38420	FJ621385.1	-	4D
Sinularia sp.	RMNH:Coel. 41339	KF915753.1	KF915515.1	4D
S. ceramensis	RMNH:Coel. 38442	FJ621464.1	-	4D
S. mauritiana	NTM C13852	FJ621445.1	-	5A
S. humesi	UF 3500	FJ621431.1	-	5A

Table 7.4 continued…

***Sci*entific name**	**Voucher number**	***MutS***	***28S rRNA***	**Clade**
S. gardineri	ZMTAU CO34097	FJ621414.1	KC542819.1	5A
S. hirta	ZMTAU CO34100	FJ621428.1	KC542820.1	5B
S. australiensis	NTM C14519	FJ621438.1	KC542825.1	5C
S. australiensis	NTM C14492	FJ621437.1	KC542824.1	5C
S. leptoclados	ZMTAU CO35308	KC542857.1	KC542836.1	5C
S. leptoclados	ZMTAU CO34095	FJ621439.1	KC542837.1	5C
S. densa	RMNH:Coel. 40840	KC542851.1	-	5C
S. densa	NTM C1993	FJ621397.1	-	5C
S. abrupta	NTM C14012	KC542849.1	-	5C
S. abrupta	ZMTAU CO33623	JX991168.1	KC542822.1	5C
S. abrupta	NTM C13799	FJ621374.1	-	5C
S. molesta	NTM C3755	FJ621475.1	-	5C
S. muralis	NTM C13978	FJ621450.1	-	5C
S. erecta	ZMTAU CO34144	FJ621404.1	KC542835.1	5C
S. erecta	NTM C12557	FJ621403.1	-	5C

NTM=Museum and Art Gallery of the Northern Territory; QM = Queensland Museum; ZMTAU = Zoological Museum, Tel Aviv University. “-“ no data available.

Table 7.5 GenBank sequences for voucher specimens from previously published authors used in phylogenetic analyses for Chapter 4.

Scientific name	Voucher number	*MutS*	*28S rRNA*
Aldersladum sodwanum	ZMTAU CO31520	JX991193.1	JX991213.1
Anthelia glauca	ZMTAU CO36508	KM371369.1	KM224954.1
Anthelia glauca	ZMTAU CO34183	JX203812.1	JX203753.1
Chironephthya sp.	CSM-2010	GQ342513.1	JX203730.1
Chironephthya sp.	ZMTAU CO34203	-	JX203730.1
Chironephthya sp.	RMNH:Coel. 40900	DQ302830.1	KF915348.1
Cladiella australis	ZMTAU CO33613	-	JX991202.1
Cladiella kashmani	ZMTAU CO32334	JX991195.1	JX991215.1
Cladiella krempfi	ZMTAU CO33592	-	JX991205.1
Cladiella pachyclados	ZMTAU CO33604	JX991146.1	JX991206.1
Dendronephthya mutabilis	SAF305	MG053060.1	MG053016.1
Dendronephthya sinaiensis	ZMTAU CO34163	GQ342506.1	JX124349.1
Euplexaura rhipidalis	ZMTAU CO34220	-	JX203735.1
Euplexaura sp.	CSM-2010	GQ342518.1	-
Euplexaura sp.	RMNH:Coel. 40929	KF915617.1	-
Heteroxenia fuscescens	ZMTAU CO34118	GQ342528.1	JX203756.1
Heteroxenia fuscescens	ZMTAU CO36506	-	KM224902.1
Junceella juncea	RMNH:Coel. 40932	KF915624.1	KF915382.1
Junceella fragilis	RMNH:Coel. 40931	KF915623.1	KF915381.1
Klyxum adii	ZMTAU:CO32636	JX991199.1	JX991151.1
Klyxum utinomii	ZMTAU:CO34639	JX991151.1	JX991212.1
Leptogorgia alba	MECN Ant0024	KX721200.1	KX721238.1
Leptogorgia sp.	MECN Ant0031	KX721204.1	KX721242.1
Litophyton sp.	NTM C011318	DQ302821.1	-
Lobophytum patulum	NTM-C013965	DQ280574.1	-
Lobophytum crassum	RMNH:Coel. 40954	KF915643.1	KF915408.1
Lobophytum crassum	RMNH:Coel. 40951	KF915642.1	KF915407.1
Lobophytum venustum	NTM C014525	DQ280587.1	-
Lobophytum venustum	NTM C014529	DQ280588.1	-
Litophyton sp.	1201948	KC864870.1	-

Table 7.5 continued…

Scientific name	**Voucher number**	***MutS***	***28S rRNA***
Nephthea elatensis	ZMTAU CO34112	GQ342508.1	JX203725.1
Nephthea sp.	RMNH:Coel. 40972	KF915652.1	-
Ovabunda impulsatilla	ZMTAU CO34896	KY442339.1	KY442385.1
Ovabunda faraunensis	ZMTAU CO34886	KY442335.1	KY442381.1
Paraplexaura sp.	NTM C014494	DQ302861.1	-
Parasphaerasclera aurea	RMNH:Coel. 40205	KF728099.1	KF728082.1
Parasphaerasclera aurea	RMNH:Coel. 41535	KF728100.1	KF728083.1
Parasphaerasclera rotifera	UF3890	GQ342472.1	JX203639.1
Parasphaerasclera valdiviae	RMNH:Coel. 40206	KF728098.1	KF728086.1
Parasphaerasclera valdiviae	RMNH:Coel. 41532	KF728097.1	KF728085.1
Rumphella sp.	RMNH:Coel. 40994	KF915668.1	KF915434.1
Rumphella sp.	RMNH:Coel. 40996	KF915669.1	KF915435.1
Rumphella sp.	RMNH:Coel. 40816	JX203797.1	JX203715.1
Sansibia sp.	NTM C012955	DQ302840.1	-
Sarcophyton boettgeri	NTM C014181	DQ280501.1	-
Sarcophyton crassocaule	NTM-013916	DQ280507.1	-
Sarcophyton crassocaule	RMNH:Coel. 33076	DQ280508.1	-
Sarcophyton crassum	RMNH:Coel. 41018	KF915678.1	KF915444.1
Sarcophyton crassum	RMNH:Coel. 41022	KF915679.1	KF915445.1
Sarcophyton ehrenbergi	RMNH:Coel. 40968	KF915690.1	KF915455.1
Sarcophyton ehrenbergi	NTM C14455	-	JX203650.1
Sarcophyton elegans	RMNH:Coel. 41011	KF915694.1	-
Sarcophyton gemmatum	ZMTAU CO34057	GU356016.1	-
Sarcophyton glaucum	RMNH:Coel. 41020	KF915708.1	KF915473.1
Sarcophyton glaucum	RMNH:Coel. 41019	-	KF915472.1
Sarcophyton glaucum	RMNH:Coel. 41036	-	KF915477.1
Sarcophyton glaucum	RMNH:Coel. 33072	DQ280537.1	-
Sarcophyton glaucum	RMNH:Coel.41032	-	KF915475.1
Sarcophyton glaucum	RMNH:Coel.41028	-	KF915474.1
Sarcophyton glaucum	RMNH:Coel. 41027	-	KF915478.1
Sarcophyton glaucum	RMNH:Coel. 41033	KF915712.1	KF915476.1
Sarcophyton glaucum	ZMTAU CO34063	GU356015.1	-

