DNA barcoding of sponges (Phylum Porifera) in South African

by

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Declarations

I, Benedicta Biligwe Ngwakum, declare that the study, “DNA barcoding of sponges (Phylum Porifera) in South Africa” is my own work, which has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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Summary of the study

Marine sponges are an ecologically important and highly diverse component of marine benthic communities and are found in all oceans. Although their ecological, commercial and evolutionary importance is increasingly recognized, knowledge on their taxonomy, diversity, biogeography, and range shifts are limited. South Africa is no exception.

South Africa has a unique coastline, bathed by two opposing currents, creating a large diversity of ecosystems, and is particularly rich in biodiversity. This includes the marine invertebrates of which marine sponges form a particularly important component. Although 343 sponge species have to date been recorded from South Africa, this number is likely a gross underestimation, and more that 50% have species names derived from Northern Hemisphere sponges (see Ridley & Dendy, 1887; Kirkpatric 1902, 1903a & b; Stephens 1915; Burton 1926, 1931, 1933a & b, 1936; Lévi, 1963, 1967). This is due to the fact that sponge species are notoriously difficult to identify, because characters for comparative morphology are often scarce, and morphological plasticity due to environmental changes makes clear identifications difficult. Currently, some sponges from South Africa are considered cosmopolitan. This is because of ambiguous identifications due to insificiant morphological data and the difficulty to delineate cryptic species. These taxonomic misidentifications are a consequence of their simplicity coupled with intraspecific variability. This lumping of species with similar morphologies hinders our understanding of the actual diversity of sponges (Klautau et al. 1999; Andreakis et al. 2007).

To solve such taxonomic dilemmas, achieve sound species identifications and better understand sponge diversity, an integrative taxonomic approach that complements the morphological data with molecular data can be used (Cárdenas *et al*., 2012; Boury-Esnault *et al*., 2013). For example, Samaai *et al*. (2017) used such an approach to strengthen their argument for the misidentification of *Suberites tylobtusus* Lévi, 1958 on the west coast of South Africa, a species who type locality is in the Red Sea.

To date, South African sponges have been delineated using morphological characters, and no phylogenetic or biogeographical relationships have been established for them. In addition to the shortcomings resulting from an inadequate approach towards defining species, sponge taxonomy is hampered by the fact that 80% of South Africa's Exclusive Economic Zone (EEZ) is undersampled. Continental South Africa has a coastline of some 3,650 km and an Exclusive Economic Zone (EEZ) of just over 1 million km^2. Waters in the EEZ extend to a depth of 5,700 m, with more than 65% deeper than 2,000 m (Griffiths *et al*., 2010). Most of the region's sponge samples have been collected from depths shallower than 500 m, with the largest concentration of collection from shallow hard reefs less than 40 m. The slope, bathyal and abyssal zones remain almost completely unexplored (Samaai, pers. comm.). Considering that South Africa is widely recognized as a region of high biological diversity and considered the third most diverse country in terms of terrestrial diversity, marine species diversity is predicted to be as high due to the high number of marine habitats and ecosytems and unique coastline surrounded by three oceans (Griffith *et al*., 2010).

The aims of this study were to 1) extract and sequence sponge DNA material from various regions around South Africa, 2) establish a DNA reference library for South African sponges using DNA barcoding, 3) compare species identification based on morphological classification with genetic data derieved both as part of this study and previous studies, 4) examine genetic differences in morphospecies from different ecoregions around South Africa, 5) Define new and crptic species from distinct genetic lineages using DNA barcoding and 6) reveal cryptic diversity within the morpho species

Sponge samples were collected over the past 10 years from various locations along the South African coastline, from shallow and deep reefs and unconsolidated sediments, including various vulnerable marine ecosystems such as canyons and seamounts. DNA was extracted and a portion of the mitochondrial cytochrome oxidase subunit 1 (cox1) gene was amplified and sequenced. An additional nuclear marker was amplified in cases where cryptic species were suspected. Genetic data were compared with both morphological data and previously generated genetic data, and identifications were compared, verified, confirmed and corrected when dubious. I

found that the sponge fauna from South Africa comprises numerous endemics that likely constitute cryptic species, and there was evidence for distinct genetic groups associated with different ecoregions. This present study provides the first reference library for South African sponges against which future sequence data can be compared. It represents a first step to advance our understanding of the diversity, ecology and biogeography of South African sponges, both locally and regionally.

Publications

Some of work reported in the present dissertation has so far been incorporated into the following scientific studies:

Samaai T, Maduray S, Janson L, Gibbons MJ, Ngwakum B, Teske PR (2017) A new species of habitat-forming *Suberites* (Porifera, Demospongiae, Suberitida) in the Benguela upwelling region (South Africa). *Zootaxa* 4254:49-81.

Samaai T, Ngwakum B, Payne RB, Teske PR, Janson L, Kerwath S, Parker D, Gibbons MJ (submitted) Four new species of Latrunculiidae (Demospongiae, Poecilosclerida) from the Agulhas ecoregion, and an overview of the biodiversity and distribution of the family in temperate Southern Africa. *Zootaxa*.

Payne RP, Ngwakum B, Teske PR, Samaai T (in prep.) *Suberites ambulodomus* sp. nov. (Porifera: Demospongiae: Suberitida: Suberitidae), a new species of hermitcrab-associated sponge from South Africa

Abstract

South Africa is renowned for its biological diversity and is a hotspot for marine invertebrates (Griffith *et al*., 2010), including sponges (Porifera). Despite their pivotal role for the functionality of the marine ecosystem, a reliable estimate of the species richness of sponges in South Africa is difficult. Using morphological characters for species identification has its limitations: it is hindered by the paucity and plasticity of morphological characters (Blanquer & Uriz 2007, Sperling *et al., 2011)* which may result in numerous species being morphologically indistinguishable, i.e. “cryptic” species. The sponge fauna of South Africa is also understudied, although this issue has begun to be addressed, with more than 40 new species described from this region over the last 10 years. It is likely that the current estimate of sponge biodiversity nonetheless remains a considerable underestimate, and numerous suspected new species remain to be described (Samaai, pers. comm.). Over the last five years, increased efforts were placed on documenting South African biodiversity, with sponges as one of the focus groups. The present study is part of this initiative, and constitutes the first genetic study on South African sponges aimed at complementing morphological data to help resolve sponge taxonomy. The results indicate that South African sponges are not as widespread as previously thought, but comprise cryptic and genetically distinct evolutionary lineages. Importantly, the results show that sponges identified from South Africa as southern hemisphere are representatives of supposedly cosmopolitan species that have been misidentified. Moreover, some species assumed to be widespread in southern Africa actually turned out to be subdivided into regional evolutionary lineages with distinct distribution ranges. In some cases the molecular data corroborated the morphological species identification, whereas in other instances the combined approach revealed the presence of species complexes. This study represents a first step in constructing a reference library for South African sponges and to advance our understanding of the diversity, biogeography and evolutionary adaptability of South African sponges.

Keywords: Molecular taxonomy, *cox1*, identification, reference library

Contents

Chapter 1: General introduction

Generally, the ocean is said to have few boundaries and marine species are often considered to be widespread and randomly reproduce throughout their ranges (Caley et al., 1996, Eckman 1996, Roberts 1997). This, however, is not the case, as even high-dispersal species are often genetically structured, and some may even comprise cryptic sibling species (Pöppe *et al*., 2010, Teske *et al.,* 2011). As anthropogenic impacts increasingly alter oceans and seas (Bell *et al*., 2015, Erpenbeck *et al*., 2016, De Goeij *et al*., 2017), understanding the natural state of marine ecosystems is important to assess baselines against changes in biodiversity and the reef of the taxa over time. The use of genetic markers to define species complexes or populations has become important in estimating diversity, understand connectivity, define biogeographic and phylogenetic patterns (Wörheide *et al*., 2005). Molecular methods are tools for testing species delineations by means of traditional morphological taxonomy by providing insight into the interpretation of morphological characters (Erwin & Thacker 2007). Genetics has also contributed significantly to our understanding of sponge biodiversity in revealing that sponge populations are more structured than previously thought (Wörheide *et al*., 2005). It has facilitated a deeper understanding of the evolutionary relationships between sponge taxa and contributes towards in species delimitation and identification, and provided insight into the process that drives speciation in sponges (Wörheide & Erpenbeck 2007). Genetic studies using genetic markers used for animal barcoding and species delimitation, mitochondrial. Cytochrome c oxidase subunit 1 (*cox*1), to define species, have also shown mitochondrial DNA evolve abnormally slow in sponges (Wörheide *et al*., 2012).

Sponges are the most important filter-feeding organisms in all marine habitats, and they play an important role in shallow and deep-water food webs (De Goeij *et al*., 2017). Having said this, little is known about the sponge biodiversity around South Africa in comparison to regions such as Australia, New Zealand, Brazil, North Atlantic, India and East Africa (Van Soest & Beglinger 2008, Berumen *et al*., 2013, Erpenbeck *et al*., 2016,).

Sponges are an essential and highly diverse component of marine benthic communities (Müller *et al*., 2004, Wörheide & Erpenbeck 2007, Pöppe *et al*., 2010, Wörheide *et al*., 2012). They are found in all the world's oceans at depths ranging from euryhaline estuaries/intertidal environments to the deep-sea (Hooper & Van Soest 2002) and horizontally from the tropics to the highest latitudes, locally from rocky reef communities to muddy bottoms and ephemeral freshwater habitats (Van Soest *et al*., 2012). Their importance for global and regional ecosystems is high but not widely appreciated. Apart from the ecological roles they play in the benthic ecosystem, the pharmaceutical and evolutionary potentials of sponges are increasingly recognized (Wörheide & Erpenbeck 2007, Pöppe *et al*., 2010, Wörheide *et al*., 2012).

Despite the revision of sponges based largely on morphological characters (Hooper & Van Soest, 2002*, Systema Porifera*), Morrow & Cardenas (2015) showed that this systematic framework in general is still poorly resolved by proposing a new classification of the Demospongiae based on genetics. Over the last decade an increasing number of molecular phylogenetic studies on sponges have shaken the classical taxonomic framework, by revealing numerous polyphyletic groups, discovering new clades and by defining many cryptic and new species (Chombard *et al*., 1997, Morrow & Cárdenas 2015). Notwithstanding the above, understanding the basic diversity & biodiversity patterns or ecological and biogeographic relationships of sponges is limited; due to limited genetic datasets available to understand variation among sponge populations and species (Wörheide *et al*., 2005). The use of morphological characters in delineating species is often problematic when dealing with sponges that show plasticity, similarity in gross morphology or are cryptic in habit (Wörheide & Erpenbeck 2007, Pöppe *et al*., 2010, Patantis *et al*., 2013; Redmond *et al*., 2013, Samaai *et al*., 2017; Erpenbeck *et al*., 2017).

Main Aim

The main aim of this study was to establish a reference library for South African sponges using DNA barcoding which future research can contribute.

Objectives: The objectives were to;

a) Compare species identification based on morphological classification with genetic data derieved both as part of this study and previous studies

b) Examine genetic differences in morphospecies from different ecoregions around South Africa
c) Define new and cryptic species from distinct genetic lineages using DNA barcoding
d) To reveal cryptic diversity within the morpho-species

Notwithstanding the above, major limitations existed in identifying these sponge specimens genetically. These limitations included high contamination rates, with environmental DNA from other organisms present on the sponges co-amplifying, and the fact that sponge DNA tends to degrade easily. Further, the GenBank database (where DNA sequences are lodged) is not well populated, as sponge barcoding is still in its infancy, which makes blast searches very challenging and molecular identification very difficult.

Chapter 2: Literature review

2.1. Why study sponges?

Sponges (Phylum Porifera) represent the phylogenetically oldest, extant multicellular group (Metazoa) of animals on Earth (Müller *et al*., 2004, Pleše *et al*., 2011, Björk *et al*., 2013, Patantis *et al*., 2013, Redmond *et al*., 2013, Pisani *et al*., 2015, Vargas *et al*., 2015, Metabole *et al*., 2017), as the sponge clade is the first to branch off the metazoan tree of life. They have great significance in the reconstruction of early metazoan evolution (Wörheide *et al*., 2012; Pisani *et al*., 2015). Sponges are exclusively aquatic, and mostly inhabit marine habitats (Van Soest *et al*., 2012; Pronzato *et al*., 2017). Freshwater bodies, such as lakes and rivers, are inhabited only by a small minority of species of the Order Spongillida (Demospongiae), most of which produce gemmules (specialized resting bodies), to survive harsh terrestrial environmental conditions (Pleše *et al*., 2011, Pronzato *et al*., 2017). Sponges are among the most diverse and successful aquatic invertebrate taxa in terms of species number, morphological characters (Hooper & Van Soest 2002), and evolutionary and habitat adaptability.

Marine sponges are fixed on substrates (sessile) or anchored to the bottom of soft substrates (e.g. some hexactinellids) (Müller *et al*., 2004, Vargas *et al*., 2012, Qu *et al*., 2012, Yang *et al*., 2017) and they feedby drawing water and filtering microscopic food particles from it (Taylor *et al*., 2007, Van Soest *et al*., 2012; Patantis *et al*., 2013, Yang *et al*., 2017). They have the ability to filter 4–5 times their own volume every minute (Vinod *at al*., 2014). Their ability to survive in a competitive environment could be due to their adaptability to environmental changes (Hooper & Van Soest 2002, Müller *et al*., 2004, Van Soest *et al*., 2012, Vinod *et al*., 2014), or perhaps because of microbial communities they harbor which provide chemical defense and can account for up to 40% of a sponge’s wet weight (Müller *et al*., 2004, Patantis *et al*., 2013, Metobole *et al*., 2017). Among marine invertebrates, sponges have the most abundant microbial communities (Müller *et al*., 2004, Björk *et al*., 2013, Patantis *et al*., 2013). They also form close associations with a wide variety of other organisms (Müller *et al*., 2004) such as bryozoans, hydroids, fish, shrimps, polychaetes, crabs and other sponges (Van Soest *et al*., 2012, Björk *et al*., 2013).

Sponges play numerous important ecological roles in their ecosystems, including in nutrient cycling (Lesser, 2006) or as bioeroding organisms in coral reefs (Lopez-Victoria & Zea, 2005, Van Soest *et al*., 2012). Their significant commercial importance to the pharmaceutical and biomaterials industry has been recognized for decades, e.g. as producers of potent secondary metabolites (Mehbub *et al*., 2014, Mehbub et al., 2016), useful for drug development (Munro *et al*., 1999). Should Southern Africa be in the position to exploit its sponge resources for the production of pharmaceutical products, it is vitally important that the the taxonomy of the region, sponges should be well understood. Sponges are highly diverse, but they often do not display definable morphological features, which make them difficult to be identified by non-experts.

Sponges are sensitive to the quality of the environment, and for that reason can be used effectively to assess the well-being of marine communities and ecosystems (Carballo *et al*., 1996; 2006). However, ignorance regarding the identity of sponges negates the value of these organisms as useful indicators of environmental health. This is of particular concern along the African coasts where overdevelopment of coastal areas contributes towards marine pollution. Few ecological studies have been conducted on sponges along the African coastline, but it has been predicted that the African continent harbours a high diversity of sponges (van Soest 1994; Barnes and Bell 2002).

Despite their importance, less is known about the taxonomy of African sponges and the features of the environment that determine their distribution other sessile invertebrates such as corals and hydroids (Millard, 1975). Owing to their variability in form and size, and although chemically (Faulkner, 1998, 2000; Blunt & Munro, 2003; Erpenbeck & van Soest, 2006) and ecologically important (Carballo *et al*. 1996; Olson & McCarthy, 2005), sponges have attracted less attention than other economically important species such as fish, mollusks and echinoderms (Sarà and Vacelet 1973, Bell 2008), possibly due in part to their difficulty in identification, and paucity of worldwide expertise.

2.2. Diversity and classification of sponges

Many species of sponges were previously considered to have near cosmopolitan distribution (Downey *et al*., 2012, Van Soest *et al*., 2012). This notion resulted from lumping morphologically similar but often-evolutionary distinct lineages into single cosmopolitan morphospecies (Wörheide *et al*., 2007). Knowledge of sponge biodiversity is still far from complete and many regions such as the deepsea remain severely undersampled. To date, about 11,000 species have been formally described of which approximately 8,500 are presently valid (Van Soest *et al*., 2012; Vargas *et al*., 2012, Redmond *et al*., 2013, Vinod *et al*., 2014, Van Soest *et al*., 2019), but as many as twice that number are thought to exist and have yet to be described (Hooper & Van Soest, 2002; Van Soest *et al*., 2012). Although many oceans and seas still remain un- or undersampled, many more specimens remain undescribed in museum collections around the world (Redmond *et al*., 2013).

Although sponges are currently divided into four distinct classes, 25 orders, 128 families and 680 genera (Hooper & Van Soest 2000, Van Soest *et al*., 2019), many of these higher taxa are disputed due to new insights obtained from molecular systematic methods and new considerations of their morphological characteristics (Morrow & Cardenas, 2015), and there are also several hundred freshwater species in the Order Spongillida (Manconi & Pronzato, 2002; Van Soest *et al*., 2018). These present knowledge on sponge diversity, together with molecular studies may unravel new and cryptic species, which will contribute in future towards describing the true diversity of sponges (Hooper & Levi, 1994).

Currently, four major classes of marine sponges are recognized (Van Soest *et al*., 2012, Redmond *et al*., 2013, Metobole *et al*., 2017; Van Soest *et al*., 2018). These are the Class Calcarea, class Demospongiae, class Hexactinellida and class Homoscleromorpha (Van Soest *et al*., 2019).

2.2.1. Demospongiae (demosponges)

The class Demospongiae is the largest, most diverse and species rich taxon in the phylum Porifera (Hooper & Van Soest, 2002; Van Soest *et al*., 2012; Redmond *et al*., 2013; Morrow & Cardenas, 2015). The Class comprises about 90% of all existing sponge species and consists of sponges with siliceous spicules (Hooper & Van Soest, 2002; Van Soest *et al*., 2012). These spicules can either be monoxonic, tetraxonic, or polaxonic in structure (Fig. 1) but they are never triaxonic and occasionally they have calcareous basal skeleton (Van Soest *et al*., 2012, Wörheide *et al*., 2012; Morrow & Cardenas, 2015). The mineral skeleton can be partially or entirely replaced by an organic skeleton consisting of spongin (Bergquest, 1967; Hooper & Van Soest, 2002; Wörheide *et al*., 2012). Demosponges inhabit most aquatic habitats, including all oceans from the intertidal to the abyss, from the tropics to the polar seas and almost all types of freshwater habitats (Van Soest *et al*., 2012; Wörheide *et al*., 2012).

The family Cladorhizidae, order Poecilosclerida (Desmospongiae) is the only carnivorous sponge family, lacking the filter-feeding (aquiferous) architecture and choanocyte cells considered to be diagnostic of the Porifera (Hooper & Van Soest, 2002; Van Soest *et al*., 2012; Wörheide *et al*., 2012; Hestetun *et al*., 2016). These typically deep-sea sponges developed the ability to trap, envelop, and digest prey items, representing a unique evolutionary strategy within the phylum Porifera (Hestetun *et al*., 2016).

2.2.2. Hexactinellida (glass sponges)

The class Hexactinellida, also called the “glass sponges”, comprises siliceous sponges that are exclusively marine and restricted to the deepsea (200–6000 m). Currently, 700 extant species are considered valid, representing 7% of all sponges described to date (Reiswig, 2002; Dohrmann *et al*., 2008, Van Soest *et al*., 2012, Redmond *et al*., 2013, Van Soest et al., 2019). This number is questionable and believes to be an underestimat of the actual diversity based on the following: 1) they are found to occupy remote habitats, 2) experts working on this group of sponges are few 3) the deepsea is still largely unexplored and 4) vast museum collections await revision (Dohrmann *et al*., 2008, Wörheide *et al*., 2012). Glass sponges are remarkably different from the other three main classes of sponges (Demospongiae, Calcarea and Homoscleromorpha) in many aspects of their biology. This includes their syncytial tissue organization and triaxonic spicule symmetry (Fig. 1) which clearly distinguish them from the other three major sponge groups and make them one of the best-supported higher level metazoan monophyla (Dohrmann *et al*., 2008, Wörheide *et al*., 2012). They also differ from the other groups because they generally have a larger set of morphological characters, displaying a complex skeletal structure and vast array of different spicule types that provide a wealth of information for the taxonomy of the group (Dohrmann *et al*., 2008)

2.2.3. Homoscleromorpha

The class Homocleromorpha is a small group of marine sponges consisting of less than 100 described extant species (Wörheide *et al*., 2012, Van Soest *et al*., 2012, Redmond *et al*., 2013, Cruz-Barazza *et al*., 2014, Van Soest *et al*., 2019). The monophyly of this group is well accepted on the basis of their general organization (Fig.1) and the shared features of their cytology and embryology (Muricy & Diaz 2002; Cruz-Barazza *et al*., 2014). The Homoscleromorpha further differ from other sponges by their exclusive cinctoblastula larvae and the presence of flagellated exopinacocytes (Boury-Esnault *et al*. 1990, 2003). The classification of the Homoscleromorpha has changed considerably over the years, with its ranking elevated from Suborder to Order, Subclass and Class (Topsent 1895; Dendy 1905; Lévi 1973; Gazave *et al*. 2010, 2012). This was mainly due to the shared presence of siliceous tetractinal-like calthrops (Wörheide *et al*., 2012). These changes reflected the increasing knowledge of their biology and the discovery of new exclusive morphological characters within the phylum.

2.2.4. Calcarea (calcareous sponges)

Calcareous sponges (Class Calcarea) occur mostly in shallow water with just a few species known from the deepsea (Wörheide *et al*., 2012, Willenz *et al*., 2014). Approximately, 675 species have been described and validated to date, representing 7.5% of all living sponges (Hooper & Van Soest 2002, Wörheide *et al*., 2012, Redmond *et al*., 2013, Willenz *et al*., 2014, Van Soest *et al*., 2019). Calcareous sponges are different from the other three main classes of sponges in that, they are characterized by calcium carbonate spicules (Fig.1), that are excreted to the extracellular space, contrary to the intracellular formed siliceous spicules found in the other sponge classes (Wörheide *et al*., 2012). The skeleton of Calcarea sponges is exclusively composed of free spicules but some additionally possess a rigid basal skeleton of fused spicules (Wörheide *et al*., 2012).