Table 7.5 continued…

Scientific name	**Voucher number**	***MutS***	***28S rRNA***
Sarcophyton glaucum	RMNH:Coel. 33074	DQ280538.1	-
Sarcophyton glaucum	RMNH:Coel. 33070	DQ280536.1	-
Sarcophyton glaucum	NTM C014900	DQ280539.1	-
Sarcophyton glaucum	NTM C014907	DQ280540.1	-
Sarcophyton glaucum	NTM C014153	DQ280534.1	-
Sarcophyton mililatensis	RMNH:Coel. 40969	-	KF915479.1
Sarcophyton nanwanensis	RMNH:Coel. 33078	DQ280542.1	-
Sarcophyton patulum	NTM C013965	DQ280574.1	-
Sarcophyton pauciplicatum	ZMTAU CO34135	GU356014.1	-
Sarcophyton regulare	NTM C013982	DQ280543.1	-
Sarcophyton tumulosum	ZMTAU CO34698	JX991166.1	-
Sarcophyton tumulosum	ZMTAU CO34710	JX991167.1	-
Sarcophyton tortuosum	NTM C013896	DQ280545.1	-
Sarcophyton trocheliophorum	RMNH:Coel. 41035	-	KF915483.1
Sarcophyton trocheliophorum	RMNH:Coel. 41034	KF915718.1	KF915482.1
Scleronephthya sp.	NTM C011489	DQ302825.1	-
Sinularia brassica	RMNH:Coel. 41306	KF915730.1	KF915494.1
Sinularia brassica	RMNH:Coel. 41305	KF915728.1	KF915492.1
Sinularia brassica	RMNH:Coel. 41304	KF915727.1	KF915491.1
Sinularia brassica	RMNH:Coel. 41308	KF915726.1	KF915490.1
Sinularia brassica	RMNH:Coel. 41303	KF915725.1	KF915489.1
Sinularia dura	NTM C13808	FJ621402.1	-
Sinularia erecta	ZMTAU CO34144	FJ621404.1	KC542835.1
Sinularia grandilobata	RMNH:Coel. 41313	KF915745.1	KF915507.1
Sinularia heterospiculata	RMNH:Coel. 41320	KF915747.1	KF915508.1
Sinularia leptoclados	ZMTAU CO35308	KC542857.1	-
Sinularia notanda	NTM C14555	FJ621452.1	-
Sinularia polydactyla	ZMTAU CO34142	FJ621387.1	-
Sinularia variabilis	NTM C14134	FJ621485.1	-
Stereonephthya sp.	RMNH:Coel. 41062	KF915788.1	KF915542.1
Stereonephthya cundabiluensis	ZMTAU CO34204	GQ342512.1	JX124351.1
Sympodium caeruleum	ZMTAU CO34185		JX203758.1

Table 7.5 continued…

Scientific name	**Voucher number**	***MutS***	***28S rRNA***
Sympodium sp.	CSM-2016	KT861619.1	-
Verrucella sp.	RMNH Coel.40030	-	JX203704.1
Verrucella sp.	NTM C014982	DQ302864.1	-
Xenia puertogalerae	CAS:IZ:184545	KJ511364.1	KJ511326.1
Xenia puertogalerae	CAS:IZ:184539	KJ511363.1	KJ511325.1

NTM=Museum and Art Gallery of the Northern Territory; ZMTAU = Zoological Museum, Tel Aviv University; RMNH:Coel Regional Museum of Natural History, New Delhi. “-“ no data available.

APPENDIX C: PAIRWISE DISTANCES (UNCORRECTED *P*)

The number of base substitutions per site averaging over all sequence pairs (uncorrected *p*) between *Aldersladum*, *Cladiella*, Beta and *Klyxum* specimens in Chapter 2 are shown for DNA barcodes *COI*, *MutS*, *28S rRNA* and *COI*+*MutS*+*28S* respectively.

Table 7.6 Estimates of evolutionary divergence over sequence pairs (uncorrected *p*) between taxa for *COI* (Chapter 2).

Sample	61	79	84	112	113	134	135	142	157	160	165	192	194	195	198	209	215	220	221	248	252	Ri1	Ri2	Ri3	Ro1	Ro2	Ro3	Ro4	*A. sodwanum* ZMTAU CO31520	*C. pachyclados* ZMTAU CO35507	*C. pachyclados* ZMTAU CO33604	*C. australis* ZMTAU CO33613	*C. kashmani* ZMTAU CO32246	*C. kashmani* ZMTAU CO32334	*C. tuberosa* ZMTAU CO34669	*C. krempfi* ZMTAU CO33592	*C. tuberculoides* ZMTAU CO34686	*C. bottai* ZMTAU CO34648	*C. tuberculoides* ZMTAU CO34642	*C. pachyclados* ZMTAU CO35507	*C. pachyclados* ZMTAU CO33604	*K. utinomii* ZMTAU CO34639	*K. adii* ZMTAU CO32636 I
79	0,000																																										
84	0,004	0,004																																									
112	0,004	0,004	0,007																																								
113	0,000	0,000	0,004	0,004																																							
134	0,001	0,001	0,002	0,005	0,001																																						
135	0,000	0,000	0,004	0,004	0,000	0,001																																					
142	0,000	0,000	0,004	0,004	0,000	0,001	0,000																																				
157	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000																																			
160	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000																																		
165	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000																																	
192	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000																																
194	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000																															
195	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000																														
198	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																													
209	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																												
215	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																											
220	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																										
221	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																									
248	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																								

Table 7.6 continued…

Sample	**61**	**79**	**84**	**112**	**113**	**134**	**135**	**142**	**157**	**160**	**165**	**192**	**194**	**195**	**198**	**209**	**215**	**220**	**221**	**248**	**252**	**Ri1**	**Ri2**	**Ri3**	**Ro1**	**Ro2**	**Ro3**	**Ro4**	***A. sodwanum* ZMTAU CO31520**	***C. pachyclados* ZMTAU CO35507**	***C. pachyclados* ZMTAU CO33604**	***C. australis* ZMTAU CO33613**	***C. kashmani* ZMTAU CO32246**	***C. kashmani* ZMTAU CO32334**	***C. tuberosa* ZMTAU CO34669**	***C. krempfi* ZMTAU CO33592**	***C. tuberculoides* ZMTAU CO34686**	***C. bottai* ZMTAU CO34648**	***C. tuberculoides* ZMTAU CO34642**	***C. pachyclados* ZMTAU CO35507**	***C. pachyclados* ZMTAU CO33604**	***K. utinomii* ZMTAU CO34639**	***K. adii* ZMTAU CO32636 I**
252	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																							
Ri1	0,036	0,036	0,035	0,040	0,036	0,035	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036																						
Ri2	0,039	0,039	0,037	0,042	0,039	0,037	0,039	0,039	0,039	0,039	0,039	0,039	0,039	0,039	0,039	0,039	0,039	0,039	0,039	0,039	0,039	0,002																					
Ri3	0,037	0,037	0,036	0,041	0,037	0,036	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,001	0,001																				
Ro1	0,037	0,037	0,036	0,041	0,037	0,036	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,001	0,001	0,000																			
Ro2	0,036	0,036	0,035	0,040	0,036	0,035	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,000	0,002	0,001	0,001																		
Ro3	0,036	0,036	0,035	0,040	0,036	0,035	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,000	0,002	0,001	0,001	0,000																	
Ro4	0,036	0,036	0,035	0,040	0,036	0,035	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,000	0,002	0,001	0,001	0,000	0,000																
A. sodwanum ZMTAU CO31520	0,036	0,036	0,035	0,040	0,036	0,035	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,000	0,002	0,001	0,001	0,000	0,000	0,000															
C. pachyclados ZMTAU CO35507	0,037	0,037	0,036	0,041	0,037	0,036	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,001	0,001	0,000	0,000	0,001	0,001	0,001	0,001														
C. pachyclados ZMTAU CO33604	0,036	0,036	0,035	0,040	0,036	0,035	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,000	0,002	0,001	0,001	0,000	0,000	0,000	0,000	0,001													
C. australis ZMTAU CO33613	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,036	0,039	0,037	0,037	0,036	0,036	0,036	0,036	0,037	0,036												
C. kashmani ZMTAU CO32246	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,036	0,039	0,037	0,037	0,036	0,036	0,036	0,036	0,037	0,036	0,000											
C. kashmani ZMTAU CO32334	0,000	0,000	0,004	0,004	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,036	0,039	0,037	0,037	0,036	0,036	0,036	0,036	0,037	0,036	0,000	0,000										
C. tuberosa ZMTAU CO34669	0,037	0,037	0,036	0,041	0,037	0,036	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,001	0,004	0,002	0,002	0,001	0,001	0,001	0,001	0,002	0,001	0,037	0,037	0,037									
C. krempfi ZMTAU CO33592	0,036	0,036	0,035	0,040	0,036	0,035	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,000	0,002	0,001	0,001	0,000	0,000	0,000	0,000	0,001	0,000	0,036	0,036	0,036	0,001								
C. tuberculoides ZMTAU CO34686	0,037	0,037	0,036	0,041	0,037	0,036	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,001	0,001	0,000	0,000	0,001	0,001	0,001	0,001	0,000	0,001	0,037	0,037	0,037	0,002	0,001							
C. bottai ZMTAU CO34648	0,037	0,037	0,036	0,041	0,037	0,036	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,001	0,001	0,000	0,000	0,001	0,001	0,001	0,001	0,000	0,001	0,037	0,037	0,037	0,002	0,001	0,000						
C. tuberculoides ZMTAU CO34642	0,037	0,037	0,036	0,041	0,037	0,036	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,001	0,001	0,000	0,000	0,001	0,001	0,001	0,001	0,000	0,001	0,037	0,037	0,037	0,002	0,001	0,000	0,000					
C. pachyclados ZMTAU CO35507	0,036	0,036	0,035	0,040	0,036	0,035	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,000	0,002	0,001	0,001	0,000	0,000	0,000	0,000	0,001	0,000	0,036	0,036	0,036	0,001	0,000	0,001	0,001	0,001				
C. pachyclados ZMTAU CO33604	0,036	0,036	0,035	0,040	0,036	0,035	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,036	0,000	0,002	0,001	0,001	0,000	0,000	0,000	0,000	0,001	0,000	0,036	0,036	0,036	0,001	0,000	0,001	0,001	0,001	0,000			
K. utinomii ZMTAU CO34639	0,001	0,001	0,005	0,005	0,001	0,002	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,037	0,040	0,039	0,039	0,037	0,037	0,037	0,037	0,039	0,037	0,001	0,001	0,001	0,039	0,037	0,039	0,039	0,039	0,037	0,037		
K. adii ZMTAU CO32636 I	0,001	0,001	0,005	0,005	0,001	0,002	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,037	0,040	0,039	0,039	0,037	0,037	0,037	0,037	0,039	0,037	0,001	0,001	0,001	0,039	0,037	0,039	0,039	0,039	0,037	0,037	0,000	
K. utinomii ZMTAU: CO34127	0,001	0,001	0,005	0,005	0,001	0,002	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,037	0,040	0,039	0,039	0,037	0,037	0,037	0,037	0,039	0,037	0,001	0,001	0,001	0,039	0,037	0,039	0,039	0,039	0,037	0,037	0,000	0,000

Table 7.7 Estimates of evolutionary divergence over sequence pairs (uncorrected *p*) between taxa for *MutS* (Chapter 2)

Sample	**61**	**79**	**84**	**112**	**113**	**134**	**135**	**142**	**157**	**160**	**165**	**192**	**194**	**195**	**198**	**209**	**215**	**220**	**221**	**236**	**248**	**252**	**Ri1**	**Ri2**	**Ri3**	**Ro1**	**Ro2**	**Ro3**	**Ro4**	***C. pachyclados* ZMTAU CO34129**	***C. sphaerophora* ZMTAU CO34132**	***C. pachyclados* ZMTAU CO35508**	***C. pachyclados* ZMTAU CO35507**	***C. kashmani* ZMTAU CO32482**	***C. kashmani* ZMTAU CO32334**	***C. kashmani* ZMTAU CO32246**	***C. tuberculoides* ZMTAU CO34686**	***C. tuberculoides* ZMTAU CO34642**	***C. pachyclados* ZMTAU CO33604**	***A. sodwanum* ZMTAU CO31520**	***K. utinomii* ZMTAU CO34639**	***K. adii* ZMTAU CO32636 I**
79	0,000																																									
84	0,000	0,000																																								
112	0,000	0,000	0,000																																							
113	0,000	0,000	0,000	0,000																																						
134	0,000	0,000	0,000	0,000	0,000																																					
135	0,000	0,000	0,000	0,000	0,000	0,000																																				
142	0,000	0,000	0,000	0,000	0,000	0,000	0,000																																			
157	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																																		
160	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																																	
165	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																																
192	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																															
194	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																														
195	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																													
198	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																												
209	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																											
215	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008																										
220	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008																									
221	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008	0,000																								
236	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,031	0,023	0,023																							
248	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008	0,000	0,000	0,023																						
252	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008	0,000	0,000	0,023	0,000																					
Ri1	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,035	0,027	0,027	0,004	0,027	0,027																				
Ri2	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,035	0,027	0,027	0,004	0,027	0,027	0,000																			
Ri3	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,035	0,027	0,027	0,004	0,027	0,027	0,000	0,000																		
Ro1	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,031	0,023	0,023	0,000	0,023	0,023	0,004	0,004	0,004																	
Ro2	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,031	0,023	0,023	0,000	0,023	0,023	0,004	0,004	0,004	0,000																
Ro3	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,031	0,023	0,023	0,000	0,023	0,023	0,004	0,004	0,004	0,000	0,000															

Table 7.7 continued...