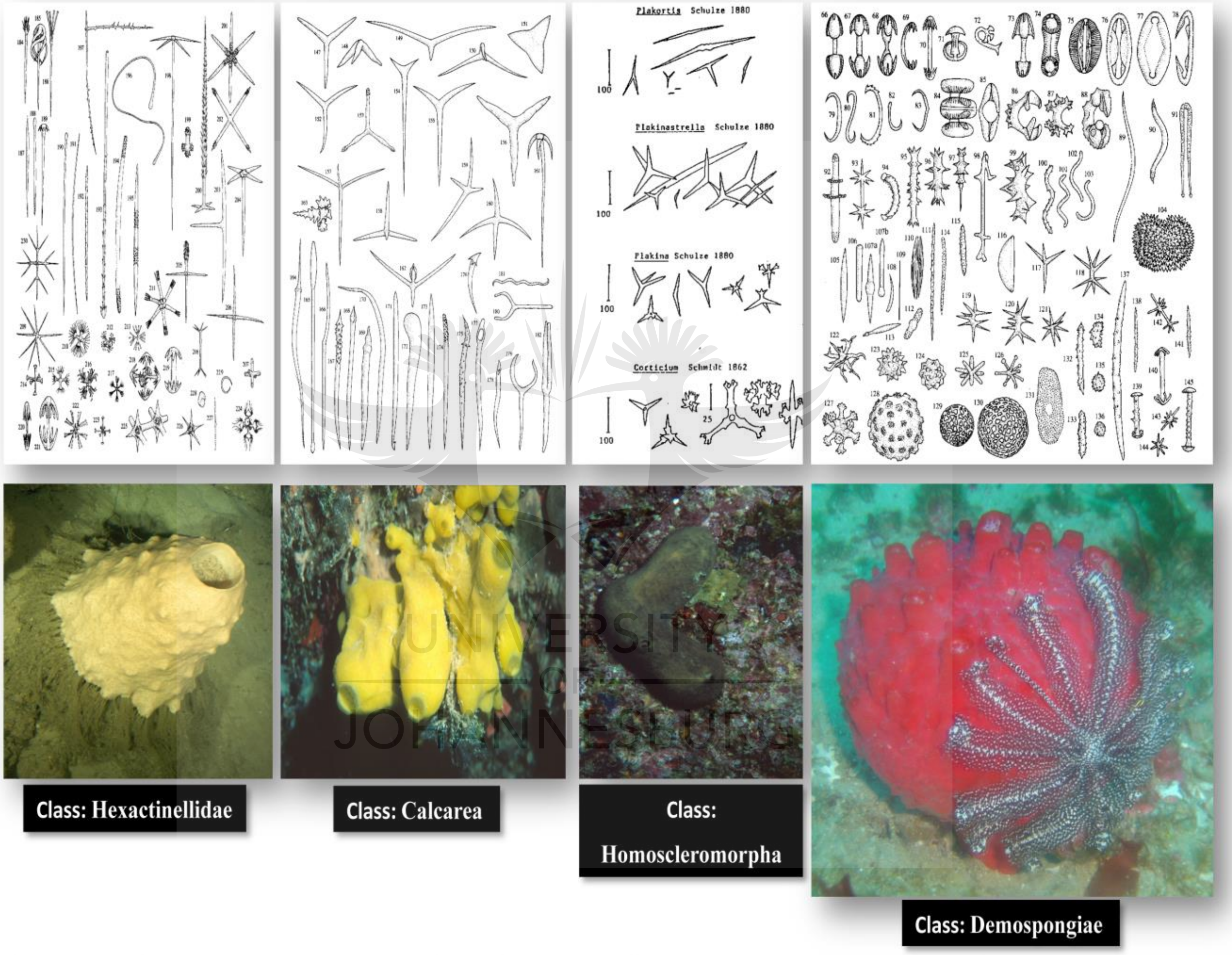

Fig. 1: Spicule plates representing the four different Classes of the phylum Porifera Hexactinellida, Calcarea, Homoscleromorpha and Desmospongiae).

2.3. Global sponge diversity

Sponges have been well represented globally in aquatic habitats. They are highly abundant in temperate, tropical and polar aquatic ecosystem (see Bell & Smith 2004, McClintock *et al*., 2005, Van Soest etal., 2012, Manconi *et al*., 2013). Even though they are very difficult to identify to any operational taxonomic unit (OTU) based on morphological characters, more than 9,125 (Van soest *et al*., 2019) sponge species (marine and non marine) have been described to date, with an estimated real diversity exceeding 15,000 worldwide (Hooper & Van Soest, 2002; Van Soest *et al*., 2012. Van Soest et al., 2019). The majority of studies on sponges has been carried out in temperate Northern Atlantic (including the Mediterranean), western tropical Atlantic, temperate Australasia and Indo-Pacific, but very little has been done on sponges from Africa, Southern Ocean and Western Indian Ocean (Bell *et al*., 2015). Hotspots for sponge research are the Mediterranean Sea, the Caribbean Sea and the Great Barrier Reef (Bell *et al*., 2015). However, sponge studies have been poorly carried out from other areas, such as temperate Southern Africa (see further Van Soest *et al*., 2012).

2.3.1. Overview of Global sponge diversity

Sponge taxonomist has reported various sponge richness's, for example 681, 432, 530, 200 and 85 from different locations or regions (see Coll *et al*., 2010; Voultsiadou, 2005b; Evcin & Cinar 2005). This local or regional diversity richness changes are due to increased surveys at a location or using new tools such as genetics to separate cryptic or morphologically similar species. Globally, the numbers of known or valid sponges to date is 9,125 (Van Soest *et al*., 2019), with the vast majority, 83%, belonging to the class Demospongiae (Van Soest *et al*., 2012, 2019). Global species richness however, as indicated in Van Soest *et al*. (2012) is biased towards collection and taxonomy efforts (see figure 11 in Van Soest *et al*. (2012). Having said this, knowledge of sponge biodiversity is still largely incomplete and estimated that twice the number is thought to exist.

2.3.2. Sponge diversity along the coasts of the Mediterranean Sea and temperate North-eastern Atlantic

Studies carried out on sponges along the coasts of Turkey by Colombo *et al*. (1885), reported five species [*Leucandra aspera*, *Geodia gigas*, *Suberites domuncula*, *Petrosia* (*Petrosia*) *ficiformis*, and *Siphonochalinacoriacea*] in the Çanakkale Strait. Later Ostroumoff *et al*. (1896) listed 31 species of sponges from different depths of the Marmara Sea and the İstanbul Strait. In a study to assess the sponge diversity in shallow-water benthic habitats of the southern coast of Turkey by Evcin & Cinar (2005) a total of 29 sponge species belonging to two classes and 19 families were recorded. All of these species were new to the Mediterranean coast of Turkey. *Phorbas plumosus* was new to the eastern Mediterranean fauna, eight species (*Clathrina clathrus*, *Spirastrella cunctatrix*, *Desmacella inornata*, *Phorbas plumosus*, *Hymerhabdia intermedia*, *Haliclona* (*Halichoclona*) *fulva*, *Petrosia* (*Strongylophora*) *vansoesti*, and *Ircinia dendroides*) were new to the marine fauna of Turkey, and 19 species (*C. clathrus*, *Sycon raphanus*, *Erylus discophorus*, *Alectona millari*, *Cliona celata*, *Diplastrella bistellata*, *Mycale* (*Aegogropila*) *contareni*, *Mycale* (*A.*) *cf. rotalis*, *Mycale* (*Mycale*) *lingua*, *D. inornata*, *Phorbas plumosus*, *P. fi ctitius*, *Lissodendoryx* (*Lissodendoryx*) *isodictyalis*, *Hymerhabdia intermedia*, *H.* (*H.*) *fulva*, *P.* (*S.*) *vansoesti*, *I. dendroides*, *Sarcotragus spinosulus*, and *Aplysina aerophoba*) were new to the Levantine fauna.

Topaloğlu et al. (2016) documented 30 sponge species (two classes and 21 families) from the Sea of Marmara. The class Calcarea was represented by three species (*Sycon raphanus*, *Sycon ciliatum* and *Paraleucilla magna*) and the class Demonspongiae by 25 species. Some families including Chalinidae (four species) and Dysideidae (three species) had the highest number of species. Four species (*Ascandra contorta*, *Paraleucilla magna*, *Polymastia penicillus* and *Raspailia* (*Parasyringella*) *agnata*) were found to be new records from the eastern Miditerranean and six species (*A. contorta*, *P. magna, Chalinula renieroides*, *P. penicillus*, *R.* (*P.*) *agnata* and *Spongia* (*Spongia*) *nitens*] were new records for Turkey. Also 12 species (*Ascandra contorta*, *P. magna, P. penicillus*, *C. renieroides*, *Haliclona* (*Halichoclona*) *fulva*, *Haliclona* (*Rhizoniera*) *sarai*, *R.* (*P.*) *agnata*, *Timea stellata*, *Crambe crambe, Pleraplysilla spinifera* and *S.* (*S.*) *nitens*, *Aplysilla sulfurea*

Schulze, 1878 was a new records for the Marmara Sea. Previous studies reported a total of 132 sponges from the coasts of Turkey. A total of 63 from the Sea of Marmara, 13 from the Black sea, 83 from the Aegean sea and 51 from the Levantine sea (Turkish coast). As of 2016, 138 sponge species were known along the coast of Turkey and 75 from the Sea of Marmara (Topaloğlu *et al*., 2016; Van soest *et al*., 2019)

In the Aegean Sea (Mediterranean), Sarıtaş *et al*. (1972), Sarıtaş *et al*. (1973) and Sarıtaş *et al*. (1974) recorded a total of 50 sponge species in İzmir Bay. Sponge species from the Aegean Sea were also reported in faunistic and ecological works by Geldiay & Kocataş (1972), Kocataş *et al*. (1978), Ergüven *et al*. (1988), Katagan *et al*. (1991), Ergen et al. (1994), Cinar & Ergen (1998), Kocak *et al*. (1999), Topaloğlu (2001a, b) and, Çinar *et al*. (2002). A total of 108 sponges are reported from the Sea of Marmara and Aegean Seas, collectively. However, from the Sea of Marmara and the Mediterranean coast of Turkey (Aegean Sea) a total of 56 and 80 sponge species were reported respectively.

In another study on eastern Mediterranean Demospongiae, Voultsiadou (2005) provided information on the sponge fauna of the Aegean Sea and presented the first sponge checklist of this region. Twenty-five new species were recorded for the eastern Mediterranean and therefore added an additional 25 species to the Aegean demosponge fauna. The 200 species recorded from this area are classified in 103 genera and 54 families, within the class Demospongiae. Although all species reported by the authors were included in the list, it is questionable because some of the records were of rare species recorded for the first time from the Eastern Mediterranean, without any confirmation of their identity or discussion on their distribution (e.g. the species *Cerbaris curvispiculifera* (Carter), *Eurypon major* Sarà, *Petrosia clavata* (Esper), *Spongosorites intricatus* (Topsent), listed by Kefalas *et al.,* 2003).

The diversity of sponges of shallow-water Northeast Atlantic and Western Mediterranean, representing 745 demosponge species are recorded from 187 genera in 64 families (Xavier & Van Soest, 2012). Overall, the Mediterranean is more species rich than the Northeast Atlantic, habouring 539 and 480 species, respectively. Sponge species hotspots in the Mediterranean are the south coasts of Spain, France and Italy, each harbouring over 230 shallow-water demosponge species. In the Northeast Atlantic the highest species-richness values were found on the North coast of Iberia, the English Channel, and the Macaronesian archipelagos of the Canaries, Madeira (CAN) and Cape Verde (CAP), with over 160 species reported for each of these areas. The Northeast Atlantic and the Mediterranean Sea appear to be a diversity hotspot for shallow-water demosponges, containing approximately 11% (> 700 species), of the currently known demosponge species at a global scale (Van Soest *et al*., 2012).

In the South Atlantic Bight (SAB), a region in temperate northwestern Atlantic, which include coastal Georgia (USA) (see Freeman *et al*., 2007, Spalding *et al*., 2009), 52 species of sponges are recorded from coastal Georgia and neighbouring hard-bottom reefs. Forty-eight of the 52 species were known species, two were new to the genera *Raspailia* and *Coelosphaera* while nine species were previously recorded in the tropical Indian Ocean , eight from the Atlantic coast (temperate region), and 31 were classified as cosmopolitan or widespread.

2.3.3. Sponge diversity of the Caribbean Sea

The diversity and abundance of sponges in the Caribbean Sea is relatively well known (Van Soest, 1978, 1980, 1981, 1984). A number of the Caribbean localities were sampled, which included Curaçao, Bonaire and Puerto Rico. Collections were made over a number of years and all material is stored at the Naturalis Biodiversity Center (NBC) The Keratosa collection was found to contain 33 species (Van Soest 1978). The Haplosclerida collection consisted of 36 species but the estimated number of haplosclerid species predicted for the Caribbean is estimated to comprise 60 species (review in Van Soest, 1980). Furthermore, 56 species poecilosclerids were described and fully illustrated from the Caribbean by Van Soest (1984). In addition, 29 new species were were found. These are *Mycale arndti*, *M. diversisigmata*, *M. americana*, *M. magnirhaphidifera*, *Strongylacidon poriticola*, *S. viridis*, *S. rubra*, *Batzella rosea*, *Hemitedania baki*, *Lissodendoryx strongylata*, *Forcepia grandisigmata*, *Coelosphaera hechteli*, *Crella chelifera*, *Hymedesmia jamaicensis*, *H. palmatichelifera*, *H. agariciicola*, *H. curacaoensis*, *Acanthancora coralliophila*, *Clathria simpsoni*, *C. bulbotoxa*, *C. hymedesmioides*, *Rhaphidophlus minutus*, *R. raraechelae*, *R. isodictyoides*, *R. oxeotus*, *Artemisina melana*, *Plocamilla barbadensis*, *Desmacella polysigmata*, and *Didiscus flavus* (Van Soest, 1984).

Twenty-three sponges were decribed from the intertidal rocky shores and subtidal reefs in the Gulf of Urabá, located in the southernmost part of the Caribbean Sea, by Zea (1987). Subsequent studies were carried out by Valderrama and Zea (2003) who recorded 65 demosponge species and one calcareous sponge species, and by Valderrama (2004). A checklist of sponges for the Gulf of Urabá, based on unpublished and published data, recoded a total of 77 demosponge species, three homoscleromorph sponge species and one calcareous sponge species, representing 46 genera, 31 families, 11 orders and three classes (Valderrama & Zea, 2013). The sponges fauna of Bonaire (Caribbean Netherlands) and Klein Curaçao (Curaçao), collected from the lower mesophotic and upper dysphotic zones (Van Soest *et al*., 2014), yielded 31 species belonging to three classes of Porifera (Demospongiae, Hexactinellida and Homocleromorpha). Thirteen of the 31 species described were new to science while the remaining 18 species were described previously.

The western Caribbean along the coast of Panama, particularly the archipelago of Bocas del Toro, which comprises more than 68 islands, have a well documented shallow-water fauna. Guzmán & Guevara (1998, 1999), and Guzmán (2003), listed 63 sponge species from the open reef habitats, while Nicholas and Barnes (unpublished) recorded 86 species from reefs, mangroves, and seagrass beds in the Bocas del Toro. In their collection, 12 sponge taxa could only be identified to family level and 11 to genus level. During a number of surveys around Bocas del Toro islands, 104 species were encountered of which 41 species were new to this area (review in Diaz, 2005). To date, approximately 120 sponge species are recorded from Bocas del Toro and Panama in the Caribbean. Fifteen species were reported previously for other localities in the Caribbean. Collectively, 590 sponge species are recorded from the Tropical Northwestern Atlantic which include the Southern Caribbean (156 species records), Southwestern Caribbean, (139 species records)

Western Caribbean 170 species records) and Eastern Caribbean (227 species records (Van soest *et al*., 2019)

2.3.4. Sponge diversity in the Northwestern Australia

A total of 1164 sponge species were recorded from the Pilbara region, Northwestern Australia (Fromont *et al*., 2016), comprising 12 Hexactinellida, 15 Calcarea, eightHomoscleromorpha and 1129 Demospongiae, from 209 genera and 78 families. The class Demospongiae representing the largest number of species was dominated by the orders Axinellida, Haplosclerida, Poecilosclerida, Tetractinellida and Dictyoceratida (with >100 species within each order). Seventy eight percent of the sponge species recorded are apparent endemics to the Pilbara region. Ten percent of the species were considered widely distributed across three ecoregions while less than 1% of the species, viz. *Echinodictyum clathrioides*, *Acanthella cavernosa*, *Clathria* (*Thalysias*) *abietina* and *Clathria* (*Thalysias*) *lendenfeldi*, were widely distributed across five ecoregions in temperate Australasia (Fromont *et al*., 2016. Collectively, 1015 valid sponge species are recorded from temperate Australasia that includes the East Central Australian Shelf (237 species records), Southeast Australian Shelf (318 species records), southwest Australian Shelf (134 species records) and west central Australian Shelf (116 species records). For the northeast Australian Shelf and orthwest Australian Shelf regions, 224 and 81 sponge species are recorded, respectively (Van Soest *et al*., 2019).

2.3.5. Sponge diversity in Singapore (Southeast Asia)

Hardwicke (1822) described the first *Cliona* species (as *Spongia patera*) from Singapore (Southeast Asia) in the 19th century. This was followed by the species *Leucosolenia flexilis* (Haeckel, 1872), *Coelocarteria singaporensis* (Carter, 1883) (as *Phloeodictyon*), and *Callyspongia* (*Cladochalina*) *diffusa* Ridley (1884). Later Dragnewitsch (1906) recorded 24 species from Tanjong Pagar and Pulau Brani in the Singapore Strait. A further six species were reported from Singapore in the 1900s by Gray (1873). Additional species lists, based on observations (no vouchers material) were made by Chuang (1961, 1973, 1977) and Chou & Wong (1985). Hooper et al. (2000) recorded eighty species, followed by a study by de Voogd and Cleary (2009), in which they recorded 80 species. Lim *et al*. (2009) recorded 62 species of fouling sponges on navigational buoys. Two new species *Tethycometes radicosa* and *Suberites diversicolor*, were collected by dredge from the muddy seabed and collected by snorkeling from an estuary in the Singapore Strait, respectively *(*Lim & Tan, 2008; Becking & Lim, 2009)

Lim et al. 2012 did a comprehensive inventory of the shallow-water sponges of Singapore (See Lim *et al*., 2012) based on an eight-year survey of intertidal and subtidal habitats. A total of 197 species from 16 orders, 50 families and 81 genera were recorded from Singapore, 23 being new records. A total of 99 species were recorded from the intertidal zone, 143 species from the subtidal zone and 45 species occurred in both habitats. A total of 53 species were recorded exclusively from intertidal habitats and 98 species were confined exclusively to subtidal habitats.

2.4. African sponge diversity

Our knowledge of the sponge fauna of the African continent is comparatively poor. Very little has been documented on the sponges of Sub-Saharas Africa. Most of the information is documented in the older literature with very few recent publications documenting sponges.

2.4.1. Overview of sponges of the Western Indian Ocean

The tropical western Indian Ocean (WIO) (excluding South Africa) has received little attention from sponge researchers in the last hundred years. This is evident from the literature on sponges of the Western Indian Ocean (WIO) and the diverse list of Porifera within the World Porifera Database (Van Soest et al., 2019). This list however, is incomplete as more than 80% of the species collected were from shallow coral reef areas. The outer shelf, slope, bathyal and abyssal zones remain almost completely unexplored.

Lendenfeld (1897), Baer (1906) and Jenkin (1908) described the first coastal sponges from East Africa, while Wright (1881), Ridley & Dendy (1887) and Topsent (1893) focused on the deeper offshore areas off East Africa. Lendenfeld (1897) was the first to describe the sponge fauna of Zanzibar. He provided a list of 22 species distributed within the Western Indian and Pacific Oceans. Thomas (1973, 1976a, 1976b, 1976c, 1979a, 1979b, 1980a, 1980b, 1981) contributed extensively to our knowledge of the sponge fauna of this region and discovered many new species in understudied areas such as the Mozambique Channel. Thomas (1973) described 127 sponge species from Mahe Island off the Seychelles, and in 1979 provided 217 distributional records for 59 sponge species collected from Inhaca Island in the Mozambique Channel (Thomas 1979). Thomas (1981) published a second paper on the sponge fauna of the Seychelles and described 73 species. Pulitzer-Finali (1993) recorded 145 shallow water sponge species from Kenya and Mozambique, of which 52 were described as new species. Van Soest (1994) compiled a list of 240 species for the Seychelles and Amirante Islands, increasing the number of know species for this region by 73 species (previously 167 species). Hooper *et al*. 2000 indicated that 44 of the 74 Microcionidae species (Order Poecilosclerida) recorded in the WIO, are endemic to the region.

Barnes & Bell (2002) listed 98 sponge species from the coastal zones of Malindi (Kenya), Quirimbas Archipelago (Northern Mozambique), Inhaca Island (Southern Mozambique) and Anakao (SW Madagascar) within the West Indian Ocean. They also provided 209 distribution records for the 98 species recorded. Richmond (1997) hypothesized that 35% of sponge species found in the Western Indian Ocean are widely distributed from the Red Sea across the Indo-Malay region, extending into the Indo-Pacific region, while 15% extend into the warm temperate region of the Atlantic Ocean. However, these suggestions had no genetic backup (Hooper *et al*., 2000) to verify this hypothesis.

2.4.2. Overview of sponges of Tropical West Africa

Van Soest (1988) described and reported a new species of *Tetrapocillon* (*Tetrapocillon) atlanticus* from the Cape Verde islands. He also provided a comparison of species from the Indo-Pacific. Van Soest (1990) reported and described another new species *Monanchora* (*Monanchora) stocki* from the Mid-Atlantic Islands. Apart from his regional comparisons he made reference to 8 valid species with various distribution patterns within the Atlantic Ocean, Pacific Ocean and Mediterranean Sea. Van Soest (1993) described the distribution of Mauritanian continental sponges and provided a list of seven species with 13 distributional records. Van Soest (1993) also discussed the affinities of the Demospongiae fauna of the Cape Verde Islands and Tropical West Africa and listed 99 species with over 526 distributions.

Burton (1956) describing the sponge fauna of West Africa and referenced approximately 186 species recorded from this region. He recorded nine endemic species and five new species (Burton, 1956). Some species had distributional recorded from the Atlantic and the Mediterranean. Burton also made an extensive collection of the sponge fauna from the Atlantic seaboard of the African continent during the Danish Expedition (1945–1946) and recorded a total number of 65 sponge species from tropical West Africa. He had previously recorded 23 species from the Atlantic Seaboard of Europe, by which in comparison with others, he concluded that half of these species have been recorded from Tropical West Africa and also occurred in the Miditerranean. These species included *Leuconia rudifera* Polèjaeff, *Tethya aurantium* Pallas, *Suberites carnosus* (Johnston), *Haliclona angulata* (Bowerbank) and *Myxilla rosacea* (Lieberkühn). Burton (1956) also noted that some more northerly species were also to be found off West Africa.

2.4.3. Overview of other African Countries

Mustapha et al. (2003) described 96 species from Tunisia. However, these sponges were found to have a greater affinity with the Mediterranean sponge fauna than with Africa. Lévi (1965) described 45 sponge species from the Red Sea, non being restricted to the region, but with extended distributions from the Mediterranean Sea to the Pacific Ocean. Lévi (1965) recorded approxiamately 144 distributions for the described species.

Maldonado (1992), after examining a total of 107 sponges from the Alborean Sea, described a total of 58 sponge species from this area with 61 apparent distributions recorded. Two of these species were recorded as new to science (*Plakinastrella mixta* and *Leptolabis*) and one, *Rhaphidectyon spinosum* Topsent, was recorded for the first time in the Mediterranean. Some specimens belonged to poorly known species, such as *Erylus papulifer* Pulitzer-Finali, *Isops anceps* (Vosmaer), *Spongosorites flavens* Pulitzer-Finali and *Timea cumana* Pulitzer-Finali, Two controversial specimens were assigned to *Microciona spinarcus* and *Plocamilla cf. novizelanica.*

Ilan et al. (2004) describes six new species in the northern Red Sea while some were reassigned and renamed to avoid homonymy. Our current knowledge of Red Sea sponges is based largely on the works of Keller (1889, 1891), Row (1911) and Lévi (1958, 1965, 1966), as well as on contributions by several other authors (e.g., Topsent, 1892, 1906; Burton, 1952, 1959; Kelly Borges and Vacelet, 1995; Vacelet *et al*., 2001; Helmy *et al*., 2004; Ilan *et al*., 2004; Helmy & Van Soest, 2005; Gugel *et al*., 2011). Most studies have focused on the Gulfs of Suez and Aqaba, leaving large areas of the Red Sea largely underexplored (Berumen *et al*., 2013). Perez, *et al*., 2004 described two new lithistids (Demospongiae) from a shallow eastern Mediterranean cave off the coast of Lebanon. They also make reference to seven species and describe eight distribution patterns. Voultsiadou & Vafidis (2004) described a few rare sponge species (Demospongiae) from the Mediterranean Sea. In spite of the Mediterranean Sea sponges being widely studied, knowledge of the eastern basin sponges is still wanting.

For the African continent, at present 1307 sponge species are recorded from the East African region, 538 from West Africa and 343 from South Africa. For Africa the highest number of species was recorded from the Western Indian Ocean with the highest number of sponges recorded from Kenya (502), South Africa (343) and the Seychelles (235).

2.4.4 South African sponge diversity

Southern Africa has a unique coastline and straddles two great oceans, which include a substantial diversity of ecosystems, ranging from tropical coral reefs to cool-water kelp forests (Samaai 2006, Metobole *et al*., 2017). These shores are particularly rich in biodiversity and some 12,914 species of free-living marine animals have been recorded or described (Gibbons *et al*, 1999; Griffiths *et al*. 2010) since the first expeditions to the Southern Seas in the early 1800s (“Challenger”, “Valdivia” expeditions). Notwithstanding the above, many taxa still remain poorly documented (Griffiths *et al*., 2010) and unexplored (Gibbons *et al*., 1996; Griffiths *et al*., 2010; Metobole *et al*., 2017). This is particularly true of sponges (Samaai, 2006) as the current knowledge of the sponge fauna of South Africa is relative; over the last decade 45 additional sponges have been described or newly recorded in the region increasing the number of species from 298 to 343.

The history of sponge research in South Africa is brief. Esper (1797) described the first sponges from the Cape of Good Hope and since then, there have been relatively few expeditions or collections from this region (e.g. “Challenger”, “Valdivia”, “Scotia” expeditions). Reports on South African sponges included those of Carter (1876, 1879, 1881, and 1883), Vosmaer (1880), Ridley & Dendy (1887), Kirkpatrick (1902, 1903), Baer (1905), Stephens (1915) and Burton (1926, 1931, 1933, 1936). Lévi (1963, 1967) described deep-water sponges from the orders Poecilosclerida and Astrophorida, while Borojevic (1967) conducted a study on Calcarea sponges. Uriz (1984, 1985, 1988) described the sponge fauna of Namibia. Day (1974) provided the first species list and ecological notes for sponges in the False Bay, South Africa.

Studies on sponges during the late 1800s and early 1900s were initially focused on the deep-water fauna of the south and east coasts of South Africa. Although the lists that were compiled were comprehensive at the time, there were no detailed descriptions of these species. Within a period of 40 years, from 1847 to 1887, Carter published no less than 125 papers on sponges, which included species found around South Africa. Vosmaer (1880) examined the sponges from the Leyden Museum of Natural History. This collection contained a few sponges collected from the Cape of Good Hope [*Amphilectus caesper*, *Desmacidon (Myxilla?) elastica* and *Clathria lobata*], which he described.

Ridly and Dendy (1887) recorded a total of 54 genera and approximately 100 sponge species collected during the "Challenger" Expedition. Most of the species they described were from the Pacific, the Indo-Pacific and Antarctic waters, but the expedition also made a valuable contribution to understanding the sponge fauna of South Africa. Ten species were described from South Africa: *Raspailia flagelliformis*, *Raspailia rigida*, *Clathria Lobata*, *Coelosphaera navicelligerum*, *Desmacidon ramosa*, *Lissodendoryx digitata*, *Isodictya conulosa*, *Isodictya grandis* and one species belonging to the genus *Haliclona.*

Baer (1905) provided a list of 24 sponge species for Zanzibar which included a few species found off the Cape of Good Hope reported by Ridley and Dendy (1887). Kirkpatrick (1902, 1903) decribed the sponges from the “Gilchrist” collection, which where the most complete account of South African sponges at the time. Kirkpatrick's collection comprised approximately 50 species of which 28 were new and mostly collected from the east coast of South Africa (in what is now KwaZulu-Natal). Comparing Kirkpatrick's collection to those of Ridley and Dendy (1887) and Carter (1876, 1879, 1881, 1883, 1885), indicated only six species in common, which included *Tetilla casula* Carter, *Clathria typica* Carter, *Higginsia bidentifera* Ridley and Dendy, *Desmacidon ramosum* Ridley and Dendy, *Desmacidon grande* Ridley and Dendy, and *Hamacantha esperioides* Ridley and Dendy.

Stephens (1915) described 37 new species from the west coast of South Africa (False Bay to Saldanha Bay) and also expanded the range of the number of previously recorded species. Although his collection was small, it contributed much to the knowledge of the South African west coast sponges. Comparing Stephens's collection to Kirkpatrick collection, five genera were in common but no species were shared. This is because a) the west coast is bathed by the cold Benguela Current and the south and east coasts are influenced by the warm Agulhas Current, and b) Kirkpatrick's 1902-03 collection was from deeper waters than the sponges collected by the "Scotia" expedition (Stephens, 1915). All these expeditions collected large quantities of sponges, and many of the new species that were described for the west coast were not found on the Natal (i.e., east) coast (Lévi, 1963). In total, the "Gilchrist" (Kirkpatrick, 1902-03), "Challenger" (Ridley & Dendy, 1887) and "Scotia" expeditions (Stephens, 1915) collected a total of 180 Demospongiae, 16 Calcarea and six Hexactinellida sponges from around South Africa (Lévi, 1963).

Burton (1926-1936) was the first to report on Lithistid sponges from the west coast of South Africa. Burton (1926) described 21 species of "Myxospongida" and "Astrotetraxonida", and also reported on specimens from the Natal and Durban museums. The most comprehensive works covering South African sponges are those of Lévi (1963, 1967) on the orders Poecilosclerida and Astrophorida (Class Demospongiae), and by Borojevic (1967) on the class Calcarea. Most of the coastal species were collected along the east coast. Uriz (1984, 1985, and 1988) focused her attention on the Namibian (not South African) deep-water sponge fauna. Still, in view of the geographical proximity and depth of the waters considered in these works (including Lévi, 1963), they were taken as the initial references for the study of the sponge fauna along the west coast of South Africa.

While the above works provide some general information, albeit in a haphazard fashion, much more recent surveys by Samaai contributed vastly to the knowledge of the shallow-water sponges of South Africa (Samaai, 2002; Samaai *et al*., 2004; Samaai & Gibbons, 2005). During the past years, approximately 45 new species and two new genera have been described for South Africa (Samaai & Gibbons, 2005; Samaai *et al*., 2003; Samaai *et al*., 2004a&b, Samaai *et al*., 2004), with new species being discovered at an increasing rate.

Currently, the number of described sponges from South Africa (ranging from Oranjemund on the West Coast to Richards Bay on the East Coast), is 343 species (Samaai, pers. comm.) based solely on morphological characters. This is low compared to other marine invertebrates such as mollusk, snails, annelids and cnidarians identified in South Africa (Gibbon *et al*., 1999; Griffiths *et al*., 2010), and may suggest that the South African sponge biodiversity is far from fully described. This lack of knowledge of marine sponge biodiversity of South Africa threatens our ability to conserve, manage and utilize this natural resource sustainably. Some sponge species such as the Lutrunculid sponges, *Tsitsikamma* spp. and *Cyclacanthia* spp., are highly diverse and endemic to the South African coastline (Samaai *et al*. 2002; Metobole *et al*., 2017). In general, South African sponges have not yet been included in studies of phylogenetic relationships within the phylum Porifera. Also, the phylogenetic relationships between the South African sponges remain to be resolved. Therefore, DNA barcoding can provide a tool to aid species discoveries and provide a deeper understanding of the evolutionary relationships and speciation of South African sponges in general.

2.5. The need for DNA based identifications of South African sponges

Many sponge species are notoriously difficult to identify, often even by taxonomic experts. This is because characters for comparative morphology are scarce and prone to homoplasies, highly variable even within the same species, or otherwise unsuitable for unambiguous identification (Van Soest *et al*., 2012). In addition, many sponges discovered in large-scale biodiversity surveys remain undescribed (Hooper

& Ekins 2004), partly also due to the lack of skilled taxonomists. Due to uncertainties in morphological systematics, sponge species have frequently been regarded as widely distributed (‘cosmopolitan’) (Klautau *et al*, Wörheide *et al*., 2007). However, genetic approaches, for example using allozymes, have shown that the idea of ‘cosmopolitan’ sponges is often problematic, and is primarily an artefact of over-conservative systematics and lumping of morphologically similar but evolutionarily distinct lineages into one widely distributed morphospecies (Klautau *et al*., 1999). Therefore, the question of how to describe and distinguish such genetically distinct and potentially reproductively isolated lineages remains complicated, due to the difficulty of relating those genetic differences to traditional morphological delineation of ‘species’.

While the use of fixed differences in “diagnostic” morphological characters (e.g. skeletal elements and architecture) is practical and has served reasonably well to catalogue diversity, it is doubtful that such a typological system reflects the real biological diversity (Van Soest *et al*., 2012). Sponge alpha-taxonomy is still quite an artificial system solely based on morphological differences without considering evolutionary history and/or reproductive isolation. Nonetheless, correctly identifying reproductive isolated and evolutionary distinct lineages of sponges remains relevant for understanding a broad range of subjects such as marine ecology, biodiversity, dispersal, animal evolution and discovery of pharmaceutically / biotechnologically valuable taxa.

Conventional morphological taxonomy alone clearly is at its limit with the task of distinguishing closely related but evolutionary distinct sponge lineages, especially in character-poor taxa. The utilization of additional characters, such as informative signature DNA sequences (also known as DNA barcodes) (Hebert, 2004; Hebert, 2003), and the establishment of a DNA sequence-aided taxonomic system are providing an opportunity to overcome these shortcomings and aid our endeavours to strive for more comprehensive species discoveries and descriptions, as well as a deeper understanding of evolutionary factors that shape species distributions in space and time. A DNA sequence-based taxonomic system should by no means replace, but rather complement, conventional taxonomy based on comparative morphology – the DNA sequences are simply regarded as additional characters to described morphological (and biochemical) features.

As indicated above, the project aims to establish a reference backbone of DNA barcodes for South African sponges. All sponge material collected over the last 10 years from South Africa is being deposited into the collections of the South African Iziko Museum, Cape Town. All sponges collected have been identified to the lowest operational taxonomic unit (OTU) possible, and stored in 96% ethanol. The specimens included in the present dissertation represent a first step in analyzing this large, comprehensive resource.

Hypotheses

I) The South African sponge fauna is as diverse as other regional sponge fauna. Assemblages with more than 40 species are considered diverse.

II) New species of South African sponges will be discovered using genetics in this study

III) Different sponge assemblages will be found in different South African ecoregions

Chapter 3: Materials and methods

3.1. Samples

Sponge samples were collected during various field trips and cruises, from 2010–2016, as part of the African Sponge Ecology Programme (ASEP). The South African Ecological Economic Zone (EEZ) can be divided into the following ecoregions: Benguela, Agulhas, Natal, Delagoa, Southeast Atlantic and Southwest Indian Ocean (Figure 2) (see also Sink *et al*., 2012). Samples were collected from the Benguela, Agulhas and Natal ecoregions and included the coast and continental shelf (Figure 2). No samples were collected from deeper-water habitats, including the shelf edge, slope, the upper, lower bathyal zones and the abyss of the Atlantic and Southwest Indian ecoregions as defined by Sink et al., 2012 (Figure 2). Collections were carried out using SCUBA up to a maximum depth of 40 m, wading in the intertidal zone and a rouged sled/dredge at depths deeper than 40 m. Upon collection, specimens were preserved in 96% ethanol. The ethanol was changed once in the lab and a small portion (5 mm^3) of the morphological sponge sample was stored separately in 96% ethanol for genetic analysis. The genetic samples were stored in a freezer, and the ethanol was completely replaced during the first days to limit DNA degradation. Approximately 2000 sponge samples collected from various locations around South Africa were preserved in this way for future genetic research.

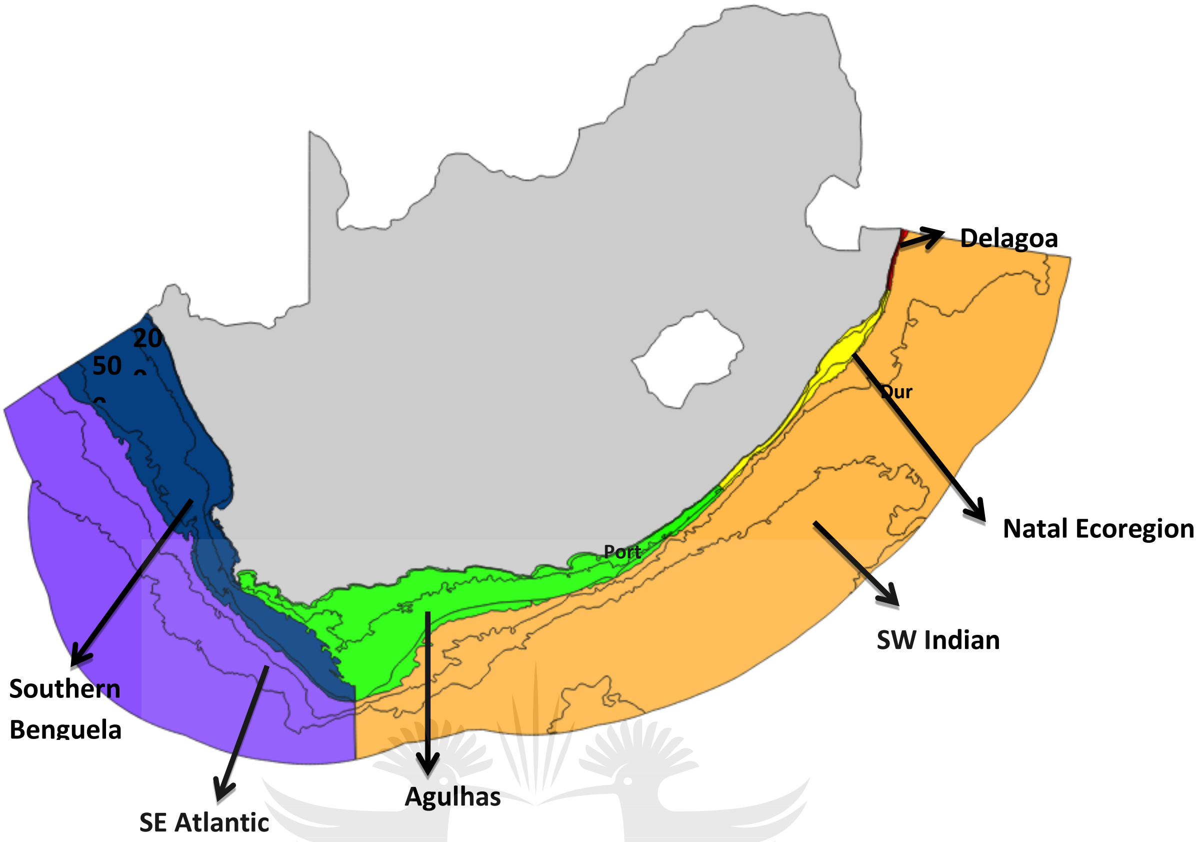

Fig 2a: A map representing the different ecoregions where sponge samples used for this study were collected during various field trips and cruises from the year 2010-2016 (Map created by Dr. Toufiek Samaai).

3.2. Sampling sites

The southern African subcontinent, located south of the 20°S latitude, forms a landmass that gradually narrows southwards (Thandar 1989). Mainland South Africa is the meeting place of two of the world’s greatest oceans and receives faunal incursions from all sides, producing a highly complex fauna. South Africa has a unique coastline, bathed by two opposing currents, with a large diversity of ecosystems offering a variety of marine habitats (Griffith *et al*., 2010). These, coupled with the narrow continental shelf, frequent upwelling and downwelling, warm and cold ocean currents and the mixing of these in the south-western corner of South Africa, have resulted in unique conditions, especially in the temperate regions, producing numerous endemic species and a highly diverse and rich fauna (Griffith *et al*., 2010)

As indicated above, South Africa’s EEZ can be divided into six ecoregions; Benguela, Agulhas, Natal, Delagoa, Southeast Atlantic and Southwest Indian (Sink *et al*., 2012). These ecoregions can further be divided into seven coastal ecozones which include the cool-temperate west coast, south-west coast, warm-temperate south coast, south-east coast, subtropical east coast and tropical north-east coasts (Figure 3). Specimens for this study were collected from different ecoregions to have a representative faunal sample.

3.2.1. Benguela Ecoregion

Deep-water samples were collected during various annual research trawls surveys undertaken by the Department of Agriculture, Forestry and Fisheries (DAFF). These samples included specimens of the newly described *Suberites dandelenae* Samaai & Maduray, 2017, which was collected from the west coast at a depth range of 80–450 m. Intertidal and shallow-water samples were collected from the Northern Cape, Cape Peninsula, Table Mountain Marine Protected Area (TMMPA) and Betty’s Bay (Koggelberg Protected Area). Samples from these shallow hard benthic reefs were collected by means of SCUBA at a depth range of 2–15 m.

3.2.2. Agulhas Ecoregion

The Agulhas ecoregion includes the south and south-east coasts of South Africa and is strongly influenced by the warm Agulhas Current. The south coast stretches from Cape Agulhas to approximately Port Elizabeth (see Figure 3) and the south-east coast stretches from Port Elizabeth to Port St Johns, and constitutes a biogeographic overlap zone, as it shares some of its fauna with the subtropical east coast (Turpie *et al*., 2000). Samples were collected from the following locations:

- **False Bay***.* Situated within the Agulhas inshore ecozone on the south coast. Intertidal samples were collected by means of wading. Shallow reef samples were collected Boulders on the western part of False Bay by means of SCUBA between depths 7–10 m.
- **Alphard Bank and associated inshore reefs.** This is a central hard benthic reef complex situated 40 nautical miles offshore of the south coast. Samples were collected by means of SCUBA diving at depths between 15–40 m and/or by dredging/sledging at depths between 10–80 m. The inshore reefs were sampled by dredging from depths between 10–30 m.
- **Plettenberg Bay and surrounding areas.** Plettenberg Bay is situated within the Agulhas inshore ecozone on the south-east coast of South Africa. Intertidal samples were collected by means of wading at the Robberg Marine protected Area (MPA) and Natures Valley. Shallow reef samples were collected from Grootbank and Blinders by means of SCUBA between depths of 10–25 m. Other locations sampled by SCUBA were Jeffreys Bay and Cape St Francis.
- **East London and Eastern Cape Area**. Sam were collected from th Amathola principality (East London and surrounds),Situated within the Agulhas inshore ecozone on the south east coast. Intertidal samples were collected by means of wading. Shallow and deep-water samples were collected off Amathola by means of dredging to a depth of 150 m.