Sample	61	79	84	112	113	134	135	142	157	160	165	192	194	195	198	209	215	220	221	236	248	252	Ri1	Ri2	Ri3	Ro1	Ro2	Ro3	Ro4	*C. pachyclados* ZMTAU CO34129	*C. sphaerophora* ZMTAU CO34132	*C. pachyclados* ZMTAU CO35508	*C. pachyclados* ZMTAU CO35507	*C. kashmani* ZMTAU CO32482	*C. kashmani* ZMTAU CO32334	*C. kashmani* ZMTAU CO32246	*C. tuberculoides* ZMTAU CO34686	*C. tuberculoides* ZMTAU CO34642	*C. pachyclados* ZMTAU CO33604	*A. sodwanum* ZMTAU CO31520	*K. utinomii* ZMTAU CO34639	*K. adii* ZMTAU CO32636 I
Ro4	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,031	0,023	0,023	0,000	0,023	0,023	0,004	0,004	0,004	0,000	0,000	0,000														
C. pachyclados ZMTAU CO34129	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,035	0,027	0,027	0,004	0,027	0,027	0,000	0,000	0,000	0,004	0,004	0,004	0,004													
C. sphaerophora ZMTAU CO34132	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,035	0,027	0,027	0,004	0,027	0,027	0,000	0,000	0,000	0,004	0,004	0,004	0,004	0,000												
C. pachyclados ZMTAU CO35508	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,035	0,027	0,027	0,004	0,027	0,027	0,000	0,000	0,000	0,004	0,004	0,004	0,004	0,000	0,000											
C. pachyclados ZMTAU CO35507	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,035	0,027	0,027	0,004	0,027	0,027	0,000	0,000	0,000	0,004	0,004	0,004	0,004	0,000	0,000	0,000										
C. kashmani ZMTAU CO32482	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008	0,000	0,000	0,023	0,000	0,000	0,027	0,027	0,027	0,023	0,023	0,023	0,023	0,027	0,027	0,027	0,027									
C. kashmani ZMTAU CO32334	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008	0,000	0,000	0,023	0,000	0,000	0,027	0,027	0,027	0,023	0,023	0,023	0,023	0,027	0,027	0,027	0,027	0,000								
C. kashmani ZMTAU CO32246	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008	0,000	0,000	0,023	0,000	0,000	0,027	0,027	0,027	0,023	0,023	0,023	0,023	0,027	0,027	0,027	0,027	0,000	0,000							
C. tuberculoides ZMTAU CO34686	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,035	0,027	0,027	0,004	0,027	0,027	0,000	0,000	0,000	0,004	0,004	0,004	0,004	0,000	0,000	0,000	0,000	0,027	0,027	0,027						
C. tuberculoides ZMTAU CO34642	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,027	0,035	0,027	0,027	0,004	0,027	0,027	0,000	0,000	0,000	0,004	0,004	0,004	0,004	0,000	0,000	0,000	0,000	0,027	0,027	0,027	0,000					
C. pachyclados ZMTAU CO33604	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,042	0,035	0,035	0,012	0,035	0,035	0,008	0,008	0,008	0,012	0,012	0,012	0,012	0,008	0,008	0,008	0,008	0,035	0,035	0,035	0,008	0,008				
A. sodwanum ZMTAU CO31520	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,023	0,031	0,023	0,023	0,000	0,023	0,023	0,004	0,004	0,004	0,000	0,000	0,000	0,000	0,004	0,004	0,004	0,004	0,023	0,023	0,023	0,004	0,004	0,012			
K. utinomii ZMTAU CO34639	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008	0,000	0,000	0,023	0,000	0,000	0,027	0,027	0,027	0,023	0,023	0,023	0,023	0,027	0,027	0,027	0,027	0,000	0,000	0,000	0,027	0,027	0,035	0,023		
K. adii ZMTAU CO32636 I	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008	0,000	0,000	0,023	0,000	0,000	0,027	0,027	0,027	0,023	0,023	0,023	0,023	0,027	0,027	0,027	0,027	0,000	0,000	0,000	0,027	0,027	0,035	0,023	0,000	
K. utinomii ZMTAU: CO34127	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,008	0,000	0,000	0,023	0,000	0,000	0,027	0,027	0,027	0,023	0,023	0,023	0,023	0,027	0,027	0,027	0,027	0,000	0,000	0,000	0,027	0,027	0,035	0,023	0,000	0,000

Table 7.8 Estimates of evolutionary divergence over sequence pairs (uncorrected *p*) between taxa for *28S rRNA* (Chapter 2).

Sample	112	113	134	135	142	157	160	165	192	194	195	198	209	215	220	221	236	248	252	61	79	84	*C. kashmani* ZMTAU CO32246	*C. kashmani* ZMTAU CO32334	*Cladiella* sp. QM:G334170	*C. sphaerophora* ZMTAU CO34132	*C. australis* ZMTAU CO33613	*C. australis* ZMTAU CO33622	*C. bottai* ZMTAU CO34648	*C. krempfi* ZMTAU CO33592	*C. pachyclados* ZMTAU CO33604	*C. tuberculoides* ZMTAU CO34686	*C. tuberculoides* ZMTAU CO34642	*C. tuberosa* ZMTAU CO34669	*C. pachyclados* ZMTAU CO35507	*K. utinomii* ZMTAU CO34639	*K. utinomii* ZMTAU: CO34127	*K. adii* ZMTAU CO32636 I	Ri1	Ri2	Ri3	*A. sodwanum* ZMTAU CO31520	Ro1	Ro2	Ro3
113	0,001																																												
134	0,032	0,031																																											
135	0,003	0,001	0,032																																										
142	0,001	0,000	0,031	0,001																																									
157	0,003	0,001	0,032	0,003	0,001																																								
160	0,001	0,000	0,031	0,001	0,000	0,001																																							
165	0,001	0,000	0,031	0,001	0,000	0,001	0,000																																						
192	0,013	0,011	0,042	0,013	0,011	0,010	0,011	0,011																																					
194	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011																																				
195	0,006	0,004	0,035	0,006	0,004	0,006	0,004	0,004	0,015	0,004																																			
198	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004																																		
209	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004	0,000																																	
215	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004	0,000	0,000																																
220	0,003	0,001	0,032	0,003	0,001	0,003	0,001	0,001	0,013	0,001	0,006	0,001	0,001	0,001																															
221	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004	0,000	0,000	0,000	0,001																														
236	0,064	0,063	0,093	0,064	0,063	0,061	0,063	0,063	0,065	0,063	0,067	0,063	0,063	0,063	0,061	0,063																													
248	0,011	0,010	0,040	0,011	0,010	0,008	0,010	0,010	0,001	0,010	0,014	0,010	0,010	0,010	0,011	0,010	0,064																												
252	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004	0,000	0,000	0,000	0,001	0,000	0,063	0,010																											
61	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004	0,000	0,000	0,000	0,001	0,000	0,063	0,010	0,000																										
79	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004	0,000	0,000	0,000	0,001	0,000	0,063	0,010	0,000	0,000																									
84	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004	0,000	0,000	0,000	0,001	0,000	0,063	0,010	0,000	0,000	0,000																								
C. kashmani ZMTAU CO32246	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004	0,000	0,000	0,000	0,001	0,000	0,063	0,010	0,000	0,000	0,000	0,000																							
C. kashmani ZMTAU CO32334	0,001	0,000	0,031	0,001	0,000	0,001	0,000	0,000	0,011	0,000	0,004	0,000	0,000	0,000	0,001	0,000	0,063	0,010	0,000	0,000	0,000	0,000	0,000																						
Cladiella sp. QM:G334170	0,065	0,064	0,093	0,065	0,064	0,063	0,064	0,064	0,069	0,064	0,068	0,064	0,064	0,064	0,063	0,064	0,018	0,068	0,064	0,064	0,064	0,064	0,064	0,064																					
C. sphaerophora ZMTAU CO34132	0,063	0,061	0,092	0,063	0,061	0,060	0,061	0,061	0,064	0,061	0,065	0,061	0,061	0,061	0,063	0,061	0,014	0,063	0,061	0,061	0,061	0,061	0,061	0,061	0,024																				
C. australis ZMTAU CO33613	0,013	0,013	0,043	0,014	0,013	0,011	0,013	0,013	0,004	0,013	0,017	0,013	0,013	0,013	0,014	0,013	0,067	0,003	0,013	0,013	0,013	0,013	0,013	0,013	0,071	0,065																			