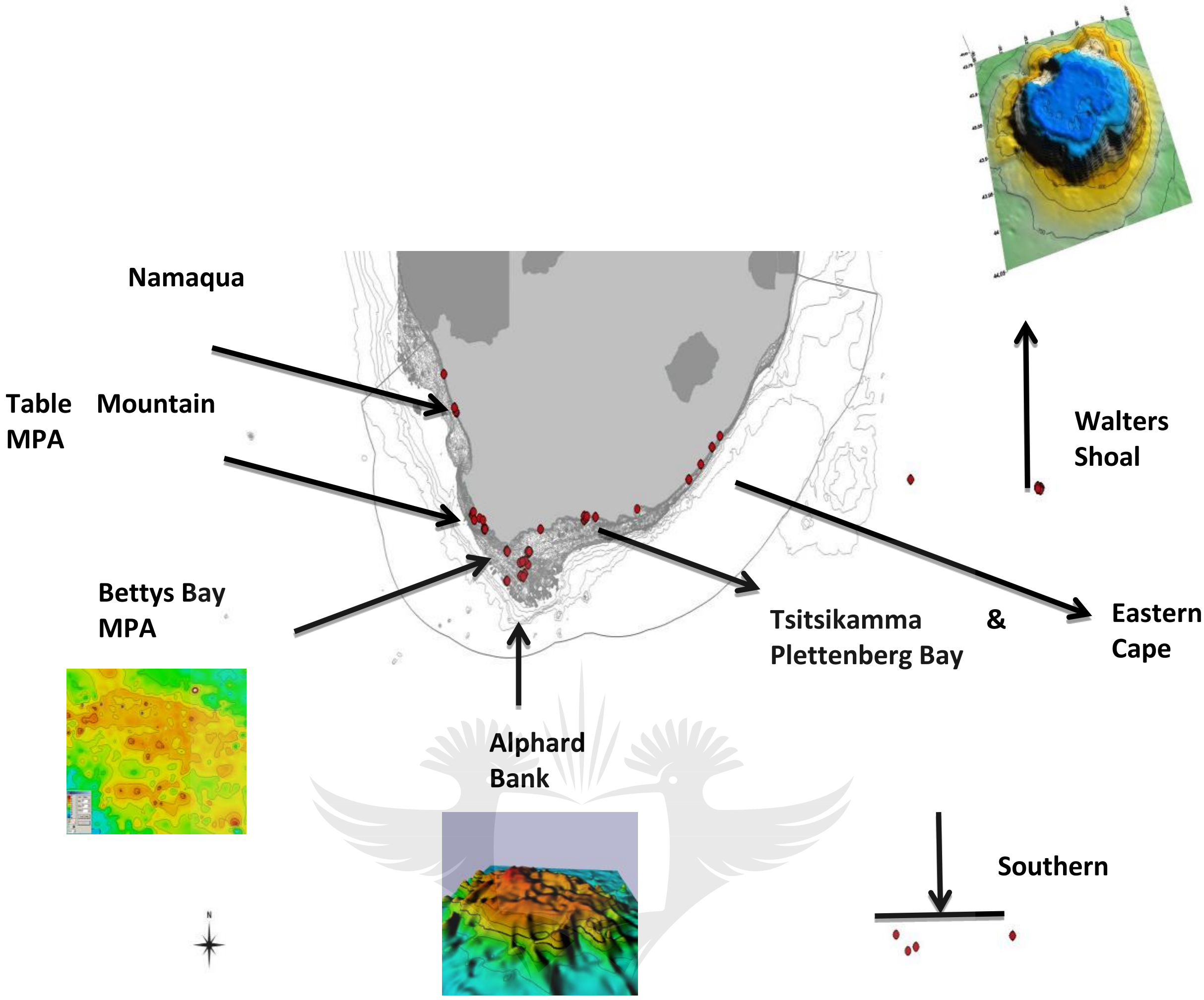

Fig 3: A Map showing the different sponge sampling locations within the different ecozones of the South African region. (Map created by Dr Toufiek Samaai)

3.3. Laboratory procedures

3.3.1. Specimens identification

Observations on appearance in life, surface structure, texture, colour, depth, latitude and longitude were recorded. However, due sampling being conducted by different individuals, photographic data and *in situ* colour were not always collected. Photographs of the samples, as well as notes on surface structure, shape and dimensions were taken after collection prior to placing them into ethanol.

3.3.2. Taxonomic procedures

Each specimen to be analysed genetically was subjected to a comparative morphological analysis that was carried out by DAFF staff. A small section (3–5 mm^3) that contained choanosome and ectosome was cut from the specimen, and placed into a test tube. The spicules were isolated from the section in a fume cupboard by digesting the sponge tissue in 100% nitric acid. Material was then washed three times (twice with distilled water and once with 70% ethanol) prior to microscopic examination. Between each rinse the material was centrifuged for 3–5 min at 4000 rpm. Clean spicule samples were then stored in 100% ethanol at room temperature. For examination purposes the spicules were re-suspended and pipetted onto a microscopic slide, and the ethanol was evaporated off on a heated tray at 40 °C. After the slides were completely dry, a few drops of Entellan or DpX were added, and a cover slip was put in place. The slides were then allowed to air dry at room temperature for at least two days, or until the mountant had hardened.

In order to examine the skeletal arrangement of the sponge, a perpendicular section (~5 mm^3) of tissue was cut from the voucher material, and embedded in paraffin wax, after it had been processed automatically through a series of dehydrating and embedding agents. Histological sections of ~75 µm were cut using a microtome. The wax was removed from the section by washing it in xylene. Sections were placed and mounted on microscopic slides with Entellan or DpX and viewed under a Zeiss

AxioSkop 40 microscope. Diagnostic features, such as arrangement of spicules, for each specimen were photographed with a Carl Zeiss MRc5 camera, using the Carl Zeiss AxioVision Rel. 4.6 software, at the appropriate level of magnification.

3.3.4. DNA Extraction

Genomic DNA was extracted from ethanol-preserved sponge tissues following a cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle 1987; 1990). The tissue samples were cut into smaller pieces and put into 1.5 Eppendorf tubes. Cells were lysed in 1 ml of CTAB extraction buffer, and proteins were digested with 5 µl of proteinase K, keeping the samples at 56°C overnight. To separate proteins and polysaccharides from nucleic acids, 500 µl of a 1:24 mixture of isoamyl-alcohol and chloroform was added to the digested tissue and centrifuged at 13,000 rpm for 20 min. Centrifuging helped in separating the tube content into three visible separate layers. Unlike other animal tissue worked on in our lab, where centrifugation was done just for about 5–10 min, sponges are highly contaminated and contain comparatively little DNA, and centrifuging for as long as 20 min helped to separate the different layers completely. The upper phase of the separated components containing nucleic acid was transferred into new tubes, and DNA was precipitated with 500 µl of chilled propan-2-ol. Following a washing step with 70% ethanol, DNA was air dried and suspended in 40 µl of diluted TE (Tris-EDTA) buffer (in a 1:100 ratio of Tris-EDTA buffer and distilled water). To improve DNA quality prior to polymerase chain reaction (PCR), the extracted DNA was purified using the QIAquick PCR Purification Kit (Qiagen) following the manufacturer’s specifications. This is procedure is usually performed on PCR products after amplification, but it helped to improve amplification success of the DNA templates very significantly. The purity and quantity of the DNA was determined with a Nanodrop 2000c spectrophotometer to ensure that only high quality DNA was used for subsequent polymerase chain reaction.

3.3.5. Polymerase chain reaction (PCR) and sequencing

A portion of the cytochrome c oxidase subunit 1 (*cox*1) gene of the mitochondrial DNA genome (hereafter referred to as the 'standard region') was amplified, using universal primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer *et al*., 1994). Where cryptic speciation was suspected, an additional region (the 'extended region') of the *cox*1 gene was amplified. This was done using a nested procedure, in which the PCR product of the first reaction was used as the DNA template of the second PCR reaction. The following two pairs of universal metazoan primers were applied in a nested-PCR to target the extended portion: C1J2165 (5' GAA GTT TAT ATT TTA ATT TTA CCN GG 3') and C1Npor2760 (5' TCT AGG TAA TCC AGC TAA ACC 3') for the first reaction (Misof *et al*., 2000; Erpenbeck *et al*., 2002) and CO1porF1 (5'CCN CAN TTN KCN GMN AAA AAA CA 3'), and CO1porR1 (5'AAN TGN TGN GGR AAR AAN G 3') (Erpenbeck *et al*., 2004; Erpenbeck *et al*., 2006), for the second reaction. For the second round of the nested PCR, 5 µl of diluted amplicon (in a 1:50 ratio of amplicon and distilled water) was used as the template. In some cases where results based on cox1 were inconclusive, I also explored the use of the nuclear Internal Transcribed Spacers (ITS) by designing primers specifically for the Demospongiae that annealed to the highly conserved 18S and 28S rRNA regions, and amplified the more variable ITS1 and ITS2 regions, in addition to the conserved, centrally located 5.8S rRNA. These were RA2_Nest1-F (GTC CCT GCC CTT TGT ACA CA) and 28S5rev_Nest1-R (GAC GTG CCT T TC CAG GTC AAC TT). Polymerase chain reactions were performed in volumes of 20 µl containing 1.2 µl of 3 mM $MgCl_2$, 2 µl of 10× buffer (promega), 0.64 µl of 20 mM dNTP mixture (Sigma-Aldrich), 0.5 µl of each primer (10 µM in concentration), 0.24 µl of Bovine Serum Albumin (BSA), 0.16 µl of Super-Therm Taq polymerase (Separation Scientific, South Africa) and 3 µl of purified DNA template. The thermal cycling protocol used for both the cox 1 (standard and extended regions) and ITS fragments was as follows: 2 minutes initial denaturation at 94°C, 40 cycles of 94°C for 30 s, 50°C for 40 s and 72°C for 1min, followed by a final extension step at 72°C for 10 min. PCR products were visualized on a 2% agarose gel via electrophoresis. Samples were sequenced at the Central Analytical Facility at Stellenbosch University.

3.3.6. Data processing and analysis

The raw sequence data were checked in MEGA 7.0 (Kumar *et al*. 2016), and forward and reverse sequences were trimmed by removing sites containing ambiguous characters at the 5' ends. The forward and reverse sequences were then aligned separately by ClustalW (Thompson *et al*., 1994), and after creating reverse complements of the reverse sequences, the two portions of the gene fragment were merged. The standard and extended *cox*1 sequences were also aligned separately and were concatenated for further analysis. To confirm that each sequences originated from sponges, they were blasted against NCBI GenBank collection (http://www.ncbi.nlm.nih.gov/) and the maximum score and E-values (Altschul *et al*., 1990) were used to select closely related specimens as these values reflect the evolutionary distances of two or more aligned sequences. Taxonomic assessments were attempted by phylogenetic grouping in relation to previously published sequences and, where possible, were subsequently compared with the morphological species descriptions.

Similar sequences (from similar species) were grouped together. To determine the placement of these sequences between the different monophyletic groups a phylogenetic analysis were done from which outgroups were chosen from GenBank. These outgroup taxa were aligned with the standard fragment of the in-group. The *cox*1 sequences were checked for potential occurrence of nuclear pseudogenes by translating the nucleotide sequences using the genetic code for invertebrate mitochondrial DNA in MEGA, and searching for stop codons. To determine whether specimens from the ecoregions within South Africa formed a monophyletic clades, phylogenetic trees were constructed using the neighbour joining method (Saitou & Nei, 1987) in MEGA, and support for individual node was based on 2000 non-parametric bootstrap estimates (Felsenstein 1985). Evolutionary distances were computed using the Kimura 2-parameter (K2P) method (Kimura 1980). The K2P distances were used to compare levels of genetic differentiation between sequences from the specimens obtained from different ecoregions.In cases where possible sequences from the GenBank were shorter than the sequences generated in this study, the program was set to apply “pairwise deletion” to allow usigshorter sequences from GenBank without losing too much information.

Chapter 4: Results

A total of 131 species were identified from this study, of which one hundred and ten (110) species are potentially new. Twenty one species (21) were previously identified. All species are endemic to the various ecoregions on the west, south and south-east coasts of South Africa.

4.1. Sequencing success and OTUs (Operational Taxonomic Units)

DNA was extracted from approximately 2000 sponge specimens of which 900 yielded high quality DNA. The remaining 1100 specimens failed to yield any DNA, which could be because the samples were old as they were collected as far back as 2010. Of the high quality DNA templates, only 705 specimens were successfully amplified. These were sequenced and blasted against the GenBank database. In total 317 *cox*1 sequences were successfully sequenced (see Supplementary Table 1). In some instances, (i.e. for 297 sequences), the cox1 gene was amplified from other organisms such as snails, bacteria or polycheates. Additional sequences with quality values below threshold were disregarded (non-substandard length > 200 bp) and re-sequenced if possible. The sequences will be added to GenBank at a later stage. Ninety-one sequences were contaminated during the morphological assessment. Of the 317 specimens with total *cox*1 sequence lengths of 678 base pairs (bp) each, 253 specimens (80%) could be assigned to a total of 67 OTUs (i.e. these samples shared their genotypes with at least one other specimen). An additional 64 specimens were singletons, i.e. they had unique sequences not shared with other samples. For 30 specimens (10%), sequencing of both standard and extended fragments (to a sequence length of 1113bps) proved very valuable because it aided in the assignment of the specimens to OTUs that were based on molecular taxonomic identification. Fragments of Internal Transcribe spacer (ITS) were also generated for 36 specimens (11%) in cases where cryptic speciation was suspected, which also aided in the molecular taxonomic identification of OTUs.

For several conspicuous South African taxa, barcodes were obtained for the first time and operational taxonomic units (OTU) assigned, including *Tsitsikamma* spp.

(OTU#= 3), *Cyclacanthia* sp. (OTU#= 1), *Hymeniacidon* spp. (OTU#= 2), *Suberites* sp. (OUT#=2), *Erylus* spp. (OTU#= 2), *Sigmaxinella* spp. (OTU#= 2), *Poecillastra* spp. (OTU#= 2), *Raspailiidae* spp. (OTU= 5), Niphatidae spp. (OTU# = 2), *Cliona* spp. (OTU#= 1), *Characella* spp. (OTU#= 3), *Polymastia* spp. (OTU#= 2), *Penares* spp. (OTU#= 2), Scopalinidae spp. (OTU#= 1), *Rhabdastrella* sp. (OTU#= 1), Micrcionidae spp. (OTU#= 1), *Clathria* sp. (OTU#= 1), Phymaraphiniidae sp. (OTU#= 1), *Callipelta* sp. (OTU#= 1),*Coelosphaera (Coelosphaera) navicelligera* (OTU#= 1). Type and voucher specimens are housed at iZiko museum of Natural History, South Africa and in Toufiek Samaai's (TS) collection at DEA Oceans & Coasts. Specimen records and photos are in the TS database, thin sections and spicule preparations have been lodged in the TS collection. The database will be further expanded with molecular and morphological details of specimens that will be collected, identify and sequenced in the nearest future.

4.2. Phylogenetic analyses and identification of OTUs

Similar sequences, from a minimum of two and a maximum of 25 specimens, were recorded as monophyletic clades in the phylogenetic analyses. Most of these clades are highly supported with 100% bootstrap support. Some clades showed distinct lineages from different ecoregions (e.g. *Suberites* spp., *Erylus* spp.), while other clades formed either one or two distinct lineages from the same bioregion (e.g. *Sigmaxinella* spp., *Cyclacanthia* spp., *Homaxinella* spp., *Rhabdastrella* spp., *Characella* spp., Raspaillidae spp., *Stelleta* spp., Microcionidae spp., Phymaraphiniidae spp., *Coelosphaera* spp., *Tetilla* spp., *Theonella* spp., and *Callipelta* spp.,). In some clades, the lineages had a wide range and was found in two or more regions along the South African coastline (*Poecillastra* spp., *Tedania* spp., *Biemna* spp., *Polymastia* spp., *Antho* spp., *Cliona* spp., and *Penares* spp.), while others showed potential cryptic speciation (*Hymeniacidon stylifera*). Furthermore, some species complexes (Niphatidae spp., *Higginsia* spp., *Geodia* spp. and *Tsitsikamma* spp) have not undergone complete separation and are still in their early stages of speciation, due to incomplete lineage sorting that can be distinguished morphologically based on some divergent morphological characters. I also observed that the order Haplosclerida is polyphyletic, particularly the species that were identified morphologically in the genus *Haliclona*. This polyphyletic nature of the order Haplosclerida found in this study has also been described in other studies that considered then one of the most difficult and unstable groups of the class Demospongiae (e.g McCormack *et al*., 2002; Borchiellini *et al*., 2004; Nichols, 2005). This is due to plasticity of morphological characters, large numbers of species and major discrepancies between morphological and molecular data. Some clades showed discordance between molecular and morphological taxonomic identifications (*Sigmaxinella* spp., *Geodia* spp., *Biemna* spp., Scopalinidae spp. and *Haliclona* spp.), as well as supported lineages, which includes two morphologically distinct species. Some of the lineages Identified in the presense study are described in more detail below.

4.2.1. Suberites spp*.*

The phylogeny reconstructed from the cox1 sequences (both standard and extended cox1 fragments) of the *Suberites* specimens from the west and south coasts of South Africa formed two distinct lineages (Fig. 4). These lineages cluster together with a bootstrap support of 58%. The two lineages, although very closely related to each other, with a short genetic distance (K2P) distances of 0.003, are completely distinct, indicating that they constitute two operational taxonomic units (OTUs).

OTU #1 has two distinct haplotypes with a strong bootstrap support (100%) and occurs along the west coast of South Africa. After a revision of the west coast species, Samaai *et al*. (2017) described the specimens as *S. dandelenae* Samaai & Maduray, 2017 . This species occurs in unconsolidated sediments of depths 80–500 m in the Benguela Ecoregion. Morphologically, the sponge is straw yellow, massive, with rounded lobes and a velvety surface (Samaai *et al*., 2017). Both morphological characters and molecular markers (cox1) were used to confirm that all west coast *Suberites* specimens were the same species. The phylogenetic tree also confirms that *Suberites dandelenae* is a sister species to OTU #2.

OTU #2, collected from the south coast of South Africa, was represented by a single haplotype. This species forms an interesting symbiotic relationship with the hermit crab that carries the sponge around on its back shell. This particular species is currently been described (Payne, unpublished data).

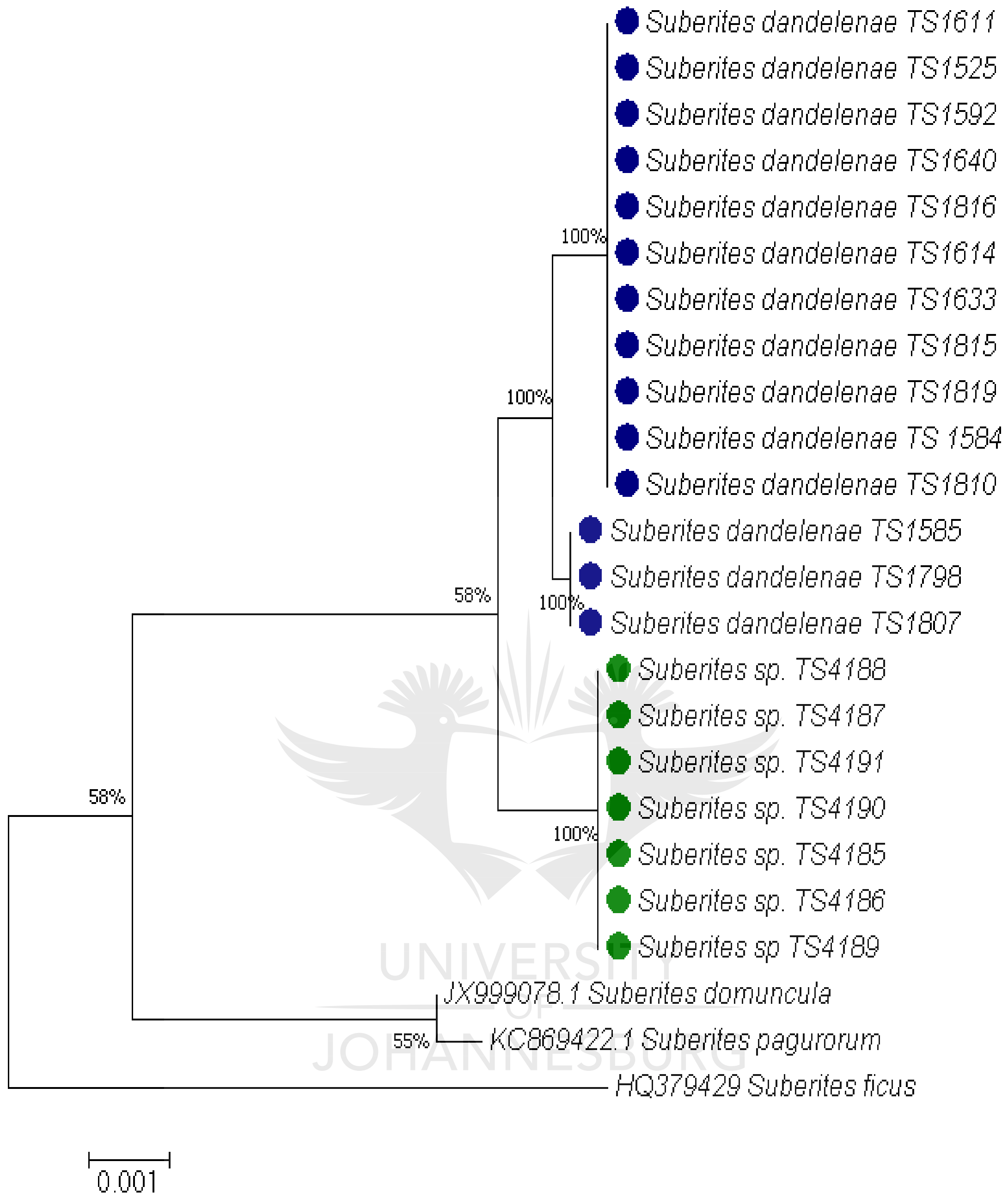

Fig. 4: Phylogeny of the Suberites species from the west (blue) and south (green) coast of South Africa based on the neighbour joining method with three haplotypes defining two distinct species (OTU=2), supported by a high bootstrap support (100%).

4.2.2. Erylus spp.

The phylogeny of *Erylus* spp., collected from the South African south-east (off East London) and south coast, forms two distinct lineages based on the cox1 sequences (Fig.5). The two lineages consist of two OTUs that clusters together with a very strong bootstrap support (100%). Each lineage is defined by a single haplotype. OTU #1 forms the lineage from the south-east coast, which is a new species whereas OTU #2 from the south coast was identified as *Erylus globulifer*.

Both lineages are closely related but completely different from each other, with a genetic distance (K2P) of just 0.003 between them. A morphological assessement also comfirmed their distinction. For example, the south-east coast *Erylus* sp. Is larger in size and consisting of multiple lobes, while *Erylus globulifer* from the south coast is a smaller pear-shaped.

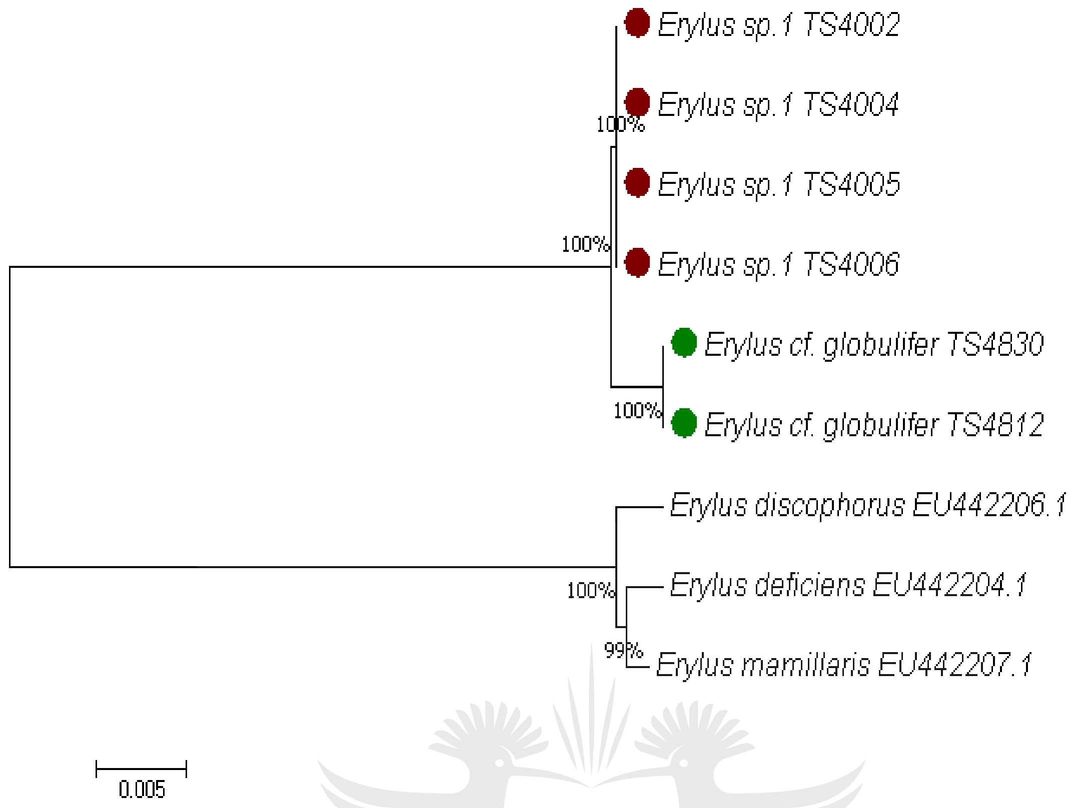

Fig 5: Phylogeny of the *Erylus* spp. from the south coast (green) and south-east coast (red) of South Africa, based on the neighbour joining method with two distinct lineages (OTU#=two) and two haplotypes strongly supported by a high bootstrap support (100%).

4.2.3. Cyclacanthia spp.

The phylogeny of *Cyclacanthia* sp. was reconstructed from the cox1 sequences generated from specimens collected on the south-east coast of South Africa, off East London. These sequences form a distinct *Cyclacanthia* lineage (Fig. 6) representing a single OTU, and I considered it a new species. *Cyclacanthia* sp. is classified seperately but as a sister taxa to *Tsitsikamma, Latrunculia* and *Sceptrella*, in the Family Latrunculiidae. The phylogeny clusters *Cyclacanthia* and *Tsitsikamma* together with strong bootstrap support of 98%.

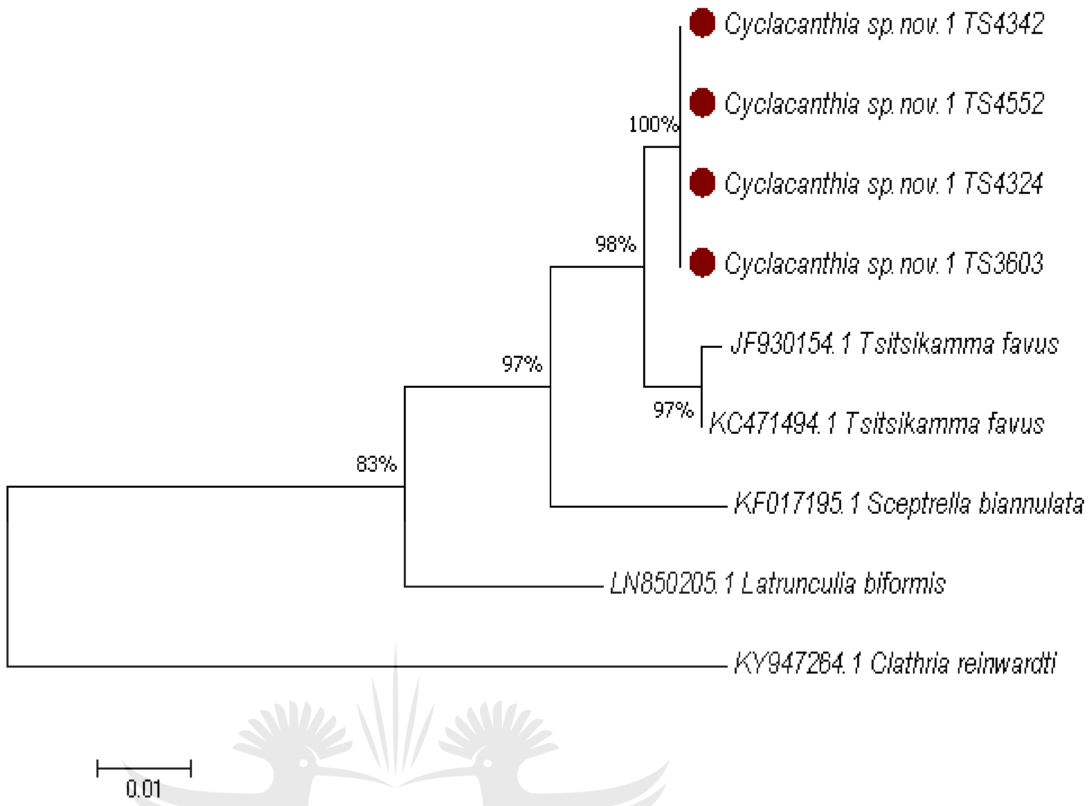

Fig. 6: Phylogeny of the *Cyclacanthia* species (OTU#= 1), from the south-east (red) coast of South Africa based on the neighbour joining method representing a single lineage defined by a single haplotypes strongly supported by a high bootstrap (100%).

4.2.4. Sigmaxinella spp.

The phylogeny of the cox1 sequences of *Sigmaxinella* spp. generated from specimens from the South African south-east coast, (East London), forms two distinct lineages (Fig. 7), which cluster together with strong bootstrap support (100%). Each lineage has a single haplotype. Morphologically, different species were identified, which are depicted as *Sigmaxinella* sp. 1, *Sigmaxinella* sp. 2 and *Sigmaxinella* sp. 3. Phylogenetic analysis recovered *Sigmaxinella sp*. 2 and *Sigmaxinella* sp. 3 as a single lineage defined by a single haplotype (with bootstrap support of 100%).

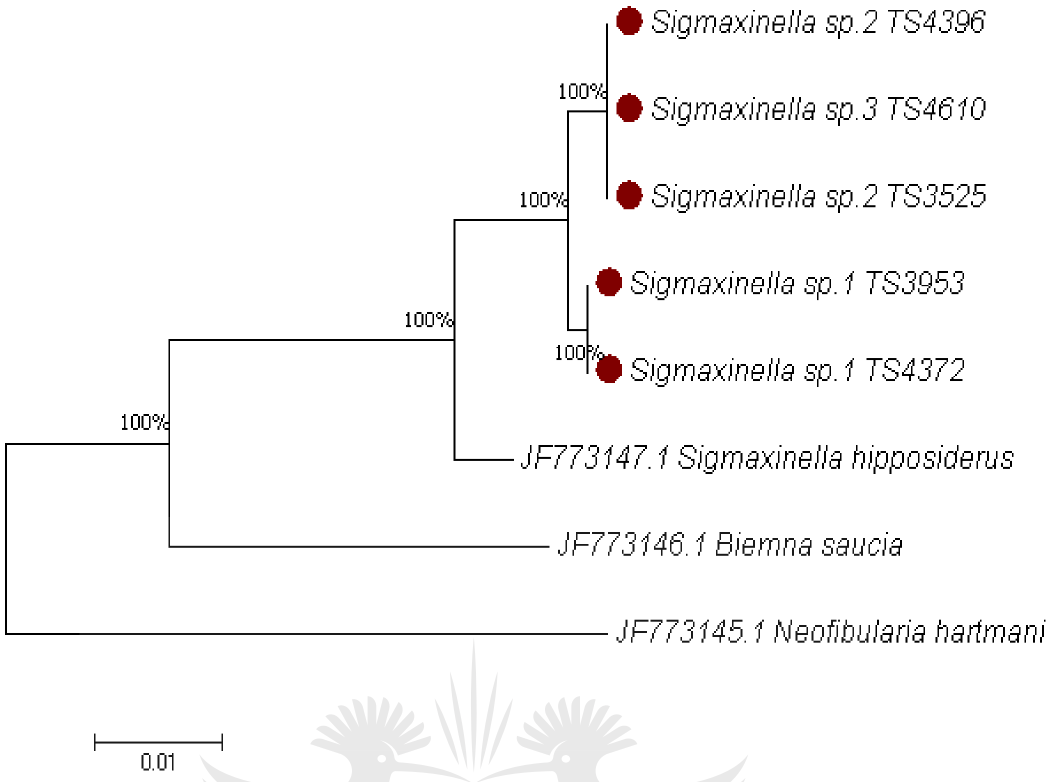

Fig 7: Phylogeny of the *Sigmaxinella* sp. based on the neighbour joining method identified along the south-east coast (red) of South Africa with two distinct lineages (OTU#=two) and two haplotypes, strongly supported by a high bootstrap support (100%).

4.2.5. Raspaillidae spp.

The cox1 sequences of Raspaillidae species (family Raspaillidae) were obtained from sponge specimens collected from the south-east coast of South Africa. The phylogeny recovered five different lineages (Fig. 8), including up to eight distinct genetic clusters, that was also identified morphologically. All eight are potentially new species . Five of the eight species are shown to have distinct operational units (OTU# 1= Raspaillidae sp. 1, OTU #2= Raspaillidae sp. 3, OTU #3= Raspaillidae sp. 7, OTU #4= Raspaillidae sp. 6 and OTU #5= Raspaillidae sp. 4*)* and three other species (Raspaillidae sp. 2*,* Raspaillidae sp. 5 and Raspaillidae sp. 8*)* were singletons i.e. their cox 1 sequences were not shared with the other Raspaillidae species.

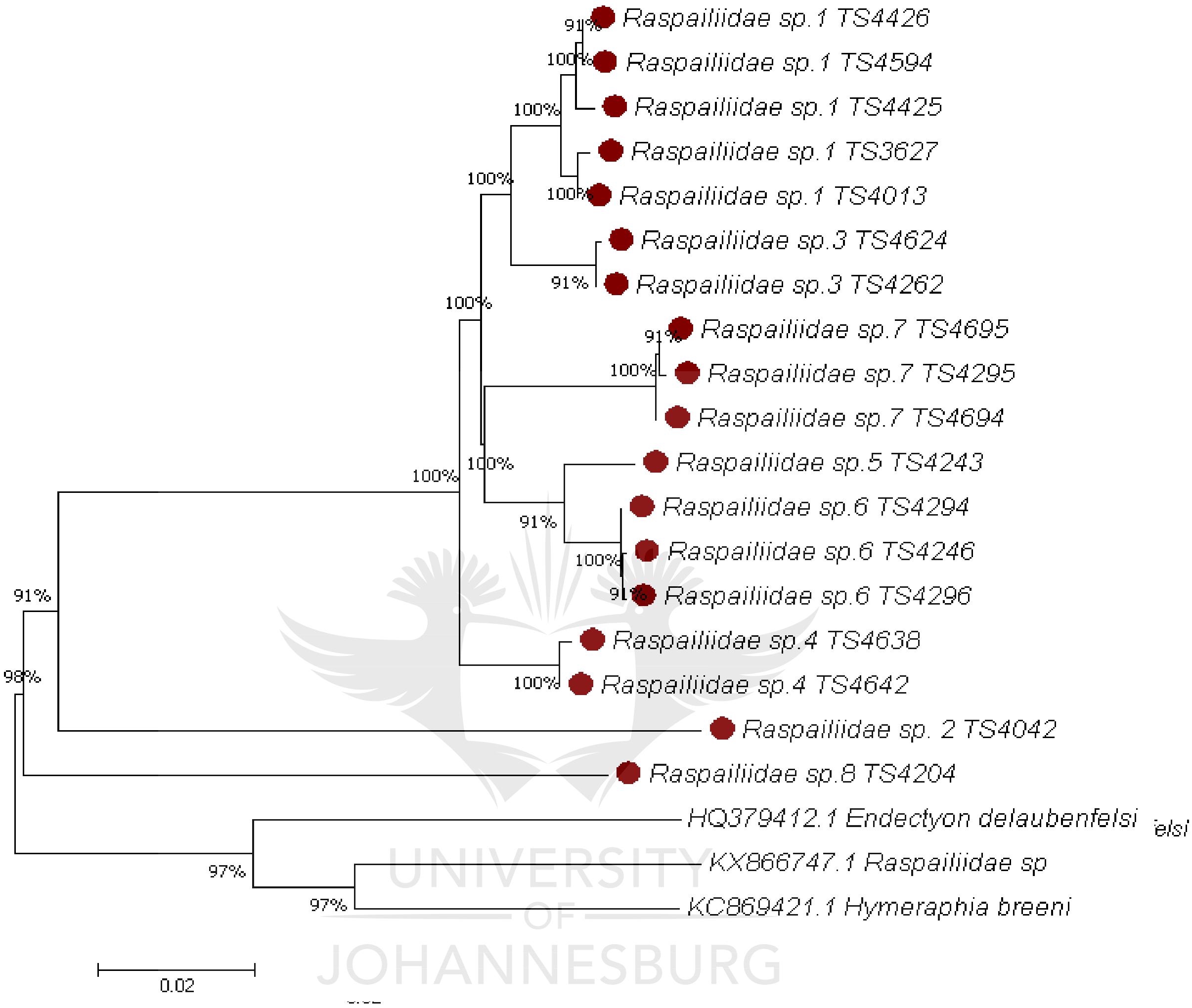

Fig 8: Phylogeny of the Raspailiidae sp. based on the neighbour joining method identified from the south-east (red) coast of South African with eight distinct lineages.

4.2.6. *Characella* spp.

The phylogeny of *Characella* sp. reconstructed from cox1 sequences of specimens collected from the south-east coast of South Africa forms up to three lineages (See Fig. 9). These lineages includes three different morphological distinct *Characella* species (OTU #1 = *Characella* sp. 1, OTU #2= *Characella* sp. 2 and OTU #3=

Characella sp. 3). These species are proposed new species for the south-east coast. Data from GenBank, from sister three genus (Theonella, Geodia and Stelleta) were used as an outgroup determine the position of these *Characella* species. Interestingly, the genus Theonella clusters more closely to the ingroup sequences than a previously generated sequence of *Characella* sp.(which was potentially misidentified).

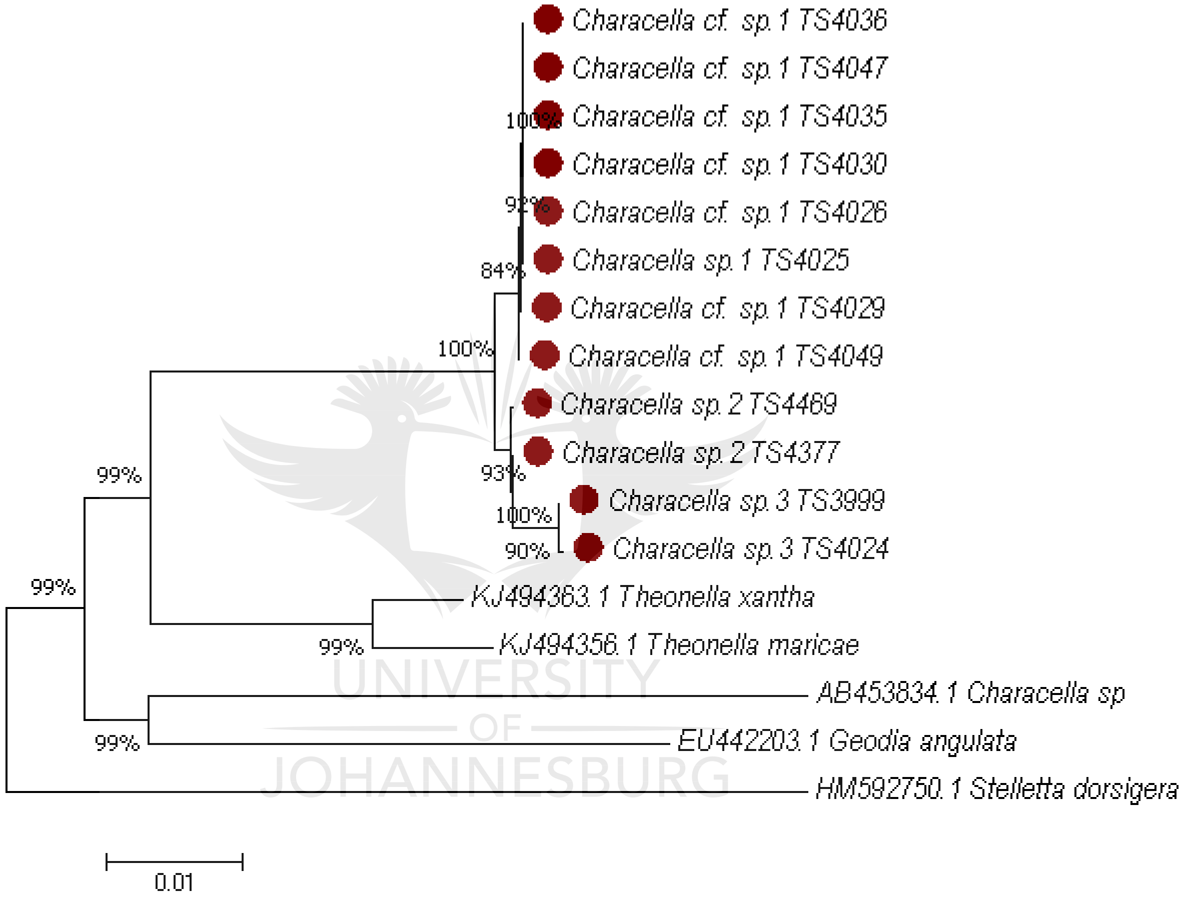

Fig 9: Phylogeny of the *Characella* species from the south-east (red) coast of South Africa based on the neighbour joining method showing three distinct lineages.

4.2.7. *Homoxinella* spp.

The cox1 sequences of *Homoxinella* species were generated from sponge specimen collected from the south-east coast of South Africa. The phylogeny forms a single lineage (Fig. 10) defined by a single haplotype and correspond to a single morphospecies (*Homaxinella* sp.).

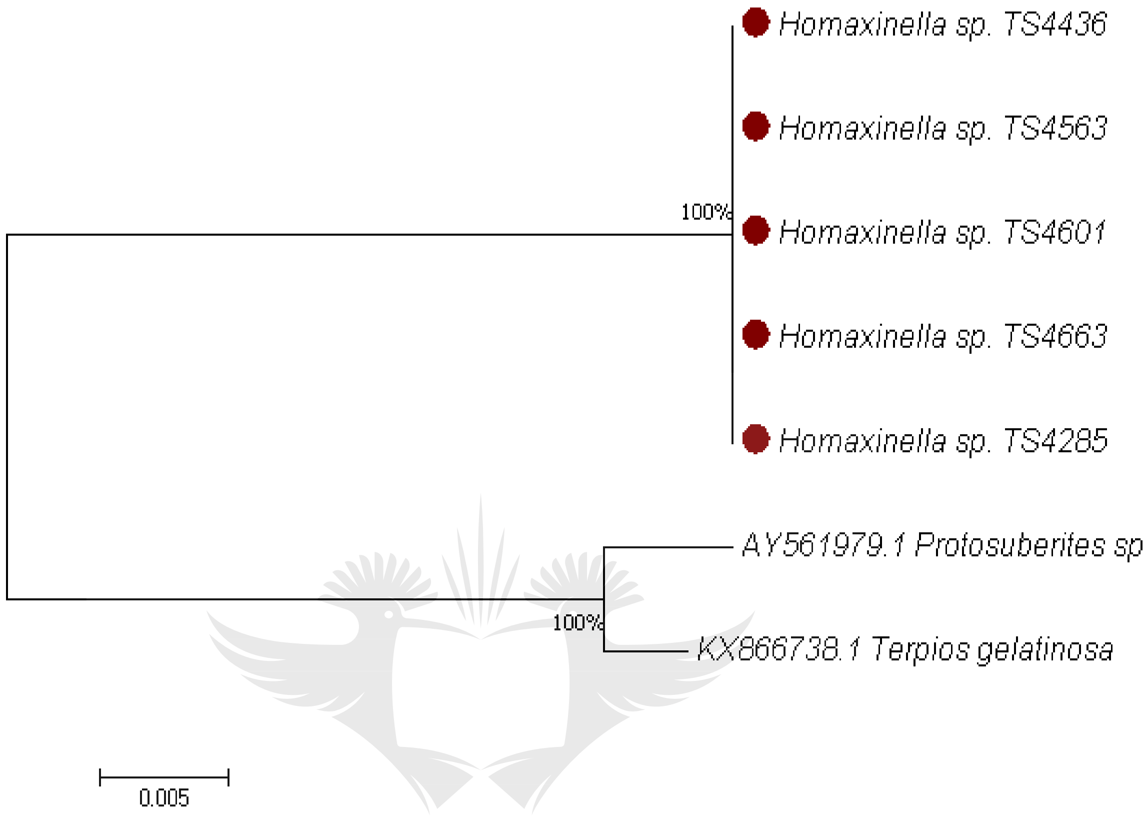

Fig 10: Phylogeny of the *Homaxinella* species from the south-east (red) coast of South Africa based on the neighbour joining method, with a single lineage defined by one highly supported (100%) haplotype representing a single morphospecies.

4.2.8. Rhabdastrella spp.

The cox1 sequences of *Rhabdastrella* spp. were generated from sponge specimen collected from the south-east coast of South Africa. The phylogeny consist of two lineages (Fig. 11) with a total of two species, which was also identified based on morphology.