Table 7.8 continued…

Sample	**112**	**113**	**134**	**135**	**142**	**157**	**160**	**165**	**192**	**194**	**195**	**198**	**209**	**215**	**220**	**221**	**236**	**248**	**252**	**61**	**79**	**84**	***C. kashmani* ZMTAU CO32246**	***C. kashmani* ZMTAU CO32334**	***Cladiella* sp. QM:G334170**	***C. sphaerophora* ZMTAU CO34132**	***C. australis* ZMTAU CO33613**	***C. australis* ZMTAU CO33622**	***C. bottai* ZMTAU CO34648**	***C. krempfi* ZMTAU CO33592**	***C. pachyclados* ZMTAU CO33604**	***C. tuberculoides* ZMTAU CO34686**	***C. tuberculoides* ZMTAU CO34642**	***C. tuberosa* ZMTAU CO34669**	***C. pachyclados* ZMTAU CO35507**	***K. utinomii* ZMTAU CO34639**	***K. utinomii* ZMTAU: CO34127**	***K. adii* ZMTAU CO32636 I**	**Ri1**	**Ri2**	**Ri3**	***A. sodwanum* ZMTAU CO31520**	**Ro1**	**Ro2**	**Ro3**
C. australis ZMTAU CO33622	0,011	0,010	0,040	0,011	0,010	0,008	0,010	0,010	0,001	0,010	0,014	0,010	0,010	0,010	0,011	0,010	0,064	0,000	0,010	0,010	0,010	0,010	0,010	0,010	0,068	0,063	0,003																		
C. bottai ZMTAU CO34648	0,064	0,063	0,093	0,064	0,063	0,061	0,063	0,063	0,063	0,063	0,067	0,063	0,063	0,063	0,064	0,063	0,014	0,061	0,063	0,063	0,063	0,063	0,063	0,063	0,024	0,003	0,064	0,061																	
C. krempfi ZMTAU CO33592	0,067	0,065	0,094	0,067	0,065	0,064	0,065	0,065	0,068	0,065	0,069	0,065	0,065	0,065	0,064	0,065	0,017	0,067	0,065	0,065	0,065	0,065	0,065	0,065	0,004	0,022	0,069	0,067	0,022																
C. pachyclados ZMTAU CO33604	0,076	0,075	0,103	0,076	0,075	0,074	0,075	0,075	0,078	0,075	0,079	0,075	0,075	0,075	0,074	0,075	0,021	0,076	0,075	0,075	0,075	0,075	0,075	0,075	0,033	0,026	0,079	0,076	0,026	0,032															
C. tuberculoides ZMTAU CO34686	0,063	0,061	0,092	0,063	0,061	0,060	0,061	0,061	0,064	0,061	0,065	0,061	0,061	0,061	0,063	0,061	0,013	0,063	0,061	0,061	0,061	0,061	0,061	0,061	0,022	0,001	0,065	0,063	0,001	0,021	0,025														
C. tuberculoides ZMTAU CO34642	0,064	0,063	0,093	0,064	0,063	0,061	0,063	0,063	0,063	0,063	0,067	0,063	0,063	0,063	0,064	0,063	0,014	0,061	0,063	0,063	0,063	0,063	0,063	0,063	0,024	0,003	0,064	0,061	0,000	0,022	0,026	0,001													
C. tuberosa ZMTAU CO34669	0,068	0,067	0,097	0,068	0,067	0,065	0,067	0,067	0,069	0,067	0,071	0,067	0,067	0,067	0,065	0,067	0,021	0,068	0,067	0,067	0,067	0,067	0,067	0,067	0,028	0,019	0,071	0,068	0,021	0,026	0,031	0,019	0,021												
C. pachyclados ZMTAU CO35507	0,065	0,064	0,093	0,065	0,064	0,063	0,064	0,064	0,069	0,064	0,068	0,064	0,064	0,064	0,063	0,064	0,018	0,068	0,064	0,064	0,064	0,064	0,064	0,064	0,000	0,024	0,071	0,068	0,024	0,004	0,033	0,022	0,024	0,028											
K. utinomii ZMTAU CO34639	0,015	0,014	0,044	0,015	0,014	0,013	0,014	0,014	0,017	0,014	0,018	0,014	0,014	0,014	0,015	0,014	0,063	0,015	0,014	0,014	0,014	0,014	0,014	0,014	0,067	0,064	0,017	0,015	0,065	0,065	0,072	0,064	0,065	0,067	0,067										
K. utinomii ZMTAU: CO34127	0,015	0,014	0,044	0,015	0,014	0,013	0,014	0,014	0,017	0,014	0,018	0,014	0,014	0,014	0,015	0,014	0,060	0,015	0,014	0,014	0,014	0,014	0,014	0,014	0,067	0,064	0,017	0,015	0,065	0,065	0,072	0,064	0,065	0,067	0,067	0,003									
K. adii ZMTAU CO32636 I	0,011	0,010	0,040	0,011	0,010	0,008	0,010	0,010	0,010	0,010	0,014	0,010	0,010	0,010	0,011	0,010	0,064	0,008	0,010	0,010	0,010	0,010	0,010	0,010	0,068	0,063	0,011	0,008	0,064	0,067	0,076	0,063	0,064	0,068	0,068	0,013	0,013								
Ri1	0,063	0,061	0,092	0,063	0,061	0,060	0,061	0,061	0,064	0,061	0,065	0,061	0,061	0,061	0,063	0,061	0,013	0,063	0,061	0,061	0,061	0,061	0,061	0,061	0,022	0,001	0,065	0,063	0,001	0,021	0,025	0,000	0,001	0,019	0,022	0,064	0,064	0,063							
Ri2	0,063	0,061	0,092	0,063	0,061	0,060	0,061	0,061	0,064	0,061	0,065	0,061	0,061	0,061	0,063	0,061	0,013	0,063	0,061	0,061	0,061	0,061	0,061	0,061	0,022	0,001	0,065	0,063	0,001	0,021	0,025	0,000	0,001	0,019	0,022	0,064	0,064	0,063	0,000						
Ri3	0,063	0,061	0,092	0,063	0,061	0,060	0,061	0,061	0,064	0,061	0,065	0,061	0,061	0,061	0,063	0,061	0,013	0,063	0,061	0,061	0,061	0,061	0,061	0,061	0,022	0,001	0,065	0,063	0,001	0,021	0,025	0,000	0,001	0,019	0,022	0,064	0,064	0,063	0,000	0,000					
A. sodwanum ZMTAU CO31520	0,064	0,063	0,093	0,064	0,063	0,061	0,063	0,063	0,065	0,063	0,067	0,063	0,063	0,063	0,061	0,063	0,000	0,064	0,063	0,063	0,063	0,063	0,063	0,063	0,018	0,014	0,067	0,064	0,014	0,017	0,021	0,013	0,014	0,021	0,018	0,063	0,060	0,064	0,013	0,013	0,013				
Ro1	0,064	0,063	0,093	0,064	0,063	0,061	0,063	0,063	0,065	0,063	0,067	0,063	0,063	0,063	0,061	0,063	0,000	0,064	0,063	0,063	0,063	0,063	0,063	0,063	0,018	0,014	0,067	0,064	0,014	0,017	0,021	0,013	0,014	0,021	0,018	0,063	0,060	0,064	0,013	0,013	0,013	0,000			
Ro2	0,064	0,063	0,093	0,064	0,063	0,061	0,063	0,063	0,065	0,063	0,067	0,063	0,063	0,063	0,061	0,063	0,000	0,064	0,063	0,063	0,063	0,063	0,063	0,063	0,018	0,014	0,067	0,064	0,014	0,017	0,021	0,013	0,014	0,021	0,018	0,063	0,060	0,064	0,013	0,013	0,013	0,000	0,000		
Ro3	0,064	0,063	0,093	0,064	0,063	0,061	0,063	0,063	0,065	0,063	0,067	0,063	0,063	0,063	0,061	0,063	0,001	0,064	0,063	0,063	0,063	0,063	0,063	0,063	0,019	0,015	0,067	0,064	0,015	0,018	0,022	0,014	0,015	0,022	0,019	0,063	0,060	0,064	0,014	0,014	0,014	0,001	0,001	0,001	
Ro4	0,064	0,063	0,093	0,064	0,063	0,061	0,063	0,063	0,065	0,063	0,067	0,063	0,063	0,063	0,061	0,063	0,000	0,064	0,063	0,063	0,063	0,063	0,063	0,063	0,018	0,014	0,067	0,064	0,014	0,017	0,021	0,013	0,014	0,021	0,018	0,063	0,060	0,064	0,013	0,013	0,013	0,000	0,000	0,000	0,001

Table 7.9 Pairwise genetic distance (uncorrected *p*) between taxa for concatenated *COI*+*MutS*+*28S* (Chapter 2).