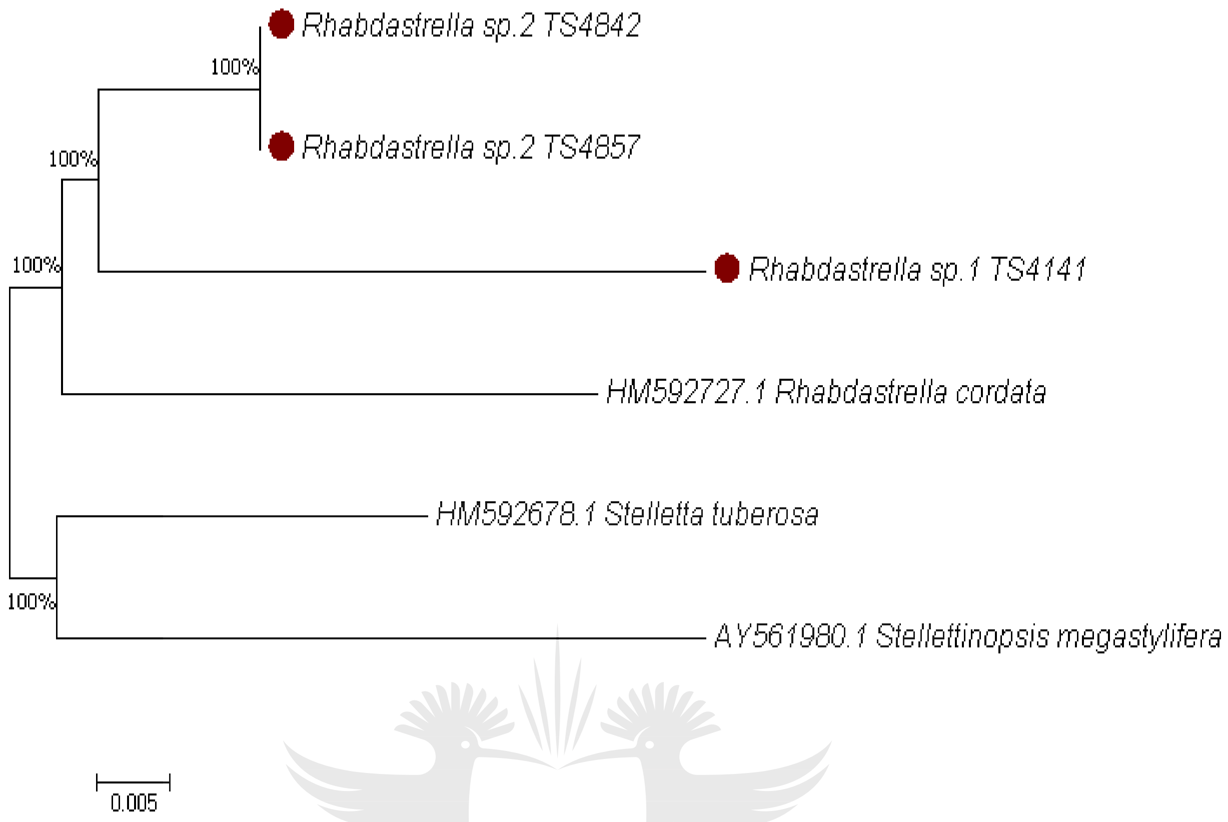

Fig 11: Phylogeny of the *Rhabdastrella* species from the south-east coast (red) of South Africa based on the neighbour joining method with two distinct lineages (OTU#=one and one singleton).

4.2.9. *Theonella* spp.

The cox1 sequences of *Theonella* species were generated from sponge specimens collected from the south-east coast of South Africa. The phylogeny forms a distinct lineage with a single species (*Theonella* sp.1), which clusters together with the out group (T. Mirabilis) with high bootstrap support of 100% (Fig. 12).

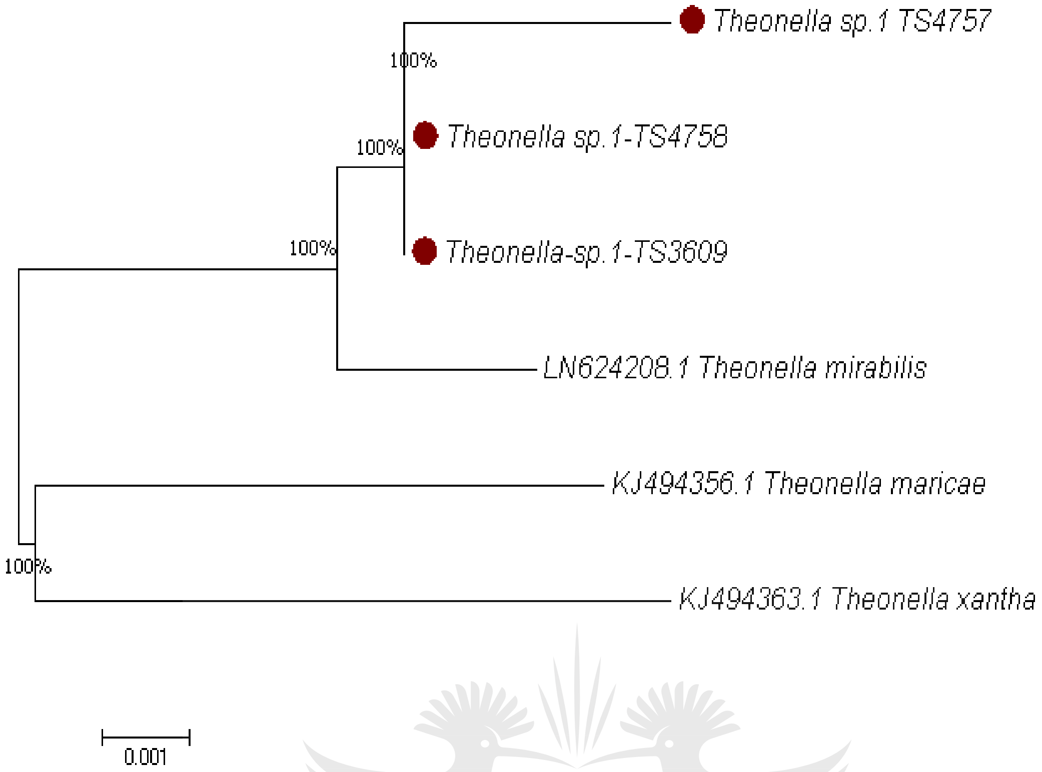

Fig 12: Phylogeny of the Theonella species from the south-east coast (red) of South Africa based on the neighbour joining method with a genetic lineage (OTU#=one) that correspond to a single morpho species.

4.2.10. Callipelta spp.

The cox1 sequences of *Callipelta* species were generated from sponge specimens collected from the south-east coast of South Africa. The phylogeny consist of two distinct lineages (Fig. 13) with two different morphospecies strongly supported with a high bootstrap support (100%).

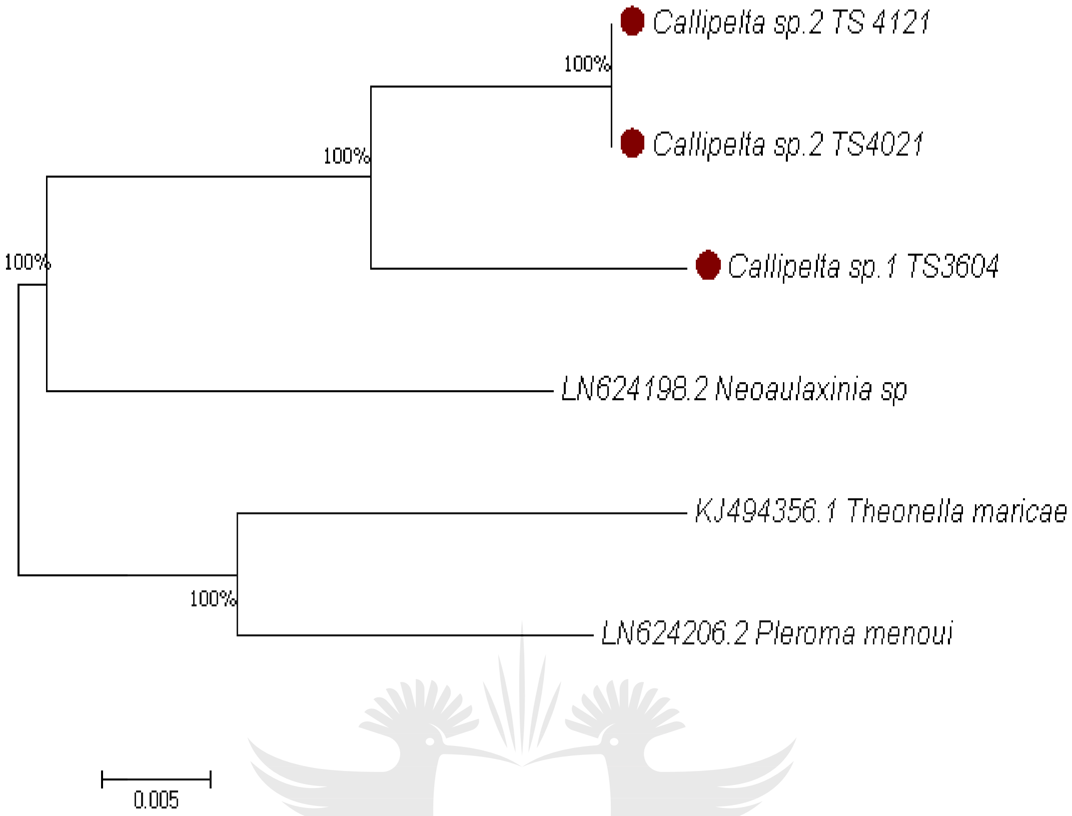

Fig 13: Phylogeny of the *Callipelta* species from the south-east coast (red) of South Africa based on the neighbour joining method with two distinct lineages (OTU#=one and one singleton).

4.2.11. Phymaraphiniidae spp.

The cox1 sequences of sponges for the family Phymaraphiniidae were obtained from specimens collected from the south-east coast of South Africa. Its phylogeny comprises two distinct lineages (Fig. 14), which clusters together with a relatively high bootstrap support (100%). These lineages consist of three distinct morphospecies (*Phymaraphiidae* sp. 1, sp. 2 and sp. 3) which are all considered new to South Africa.

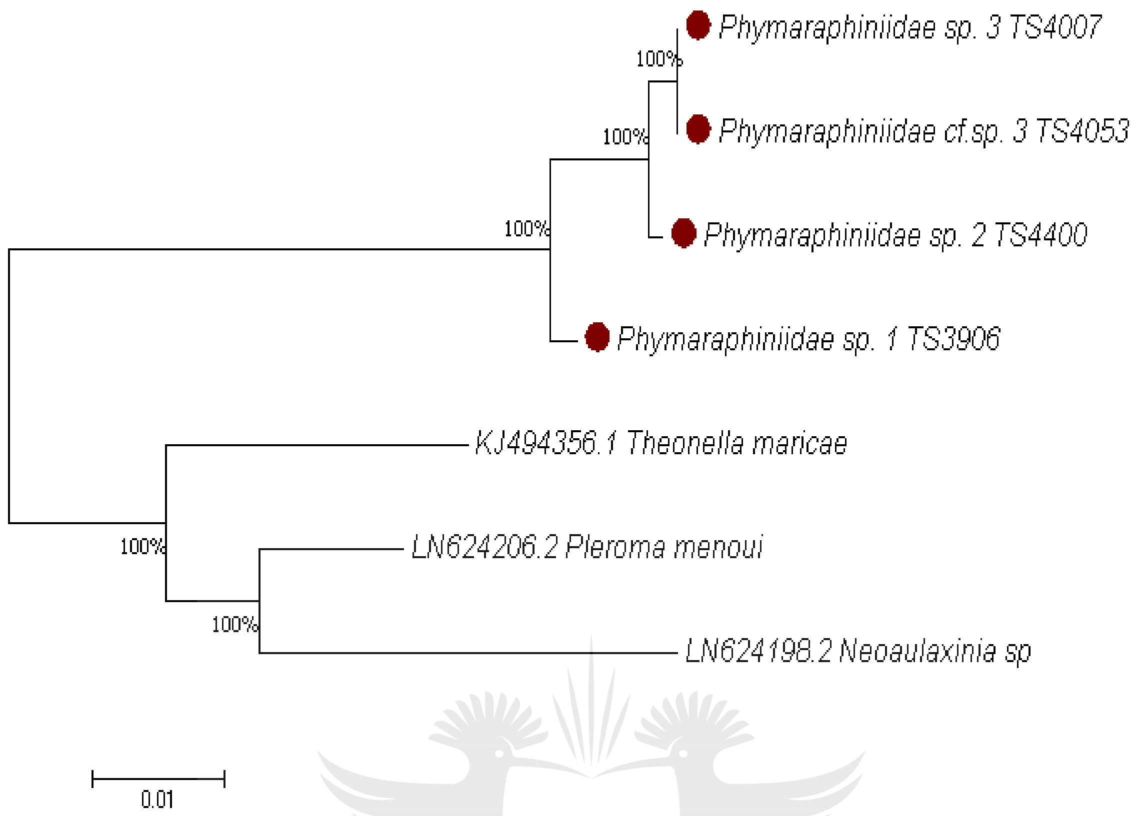

Fig 14: Phylogeny for sponges of the family Phymaraphiniidae from the south-east coast (red) of South African based on the neighbour joining method with three distinct lineages (OTU#=one and two singleton).

***4.2.12. Tetilla* spp.**

The cox1 sequences of *Tetilla* spp. was generated from sponge specimens collected on the south-east coast of South Africa. The phylogeny forms a single lineage (Fig. 15) with just one morphospecies.

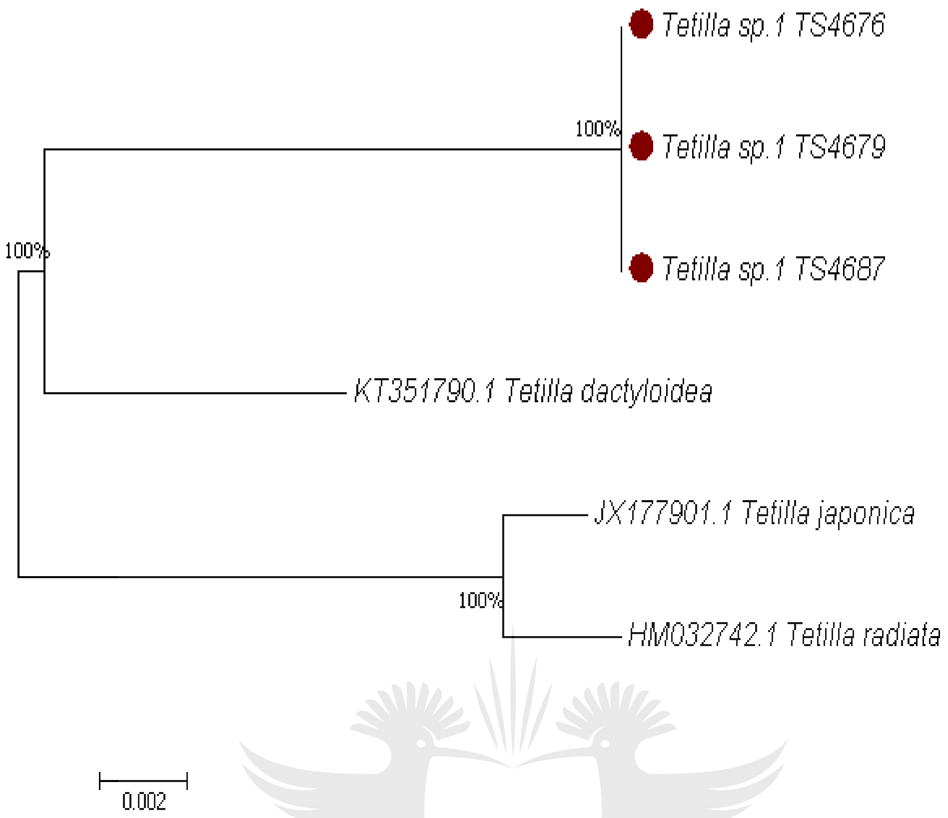

Fig 15: Phylogeny of the *Tetilla* species from the south-east coast (red) of South African based on neighbour joining method with a distinct lineage (OTU#=1) and single haplotypes.

4.2.13. Microcionidae spp.

The phylogeny reconstructed from the cox1 sequences of the family Microcionidae obtained from the specimens collected from the south-east coast, of South Africa forms three lineages (Fig. 16). These lineages consist of fpor different morphospecies (Microcionidae sp.1, sp. 2, sp. 3 and sp. 4).

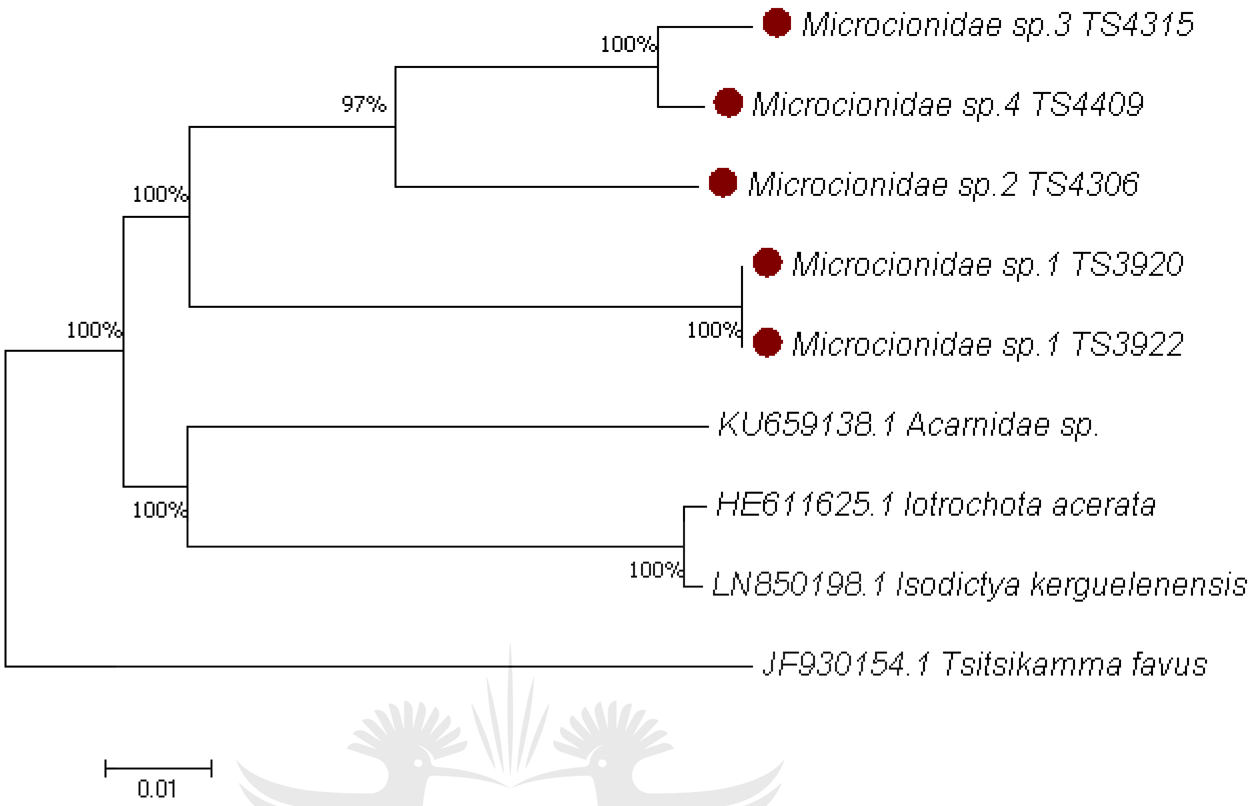

Fig 16: Phylogeny of sponges of the family Microcionidae from the south-east coast (red) of South Africa based on the neighbour joining method with three lineages (OTU#=two and one singleton) and four haplotypes.

4.2.14. Poecillastra spp.

The phylogeny of Poecillastra species was reconstructed from cox1 sequences generated from specimens collected around the south-east and west coast of South Africa. It forms three lineages with a total of two OTU's and a single singleton (Fig. 17). The two OTUs forms a lineage each (OTU#1= *Poecilastra* sp. 3 and OTU#2 = *Poescillastra* sp. 1) which clusters together with a high bootstrap support of 100%. These two highly supported lineages clusters with the third lineage represented by a single sequence (*Poecillastra* sp. 2), with a high bootstrap support of 97%. The lineage *Poecillastra* sp. 3 had a wide range and was found in two regions (west coast and south-east coast)

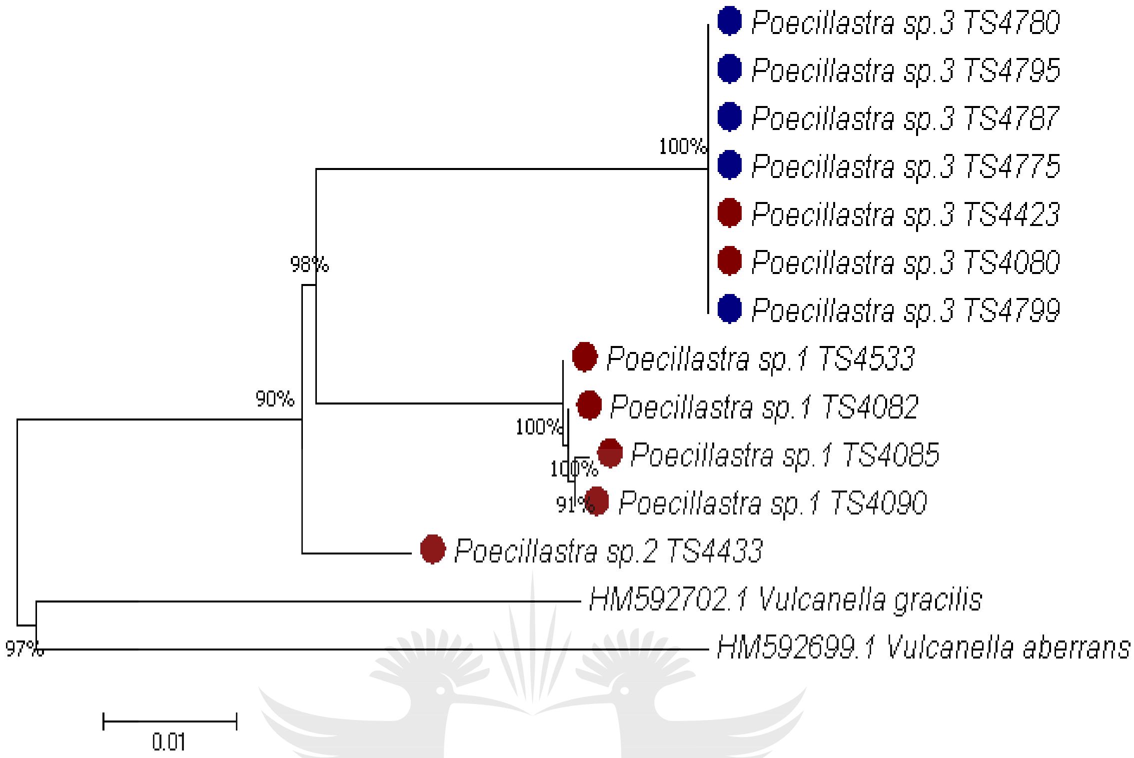

Fig 17: Phylogeny of the Poecillastra species from the west (blue) and south-east (red) coast of South Africa based on the neighbour joining method with three lineages (OTU#= 2 and 1 singleton).

***4.2.15. Tedania* spp.**

The cox1 sequences of *Tedania* spp. was generated from specimens collected from the south coast and the south-east coast of South Africa. The phylogeny comprises three distinct lineages (Fig. 18) with two highly distinct OTUs (OUT# 1= *Tedania (Tedania) tubelifera* and *Tedania (Tedaniopsis)* sp. 1) and one singleton (*Tedania (Tedania)* sp. 1), with the monophyly of the OTUs strongly supported (100% bootstrap support). Geographically, the *Tedania (Tedaniopsis)* sp. shows overlap with the ranges of the other two, as it was found both on the south coast and on the south-east coast within the Agulhas ecoregion.

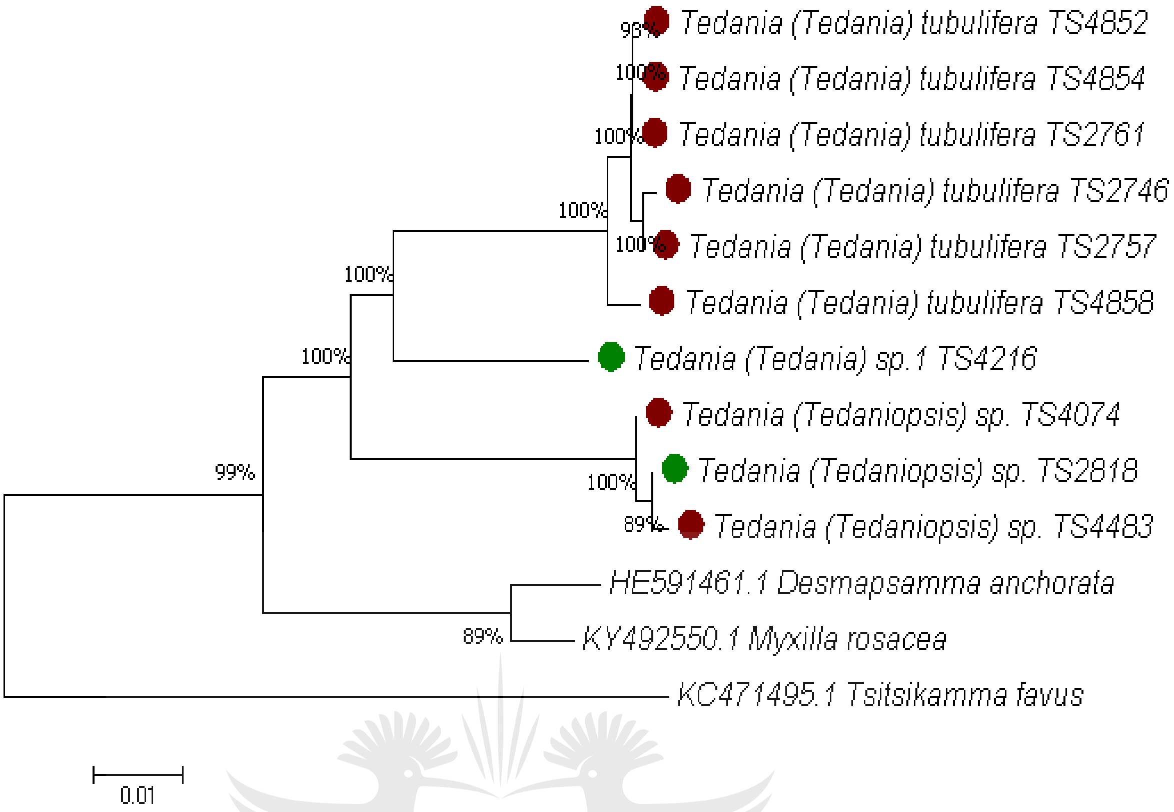

Fig 18: Phylogeny of the *Tedania* species from the south (green) and south-east (red) coast of South Africa based on the neighbour joining method with three distinct lineages (OTU#=2), strongly supported by a high bootstrap support (100%).

4.2.16. Geodia spp.

The cox1 sequences of the *Geodia* sp. were generated from specimens collected from the west coast and the south-east coast of South Africa. The phylogeny forms three different lineages with a single taxonomic operational unit (OTU) and two singletons (Fig. 19). Morphologically, different species were identified which are depicted as *Geodia* sp. 1, *Geodia* sp. 2, *Geodia* sp. 3 and *Geodia* sp. 4. Phylogenetic analysis recovered *Geodia sp*. 2 and *Geodia* sp. 3 as a single lineage defined by a single haplotype (with bootstrap support of 100%).

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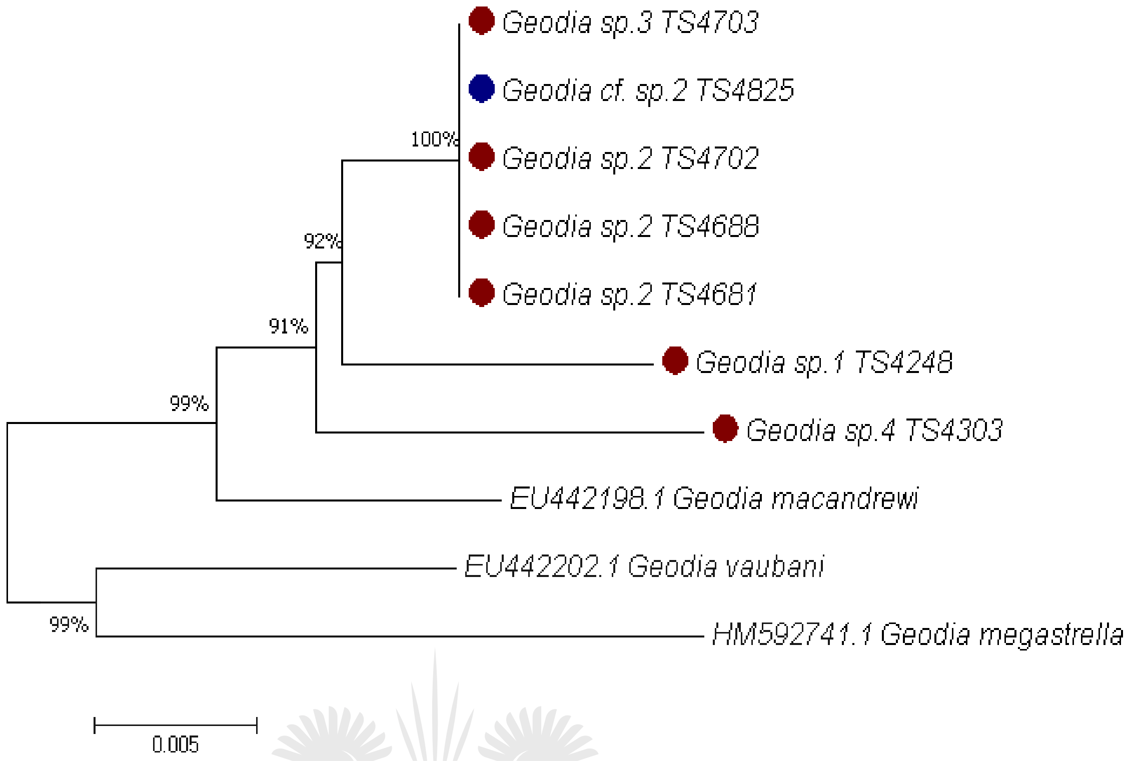

Fig 19: Phylogeny of the *Geodia* species from the west coast (blue**)** and south-east (red) coast of South Africa, based on the neighbour joining method with three lineages. The well supported lineage includes two morphospecies.

4.2.17. Biemna spp.

The cox1 sequences of *Biemna* sp. were generated from specimens collected from the south-east and south coast of South Africa. Its phylogeny comprises three lineages (Fig. 20) that are supported by a relatively strong bootstrap support (82-100%). Morphologically, different species were identified which are depicted as *Biemna* sp.1, *Biemna* sp. 2, *Biemna fistulosa* and *Biemna megalosigma var. sigmadragma*). Phylogenetic analysis recovered *Biemna* sp. 2 and *Biemna megalosigma var. sigmadragma* as a single lineage defined by a single haplotype (with bootstrap support of 100%). *Biemna* sp. 2 had a wide range as it was found in two regions (south and south-east coast)

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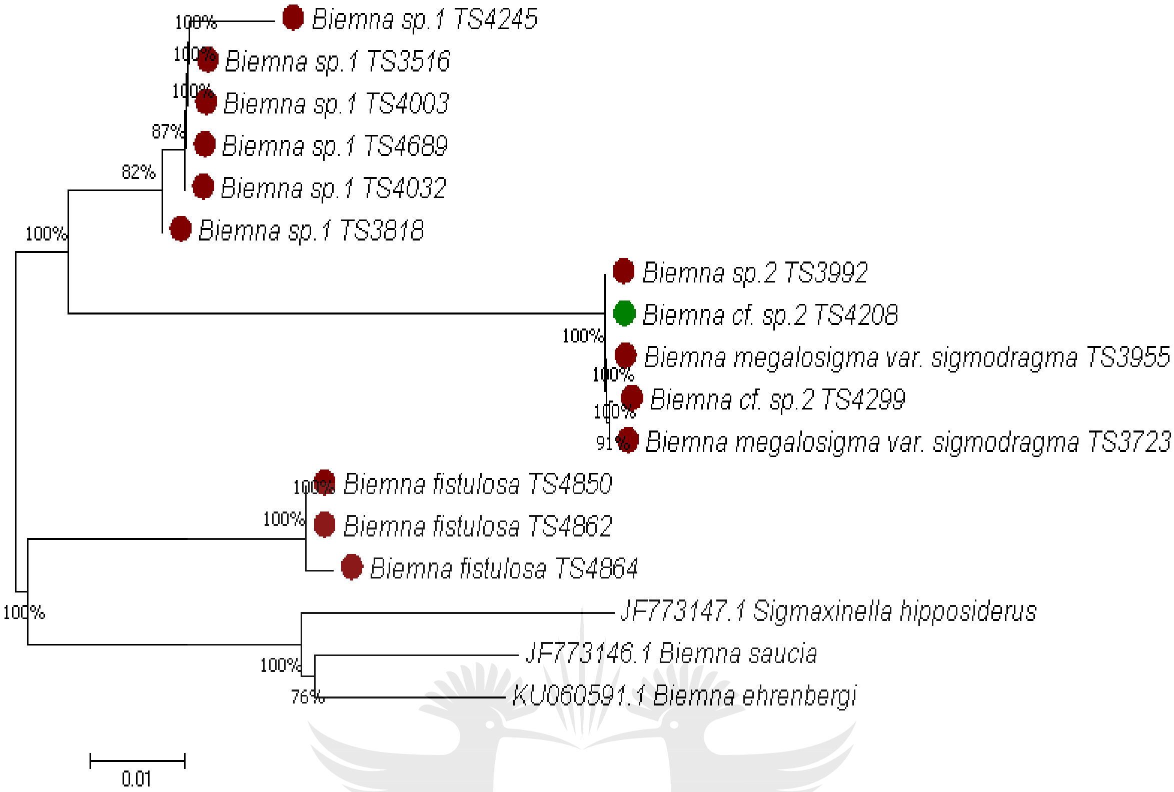

Fig 20: Phylogeny of the *Biemna* species from the south-east coast (red) and south coast (green) of South Africa based on the neighbour joining method with three lineages.

4.2.18. Cliona spp.

The cox1 sequences of the *Cliona* species were generated from specimens collected from the south coast and west coast of South Africa. The phylogeny forms a distinct lineage (Fig. 21) with a single OTU, strongly supported by 100% bootstrap support Although only two specimens are presently available, it is clear that the species has a wide range as it was found both on the west and south coast.

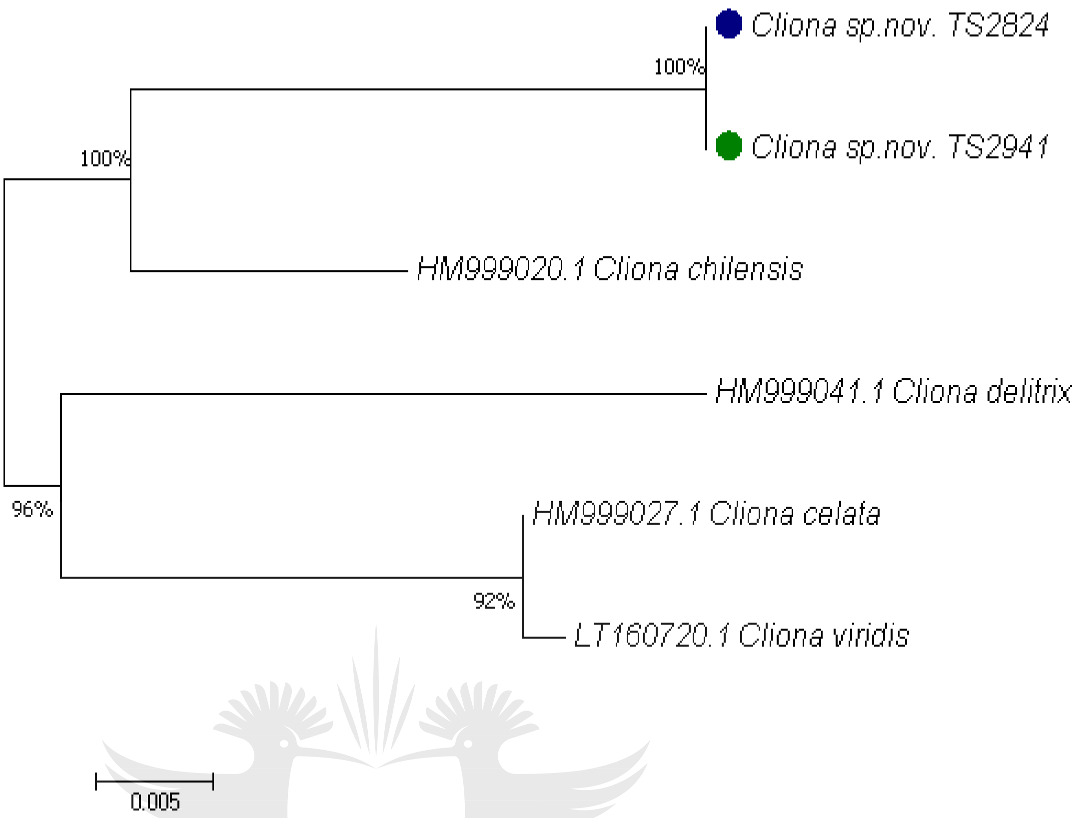

Fig 21: Phylogeny of the *Cliona* species from the west coast (blue) and south (green) coast of South Africa based on the neighbour joining method with a distinct lineage that is strongly supported (bootstrap support of 100%).

4.2.19. Polymastia spp.

The cox1 sequences of the *Polymastia sp.* were generated from samples collected around the west coast and south-east coast of South Africa. Its phylogeny recovered six lineages, with two OTUs and four singleton (Fig. 22), that forms well supported lineages with relative high bootstrap support values (84-100%). Morphologically, different species were identified which are depicted as *Polymastia* sp. 1, *Polymastia* sp. 2, *Polymastia* sp. 3, *Polymastia* sp. 4, *Polymastia* sp. 5, *Polymastia* sp. 6 and *Polymastia alantica.* Phylogenetic analysis recovered *Polymastia* sp. 2 and *Polymastia* sp. 3 as a single lineage defined by a single haplotype with a relative high bootstrap support (84%).

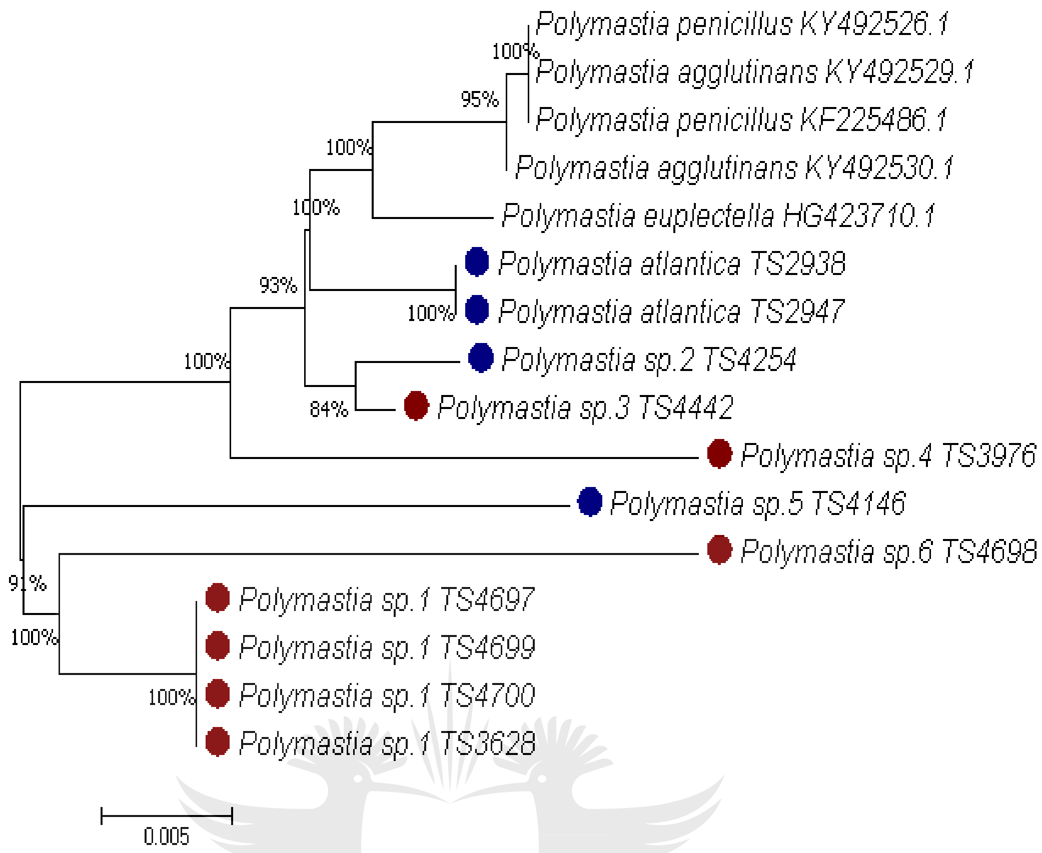

Fig 22: Phylogeny of the Polymastia species from the West (blue) and southeast (red) coast of South Africa base based on the neighbour joining method with six lineages (2twoOTUs).

4.2.20. *Clathria* spp.

The phylogeny of *Clathria* species was constructed based on the cox1 sequences, generated from specimens collected from the west coast, south coast and south-east coast of South Africa. It forms three distinct lineages (Fig. 23) which clusters together with a strong bootstrap support (100%). Morphologically, different species were identified which are depicted as *Clathria sp*. 1, *Clathria* sp. 2, *Clathria* sp. 3, Clathria sp. 4 and Clathria *sp*. 5. Phylogenetic analysis recovered *Clathria sp*. 3 and *Clathria* sp. 4 as a single lineage defined by two haplotypes which clusters together with a strong bootstrap support (100%). *Clathria* sp. 2 and *Clathria* sp. 5 was also recovered as a single lineage defined by two haplotypes with a relative high bootstrap support (84%). *Clathria* sp. 1 had a wide range as it was found in two regions (west and south coast)

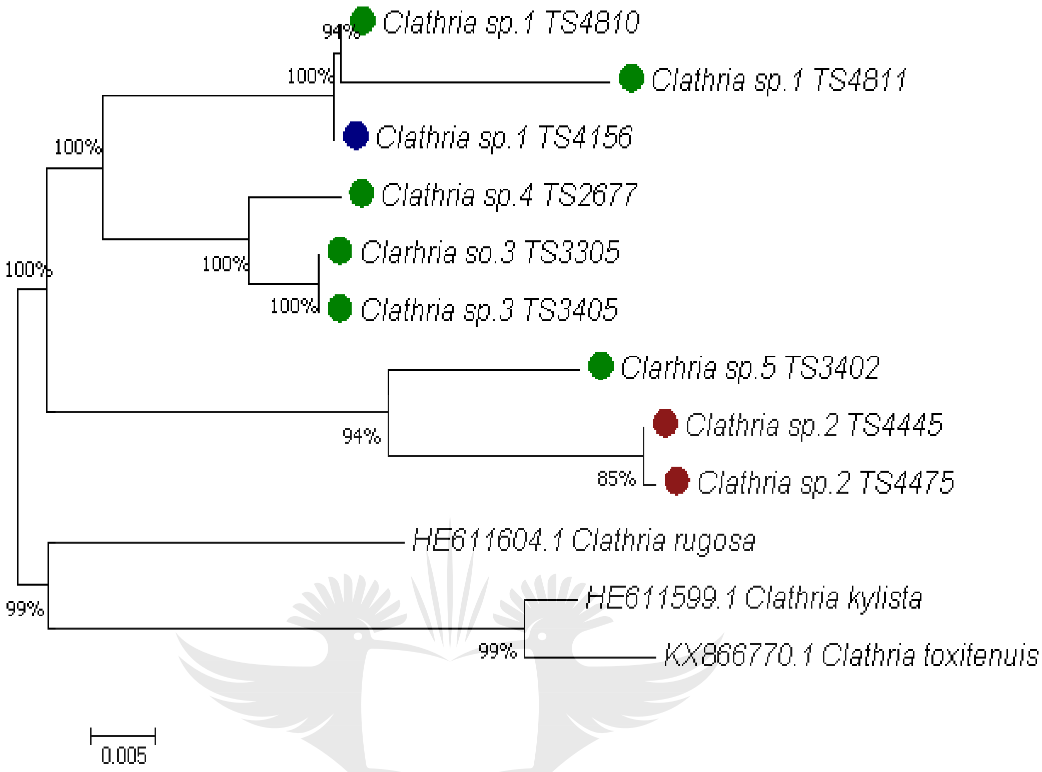

Fig 23: Phylogeny of the Clathria species from the west (blue), South (green) and south-east (red) coast of South Africa based on the neighbour joining method with three lineages which clusters together with a strong bootstrap support (100%) and includes five morphospecies.