Sample	61	79	84	112	113	134	135	142	157	160	165	192	194	195	198	209	215	220	221	248	252	Ri1	Ri2	Ri3	Ro1	Ro2	Ro3	Ro4	*A. sodwanum* ZMTAU CO31520	*C. kashmani* ZMTAU CO32246	*C. kashmani* ZMTAU CO32334	*C. tuberculoides* ZMTAU CO34642	*C. pachyclados* ZMTAU CO35507	*C. pachyclados* ZMTAU CO33604	*K. utinomii* ZMTAU CO34639	*K. adii* ZMTAU CO32636 I
79	0,000																																			
84	0,002	0,002																																		
112	0,002	0,002	0,004																																	
113	0,000	0,000	0,002	0,002																																
134	0,013	0,013	0,013	0,015	0,013																															
135	0,001	0,001	0,002	0,003	0,001	0,013																														
142	0,000	0,000	0,002	0,002	0,000	0,013	0,001																													
157	0,001	0,001	0,002	0,003	0,001	0,013	0,001	0,001																												
160	0,000	0,000	0,002	0,002	0,000	0,013	0,001	0,000	0,001																											
165	0,000	0,000	0,002	0,002	0,000	0,013	0,001	0,000	0,001	0,000																										
192	0,004	0,004	0,006	0,007	0,004	0,017	0,005	0,004	0,004	0,004	0,004																									
194	0,000	0,000	0,002	0,002	0,000	0,013	0,001	0,000	0,001	0,000	0,000	0,004																								
195	0,002	0,002	0,003	0,004	0,002	0,015	0,002	0,002	0,002	0,002	0,002	0,006	0,002																							
198	0,000	0,000	0,002	0,002	0,000	0,013	0,001	0,000	0,001	0,000	0,000	0,004	0,000	0,002																						
209	0,000	0,000	0,002	0,002	0,000	0,013	0,001	0,000	0,001	0,000	0,000	0,004	0,000	0,002	0,000																					
215	0,001	0,001	0,003	0,003	0,001	0,014	0,002	0,001	0,002	0,001	0,001	0,006	0,001	0,003	0,001	0,001																				
220	0,001	0,001	0,002	0,003	0,001	0,013	0,001	0,001	0,001	0,001	0,001	0,005	0,001	0,002	0,001	0,001	0,002																			
221	0,000	0,000	0,002	0,002	0,000	0,013	0,001	0,000	0,001	0,000	0,000	0,004	0,000	0,002	0,000	0,000	0,001	0,001																		
248	0,004	0,004	0,006	0,006	0,004	0,017	0,004	0,004	0,003	0,004	0,004	0,001	0,004	0,006	0,004	0,004	0,005	0,004	0,004																	
252	0,000	0,000	0,002	0,002	0,000	0,013	0,001	0,000	0,001	0,000	0,000	0,004	0,000	0,002	0,000	0,000	0,001	0,001	0,000	0,004																
Ri1	0,045	0,045	0,044	0,047	0,045	0,057	0,045	0,045	0,044	0,045	0,045	0,046	0,045	0,046	0,045	0,045	0,046	0,045	0,045	0,045	0,045															
Ri2	0,046	0,046	0,045	0,048	0,046	0,058	0,046	0,046	0,045	0,046	0,046	0,047	0,046	0,048	0,046	0,046	0,047	0,046	0,046	0,046	0,046	0,001														
Ri3	0,045	0,045	0,045	0,048	0,045	0,057	0,046	0,045	0,045	0,045	0,045	0,046	0,045	0,047	0,045	0,045	0,046	0,046	0,045	0,046	0,045	0,001	0,001													
Ro1	0,045	0,045	0,045	0,048	0,045	0,057	0,046	0,045	0,045	0,045	0,045	0,046	0,045	0,047	0,045	0,045	0,046	0,045	0,045	0,046	0,045	0,006	0,006	0,006												

Table 7.9 continued…

Sample	**61**	**79**	**84**	**112**	**113**	**134**	**135**	**142**	**157**	**160**	**165**	**192**	**194**	**195**	**198**	**209**	**215**	**220**	**221**	**248**	**252**	**Ri1**	**Ri2**	**Ri3**	**Ro1**	**Ro2**	**Ro3**	**Ro4**	***A. sodwanum* ZMTAU CO31520**	***C. kashmani* ZMTAU CO32246**	***C. kashmani* ZMTAU CO32334**	***C. tuberculoides* ZMTAU CO34642**	***C. pachyclados* ZMTAU CO35507**	***C. pachyclados* ZMTAU CO33604**	***K. utinomii* ZMTAU CO34639**	***K. adii* ZMTAU CO32636 I**
Ro2	0,045	0,045	0,044	0,047	0,045	0,057	0,045	0,045	0,044	0,045	0,045	0,046	0,045	0,046	0,045	0,045	0,046	0,044	0,045	0,045	0,045	0,006	0,007	0,006	0,001											
Ro3	0,045	0,045	0,044	0,047	0,045	0,057	0,045	0,045	0,044	0,045	0,045	0,046	0,045	0,046	0,045	0,045	0,046	0,044	0,045	0,045	0,045	0,006	0,007	0,007	0,001	0,001										
Ro4	0,045	0,045	0,044	0,047	0,045	0,057	0,045	0,045	0,044	0,045	0,045	0,046	0,045	0,046	0,045	0,045	0,046	0,044	0,045	0,045	0,045	0,006	0,007	0,006	0,001	0,000	0,001									
A. sodwanum ZMTAU CO31520	0,045	0,045	0,044	0,047	0,045	0,057	0,045	0,045	0,044	0,045	0,045	0,046	0,045	0,046	0,045	0,045	0,046	0,044	0,045	0,045	0,045	0,006	0,007	0,006	0,001	0,000	0,001	0,000								
C. kashmani ZMTAU CO32246	0,000	0,000	0,002	0,002	0,000	0,013	0,001	0,000	0,001	0,000	0,000	0,004	0,000	0,002	0,000	0,000	0,001	0,001	0,000	0,004	0,000	0,045	0,046	0,045	0,045	0,045	0,045	0,045	0,045							
C. kashmani ZMTAU CO32334	0,000	0,000	0,002	0,002	0,000	0,013	0,001	0,000	0,001	0,000	0,000	0,004	0,000	0,002	0,000	0,000	0,001	0,001	0,000	0,004	0,000	0,045	0,046	0,045	0,045	0,045	0,045	0,045	0,045	0,000						
C. tuberculoides ZMTAU CO34642	0,045	0,045	0,045	0,048	0,045	0,057	0,046	0,045	0,045	0,045	0,045	0,046	0,045	0,047	0,045	0,045	0,046	0,046	0,045	0,046	0,045	0,001	0,001	0,000	0,006	0,006	0,007	0,006	0,006	0,045	0,045					
C. pachyclados ZMTAU CO35507	0,046	0,046	0,045	0,048	0,046	0,057	0,046	0,046	0,045	0,046	0,046	0,048	0,046	0,048	0,046	0,046	0,047	0,045	0,046	0,048	0,046	0,009	0,010	0,010	0,008	0,008	0,008	0,008	0,008	0,046	0,046	0,010				
C. pachyclados ZMTAU CO33604	0,052	0,052	0,051	0,054	0,052	0,062	0,052	0,052	0,051	0,052	0,052	0,053	0,052	0,053	0,052	0,052	0,053	0,051	0,052	0,052	0,052	0,011	0,012	0,012	0,011	0,010	0,011	0,010	0,010	0,052	0,052	0,012	0,015			
K. utinomii ZMTAU CO34639	0,006	0,006	0,007	0,008	0,006	0,018	0,006	0,006	0,005	0,006	0,006	0,007	0,006	0,007	0,006	0,006	0,007	0,006	0,006	0,006	0,006	0,046	0,047	0,046	0,045	0,045	0,045	0,045	0,045	0,006	0,006	0,046	0,047	0,050		
K. adii ZMTAU CO32636 I	0,004	0,004	0,006	0,007	0,004	0,017	0,005	0,004	0,004	0,004	0,004	0,004	0,004	0,006	0,004	0,004	0,006	0,005	0,004	0,004	0,004	0,046	0,047	0,046	0,046	0,046	0,046	0,046	0,046	0,004	0,004	0,046	0,048	0,053	0,004	
K. utinomii ZMTAU: CO34127	0,006	0,006	0,008	0,008	0,006	0,019	0,007	0,006	0,006	0,006	0,006	0,007	0,006	0,008	0,006	0,006	0,007	0,007	0,006	0,007	0,006	0,046	0,048	0,047	0,046	0,045	0,045	0,045	0,045	0,006	0,006	0,047	0,048	0,051	0,001	0,005

The number of base substitutions per site averaging over all sequence pairs between genera (uncorrected *p*) in Chapter 4 are shown for DNA barcodes *MutS*, *28S rRNA*, and *MutS+28S* respectively.

Table 7.10 Estimates of Evolutionary Divergence (uncorrected *p*) over Sequence Pairs between genera for *MutS* (Chapter 4).

Genus	***Beta***	***Aldersladum***	***Cladiella***	***Klyxum***	***Sinularia***	***S. brassica***	***Xenia***	***Anthelia***	***Sansibia***	***Heteroxenia***	***Sympodium***	***Litophyton***	***Ovabunda***	***Sarcophyton***	***Verrucella***	***Junceella***	***Rumphella***	***Chironephthya***	***Parasphaerasclera***	***Euplexaura***	***Paraplexaura***	***Leptogorgia***	***Dendronephthya***	***Stereonephthya***
Aldersladum	0,026																							
Cladiella	0,030	0,006																						
Klyxum	0,000	0,025	0,030																					
Sinularia	0,118	0,109	0,112	0,118																				
S. brassica	0,090	0,095	0,098	0,090	0,101																			
Xenia	0,130	0,122	0,126	0,130	0,152	0,117																		
Anthelia	0,134	0,136	0,139	0,134	0,160	0,116	0,079																	
Sansibia	0,131	0,125	0,128	0,131	0,149	0,108	0,032	0,070																
Heteroxenia	0,142	0,135	0,138	0,142	0,157	0,110	0,039	0,084	0,035															
Sympodium	0,126	0,120	0,123	0,126	0,148	0,111	0,030	0,073	0,024	0,038														
Litophyton	0,106	0,111	0,114	0,106	0,122	0,073	0,136	0,129	0,124	0,134	0,125													
Ovabunda	0,124	0,115	0,119	0,123	0,150	0,120	0,020	0,078	0,037	0,051	0,031	0,136												

Table 7.10 continued...

Genus	**Beta**	***Aldersladum***	***Cladiella***	***Klyxum***	***Sinularia***	***S. brassica***	***Xenia***	***Anthelia***	***Sansibia***	***Heteroxenia***	***Sympodium***	***Litophyton***	***Ovabunda***	***Sarcophyton***	***Verrucella***	***Junceella***	***Rumphella***	***Chironephthya***	***Parasphaerasclera***	***Euplexaura***	***Paraplexaura***	***Leptogorgia***	***Dendronephthya***	***Stereonephthya***
Sarcophyton	0,117	0,120	0,124	0,117	0,103	0,113	0,154	0,165	0,146	0,160	0,147	0,136	0,152											
Verrucella	0,176	0,171	0,174	0,176	0,160	0,155	0,186	0,175	0,176	0,188	0,176	0,163	0,182	0,154										
Junceella	0,168	0,163	0,165	0,167	0,151	0,154	0,184	0,179	0,175	0,188	0,175	0,153	0,182	0,154	0,028									
Rumphella	0,101	0,106	0,110	0,101	0,104	0,061	0,110	0,111	0,103	0,109	0,103	0,078	0,109	0,117	0,150	0,138								
Chironephthya	0,085	0,090	0,095	0,085	0,114	0,062	0,112	0,118	0,104	0,115	0,106	0,078	0,111	0,116	0,155	0,142	0,068							
Parasphaerasclera	0,122	0,128	0,131	0,121	0,119	0,091	0,149	0,133	0,137	0,143	0,140	0,106	0,147	0,126	0,111	0,109	0,099	0,103						
Euplexaura	0,085	0,094	0,097	0,085	0,105	0,052	0,116	0,115	0,108	0,111	0,110	0,077	0,121	0,110	0,141	0,135	0,060	0,067	0,091					
Paraplexaura	0,093	0,098	0,101	0,093	0,124	0,068	0,120	0,115	0,111	0,121	0,115	0,086	0,119	0,128	0,156	0,147	0,065	0,076	0,092	0,066				
Leptogorgia	0,093	0,104	0,107	0,093	0,125	0,071	0,116	0,117	0,105	0,116	0,108	0,088	0,118	0,138	0,163	0,152	0,073	0,076	0,103	0,064	0,076			
Dendronephthya	0,105	0,110	0,112	0,104	0,117	0,071	0,134	0,126	0,122	0,133	0,124	0,016	0,135	0,132	0,160	0,148	0,076	0,075	0,100	0,076	0,084	0,092		
Stereonephthya	0,101	0,106	0,109	0,101	0,118	0,068	0,131	0,124	0,119	0,130	0,120	0,017	0,132	0,132	0,160	0,148	0,073	0,073	0,101	0,073	0,081	0,089	0,016	
Scleronephthya	0,093	0,098	0,101	0,093	0,109	0,060	0,119	0,114	0,107	0,117	0,110	0,017	0,119	0,127	0,156	0,144	0,061	0,065	0,090	0,066	0,069	0,077	0,016	0,012

Table 7.11 Estimates of evolutionary divergence (uncorrected *p*) over sequence pairs between genera for *28S rRNA* (Chapter 4).

Genus	***Dendronephthya***	***Litophyton***	***Stereonephthya***	***S. brassica***	***Sinularia***	**Beta**	***Aldersladum***	***Cladiella***	***Verrucella***	***Euplexaura***	***Junceella***	***Rumphella***	***Chironephthya***	***Parasphaerasclera***	***Leptogorgia***	***Xenia***	***Anthelia***	***Sansibia***	***Sympodium***	***Heteroxenia***	***Lobophytum***	***Sarcophyton***	***Ovabunda***	***Klyxum***
Litophyton	0,026																							
Stereonephthya	0,030	0,006																						
S. brassica	0,000	0,025	0,030																					
Sinularia	0,118	0,109	0,112	0,118																				
Beta	0,090	0,095	0,098	0,090	0,101																			
Aldersladum	0,130	0,122	0,126	0,130	0,152	0,117																		
Cladiella	0,134	0,136	0,139	0,134	0,160	0,116	0,079																	
Verrucella	0,131	0,125	0,128	0,131	0,149	0,108	0,032	0,070																
Euplexaura	0,142	0,135	0,138	0,142	0,157	0,110	0,039	0,084	0,035															
Junceella	0,126	0,120	0,123	0,126	0,148	0,111	0,030	0,073	0,024	0,038														
Rumphella	0,106	0,111	0,114	0,106	0,122	0,073	0,136	0,129	0,124	0,134	0,125													
Chironephthya	0,124	0,115	0,119	0,123	0,150	0,120	0,020	0,078	0,037	0,051	0,031	0,136												
Parasphaerasclera	0,117	0,120	0,124	0,117	0,103	0,113	0,154	0,165	0,146	0,160	0,147	0,136	0,152											
Leptogorgia	0,176	0,171	0,174	0,176	0,160	0,155	0,186	0,175	0,176	0,188	0,176	0,163	0,182	0,154										
Xenia	0,168	0,163	0,165	0,167	0,151	0,154	0,184	0,179	0,175	0,188	0,175	0,153	0,182	0,154	0,028									
Anthelia	0,101	0,106	0,110	0,101	0,104	0,061	0,110	0,111	0,103	0,109	0,103	0,078	0,109	0,117	0,150	0,138								
Sansibia	0,085	0,090	0,095	0,085	0,114	0,062	0,112	0,118	0,104	0,115	0,106	0,078	0,111	0,116	0,155	0,142	0,068							
Sympodium	0,122	0,128	0,131	0,121	0,119	0,091	0,149	0,133	0,137	0,143	0,140	0,106	0,147	0,126	0,111	0,109	0,099	0,103						
Heteroxenia	0,085	0,094	0,097	0,085	0,105	0,052	0,116	0,115	0,108	0,111	0,110	0,077	0,121	0,110	0,141	0,135	0,060	0,067	0,091					
Lobophytum	0,093	0,098	0,101	0,093	0,124	0,068	0,120	0,115	0,111	0,121	0,115	0,086	0,119	0,128	0,156	0,147	0,065	0,076	0,092	0,066				
Sarcophyton	0,093	0,104	0,107	0,093	0,125	0,071	0,116	0,117	0,105	0,116	0,108	0,088	0,118	0,138	0,163	0,152	0,073	0,076	0,103	0,064	0,076			
Ovabunda	0,105	0,110	0,112	0,104	0,117	0,071	0,134	0,126	0,122	0,133	0,124	0,016	0,135	0,132	0,160	0,148	0,076	0,075	0,100	0,076	0,084	0,092		
Klyxum	0,101	0,106	0,109	0,101	0,118	0,068	0,131	0,124	0,119	0,130	0,120	0,017	0,132	0,132	0,160	0,148	0,073	0,073	0,101	0,073	0,081	0,089	0,016	
Nephthea	0,093	0,098	0,101	0,093	0,109	0,060	0,119	0,114	0,107	0,117	0,110	0,017	0,119	0,127	0,156	0,144	0,061	0,065	0,090	0,066	0,069	0,077	0,016	0,012

Table 7.12 Estimates of evolutionary divergence (uncorrected *p*) over sequence pairs between genera for *MutS+28S* (Chapter 4).

Genus	*S. brassica*	*Sinularia*	Beta	*Aldersladum*	*Cladiella*	*Klyxum*	*Xenia*	*Anthelia*	*Sansibia*	*Heteroxenia*	*Sympodium*	*Ovabunda*	*Sarcophyton*	*Verrucella*	*Junceella*	*Rumphella*	*Chironephthya*	*Parasphaerascle*	*Euplexaura*	*Leptogorgia*	*Dendronephthya*	*Litophyton*	*Stereonephthya*	*Nephthea*
Sinularia	0,082																							
Beta	0,098	0,112																						
Aldersladum	0,098	0,101	0,055																					
Cladiella	0,097	0,102	0,057	0,008																				
Klyxum	0,097	0,111	0,008	0,051	0,055																			
Xenia	0,115	0,134	0,137	0,130	0,133	0,134																		
Anthelia	0,110	0,127	0,133	0,131	0,132	0,133	0,075																	
Sansibia	0,117	0,138	0,144	0,135	0,138	0,141	0,031	0,081																
Heteroxenia	0,112	0,133	0,137	0,133	0,134	0,136	0,053	0,070	0,063															
Sympodium	0,093	0,116	0,120	0,113	0,112	0,118	0,060	0,078	0,064	0,061														
Ovabunda	0,121	0,138	0,137	0,132	0,136	0,134	0,026	0,078	0,028	0,064	0,068													
Sarcophyton	0,096	0,081	0,116	0,110	0,111	0,115	0,140	0,136	0,140	0,140	0,119	0,145												
Verrucella	0,166	0,155	0,178	0,179	0,174	0,179	0,195	0,177	0,201	0,185	0,176	0,196	0,163											
Junceella	0,152	0,136	0,159	0,154	0,150	0,160	0,186	0,169	0,191	0,181	0,163	0,187	0,146	0,048										
Rumphella	0,068	0,096	0,111	0,110	0,110	0,110	0,127	0,121	0,127	0,123	0,111	0,131	0,114	0,145	0,134									
Chironephthya	0,075	0,083	0,106	0,094	0,095	0,104	0,121	0,112	0,120	0,117	0,105	0,125	0,096	0,155	0,133	0,077								

Table 7.12 continued...

Genus	***S. brassica***	***Sinularia***	***Beta***	***Aldersladum***	***Cladiella***	***Klyxum***	***Xenia***	***Anthelia***	***Sansibia***	***Heteroxenia***	***Sympodium***	***Ovabunda***	***Sarcophyton***	***Verrucella***	***Junceella***	***Rumphella***	***Chironephthya***	***Parasphaerascle***	***Euplexaura***	***Leptogorgia***	***Dendronephthya***	***Litophyton***	***Stereonephthya***	***Nephthea***
Parasphaerasclera	0,121	0,124	0,141	0,137	0,136	0,142	0,176	0,154	0,179	0,166	0,150	0,176	0,132	0,116	0,103	0,124	0,125							
Euplexaura	0,054	0,090	0,091	0,095	0,097	0,090	0,119	0,118	0,115	0,118	0,108	0,126	0,105	0,166	0,151	0,055	0,070	0,130						
Leptogorgia	0,066	0,099	0,100	0,091	0,094	0,097	0,121	0,122	0,120	0,124	0,107	0,125	0,115	0,169	0,147	0,073	0,072	0,133	0,056					
Dendronephthya	0,073	0,089	0,098	0,109	0,109	0,100	0,131	0,117	0,128	0,127	0,109	0,137	0,105	0,159	0,138	0,085	0,083	0,120	0,080	0,094				
Litophyton	0,102	0,121	0,128	0,138	0,138	0,131	0,161	0,145	0,158	0,157	0,139	0,166	0,136	0,184	0,168	0,113	0,111	0,151	0,111	0,123	0,049			
Stereonephthya	0,086	0,094	0,107	0,107	0,104	0,105	0,139	0,129	0,136	0,139	0,120	0,144	0,109	0,155	0,133	0,091	0,084	0,115	0,090	0,095	0,045	0,078		
Nephthea	0,083	0,101	0,107	0,117	0,115	0,107	0,140	0,128	0,136	0,139	0,116	0,146	0,115	0,167	0,147	0,090	0,093	0,127	0,085	0,100	0,038	0,069	0,040	
Lobophytum	0,092	0,074	0,113	0,111	0,113	0,111	0,141	0,127	0,141	0,138	0,118	0,146	0,058	0,148	0,134	0,107	0,088	0,122	0,101	0,107	0,092	0,122	0,097	0,103