4.2.21. Antho spp.

The phylogeny of *Antho* species was reconstructed based on the cox1 sequences, generated from specimens collected from the south and the south-east coast of South Africa. It forms two distinct lineages (Fig. 24) with two well supported OTUs (OTU #1= *Antho* sp. 1 and OTU #2= *Antho (Acamia) cf. prima*) that clusters together with a high bootstrap support (100%). *Antho (Acamia) cf. prima* had a wide range as it was found in two regions (south and south-east coast)

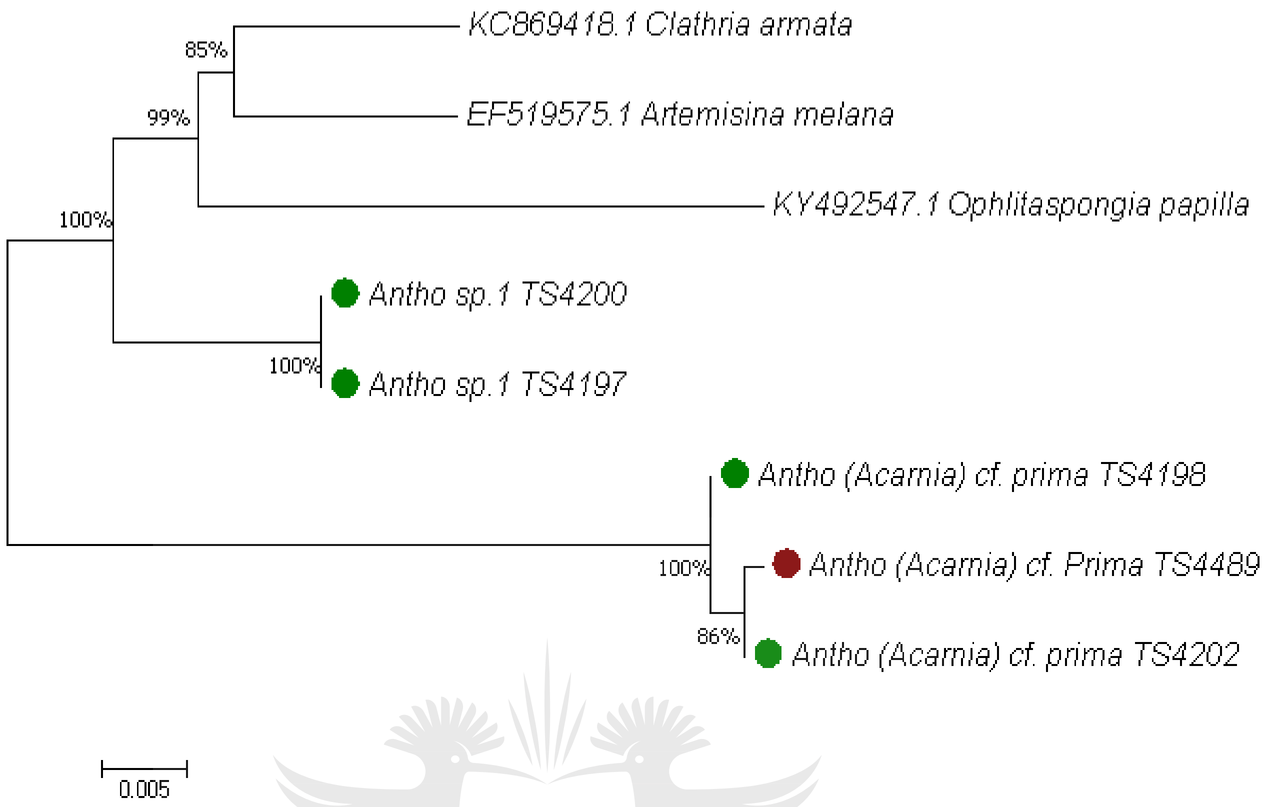

Fig 24: Phylogeny of the *Antho* species from the southeast (red) and South (green) coast of South Africa based on the neighbour joining method forms two distinct lineages strongly supported by high bootstrap supports (100%).

4.2.22. Penares spp.

The phylogeny of *Penares* species was reconstructed based on cox1 sequences, generated from specimens collected from the west and south-east coast of South Africa. It forms three different lineages (Fig. 25). Morphologically, different species with two OTUs and three singletons were identified and depicted as *Penares* sp. 1, *Penares* sp. 2, *Penares* sp. 3, *Penares sphaera* and *Penares cf. orthotriaena*. Phylogenetic analysis recovered *Penares* sp. 2, *Penares* sp. 3 and *Penares sphaera* as a single lineage defined by two haplotypes that clusters together with a high bootstrap support (100%). *Penares cf. orthotriaena*, had a wide range as it was found in two regions (west coast and south-east coast)

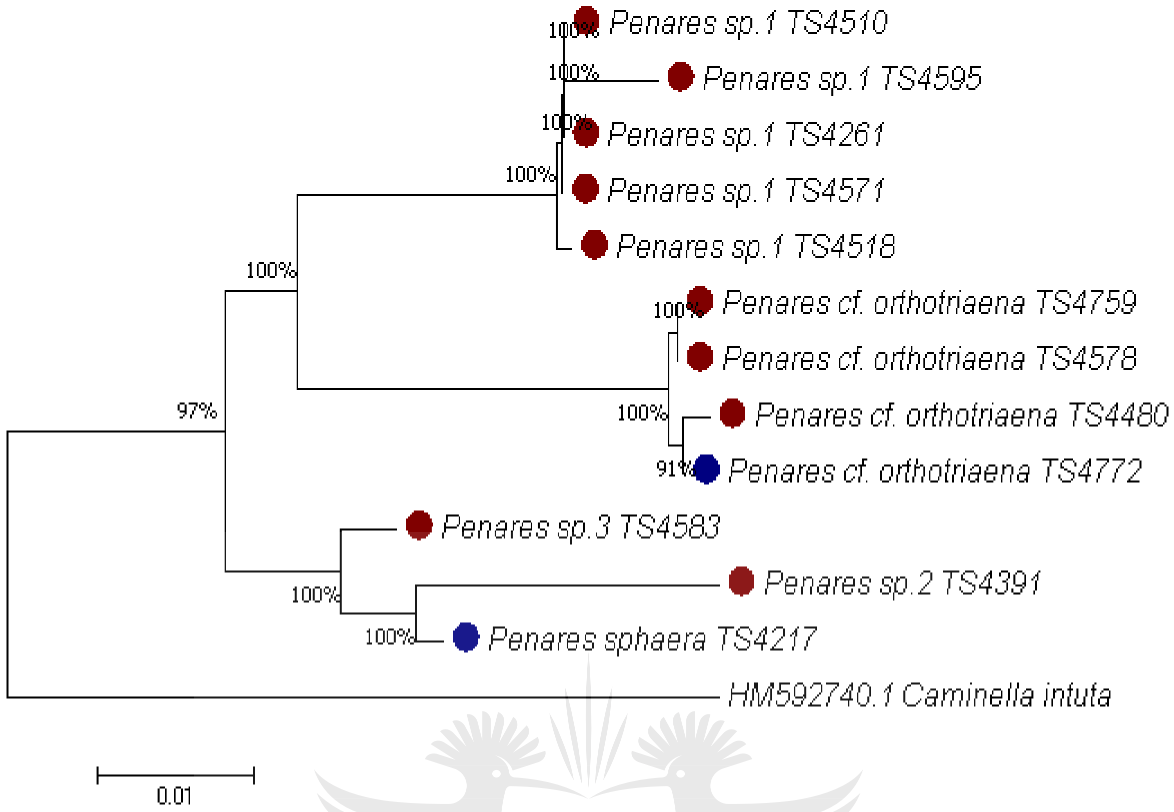

Fig 25: Phylogeny of the *penares* species from the west (blue) and south-east (red) coast of South Africa forms three lineages including five different morphospecies.

4.2.23 Hymeniacidon spp.

The phylogeny of the Hymeniacidon species was reconstructed based on the cox1 sequences, generated from specimens collected from west coast along the Cape Agulhas towards the Cape Peninsula and the south-east coast off the Eastern Cape area. Phylogenetically, it forms two distinct lineages (Fig. 26a) but also represent a cryptic species as the differentiation is not complete in the *cox*1 sequences and also divergent morphological characters are not visible yet and as a result this species was identified morphological to form a single species (*Hymeniacidon stylifera*). The ITS results shows a complete differentiation between these two lineages (Fig. 26b). But this is only because one of the Eastern Cape specimen which clusters with the west coast specimens could not be amplified and sequenced successfully.

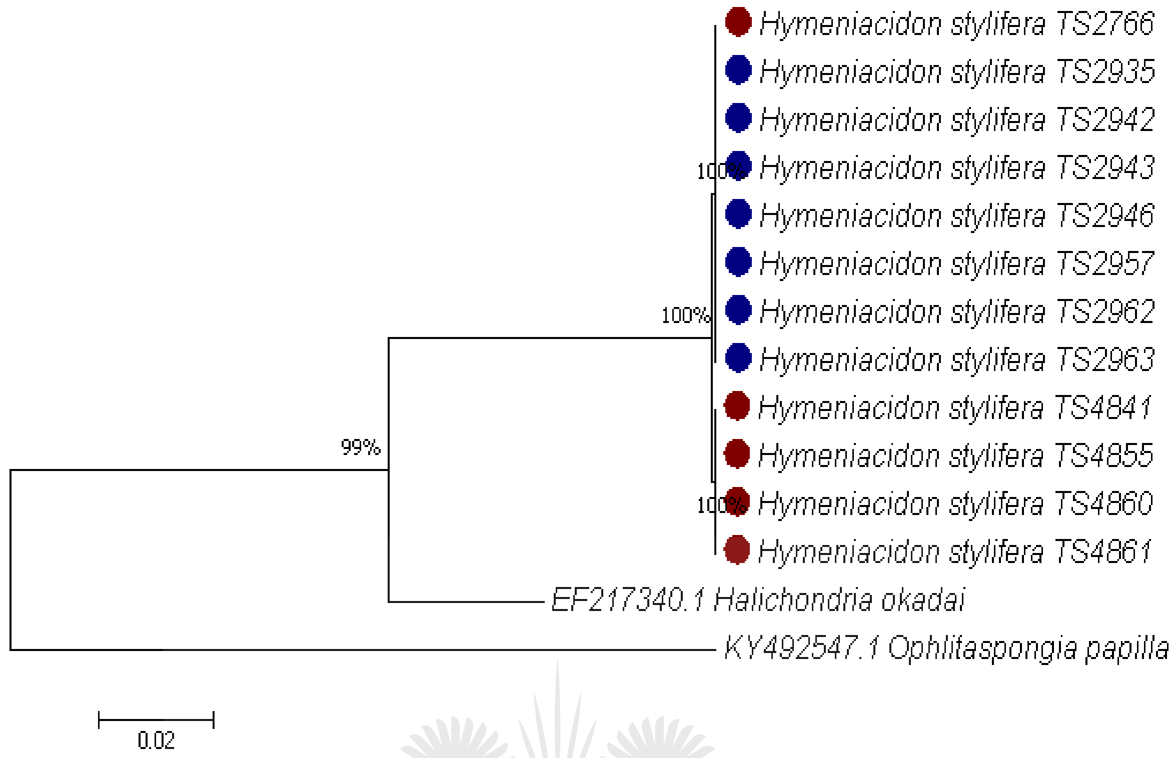

Fig 26 a: Phylogeny of the Cox 1 sequences *Hymeniacidon Stylifera* from the west (blue) and south- east(red) coast of South Africa based on the neighbour joining method with two distinct lineages and two haplotypes supported by high bootstrap support (100%).

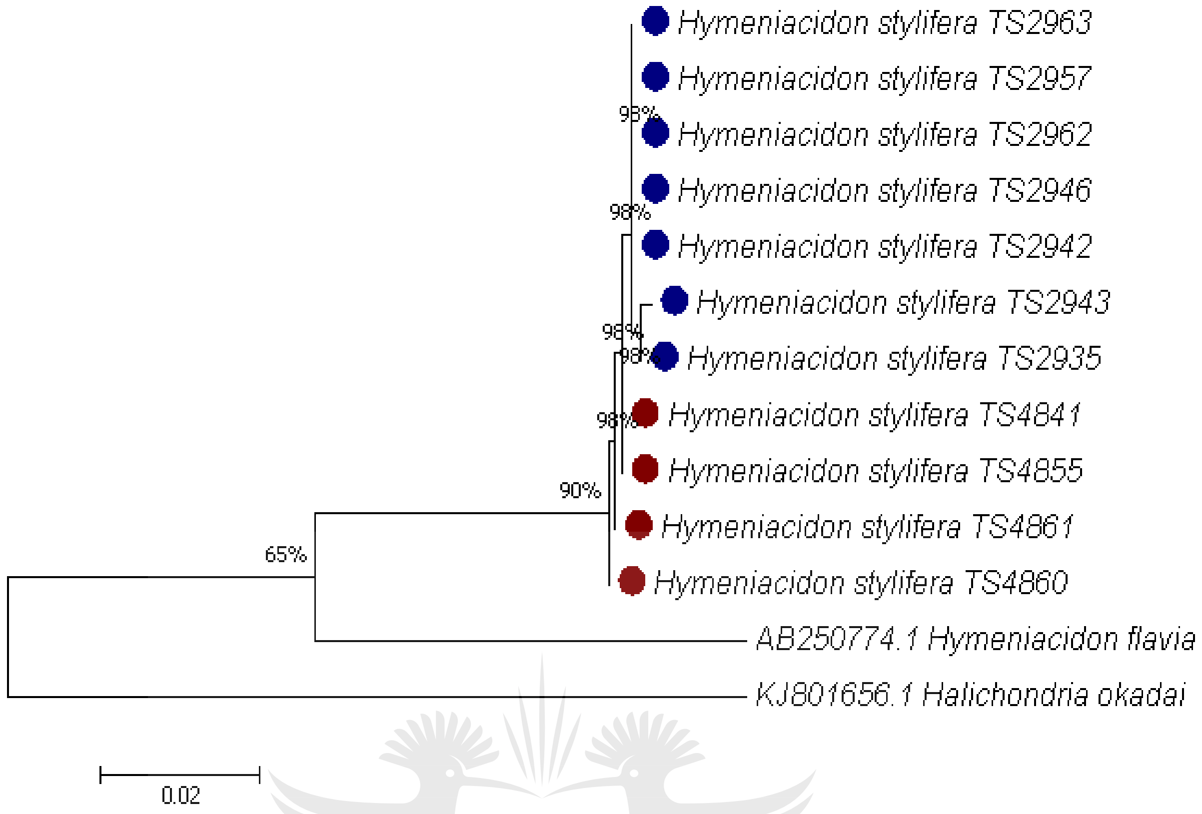

Fig 26b: Phylogeny of the Hymeniacidon species from the west (blue) and south east (red) coast of South Africa base on ITS sequences showing genetic variation among species.

***4.2.24. Coelosphaera* spp.**

The phylogeny of Coelosphara sp. was reconstructed based on cox1 sequences generated from samples collected from the South-east coast (Fig. 27). It forms a single lineages defined by two haplotypes which represent a single morphospecies.

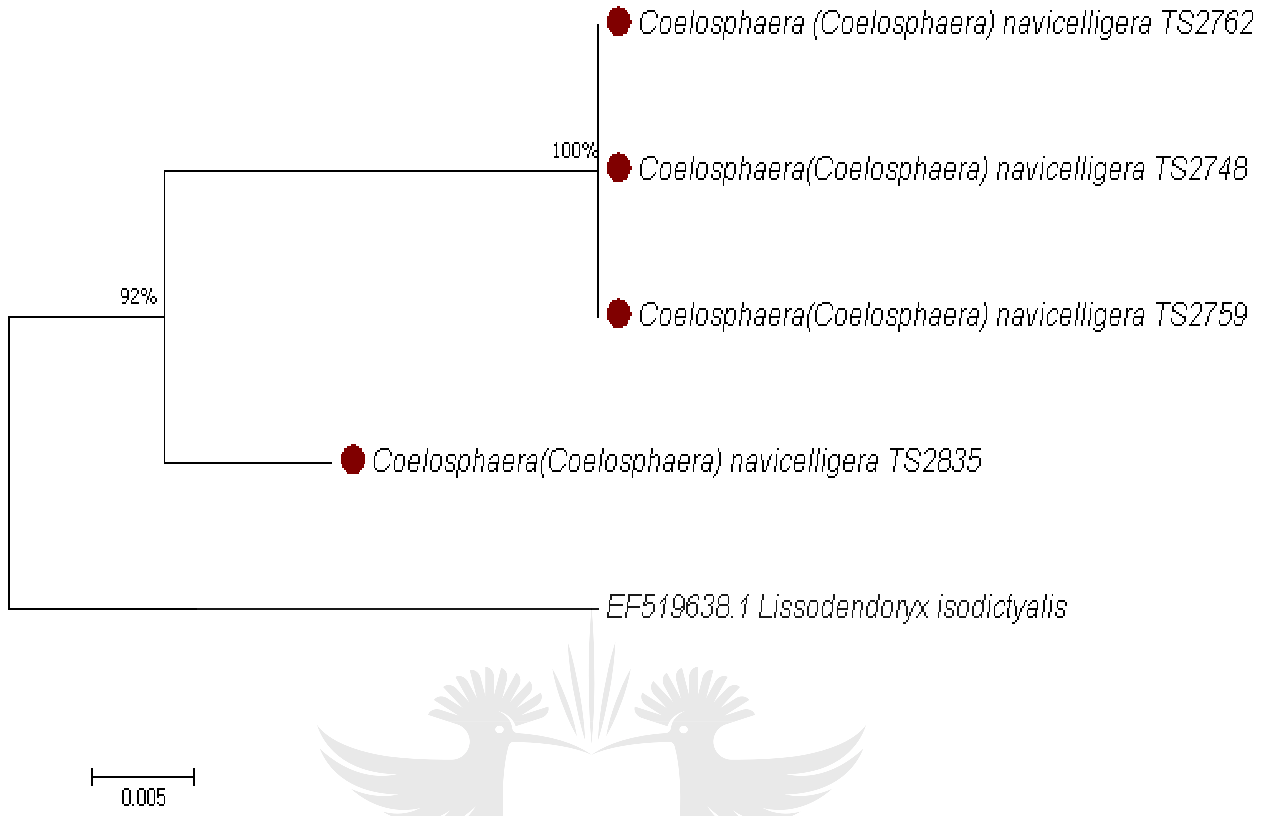

Fig 27: Phylogeny of Cox 1 sequences of *Coelosphaera* species from the south- east (red) coast of South Africa based on the neighbour joining method forms a single lineage defined by two haplotypes.

4.2.25. **Niphatidae spp.**

The cox1 sequences of the Niphatidae sp. from the family Niphatidae were generated from sponge specimens that were collected from the South east coast of South Africa. It phylogeny form five lineages Fig. 28) supported by relatively strong bootstrap support (98-100%). (Morphological identification depicted a total of seven different species (Niphatidae sp. 1, Niphatidae *sp. 2,* Niphatidea sp. 3, Niphatidae sp. 4, Niphatidae sp. 5, Niphatidae sp. 6 and Niphatidae sp. 7). However, phylogenetic analysis recovered Niphatidae sp. 5, Niphatidae sp. 6 and Niphatidae sp. 7) as a single lineage.

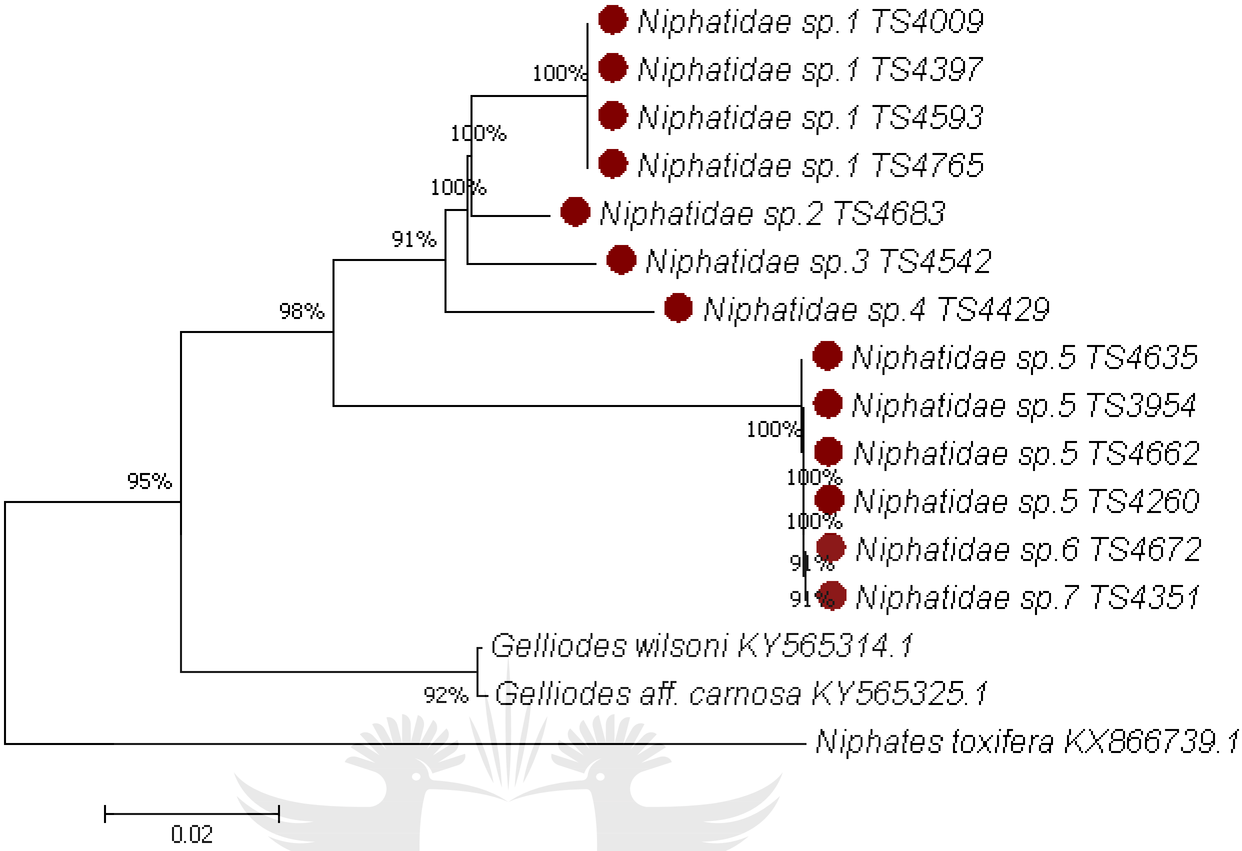

Fig 28: Phylogeny of the Niphatidae species from the south-east coast of South Africa based on the neighbour joining method with five lineages representing seven different morpho species..

4.2.26. Tsitsikamma spp.

Tsitsikamma sp forms part of the very large family Latrunculidae which is a very diverse group of sponges in South Africa and the genus *Tsitsikamma* happens to occur only in South Africa. However, specimens used in this study were collected around the south-east coast, Amathole region, around East London and Port Elizabeth

On the basis of phylogeny, the cox1 sequences of *Tsitsikamma sp* forms just one lineages (Fig. 29a) represented by a single lineage that consists of 4 morphospeciesl (*Tsitsikamma favus*, *Tsitsikamma* sp. Nov 1, *Tsitsikamma* sp.nov 2 and *Tsitsikamma* sp.nov 3). Using ITS (Fig. 29b), it shows that though it still a single lineage, there are genetic variation between the Tsitsikamma specimens.

Fig 29a: Phylogeny of cox1 sequences *Tsitsikamma* species from the south-east coast (red) of South Africa based on the neighbour joining method showing a single lineage with four morphospecies.

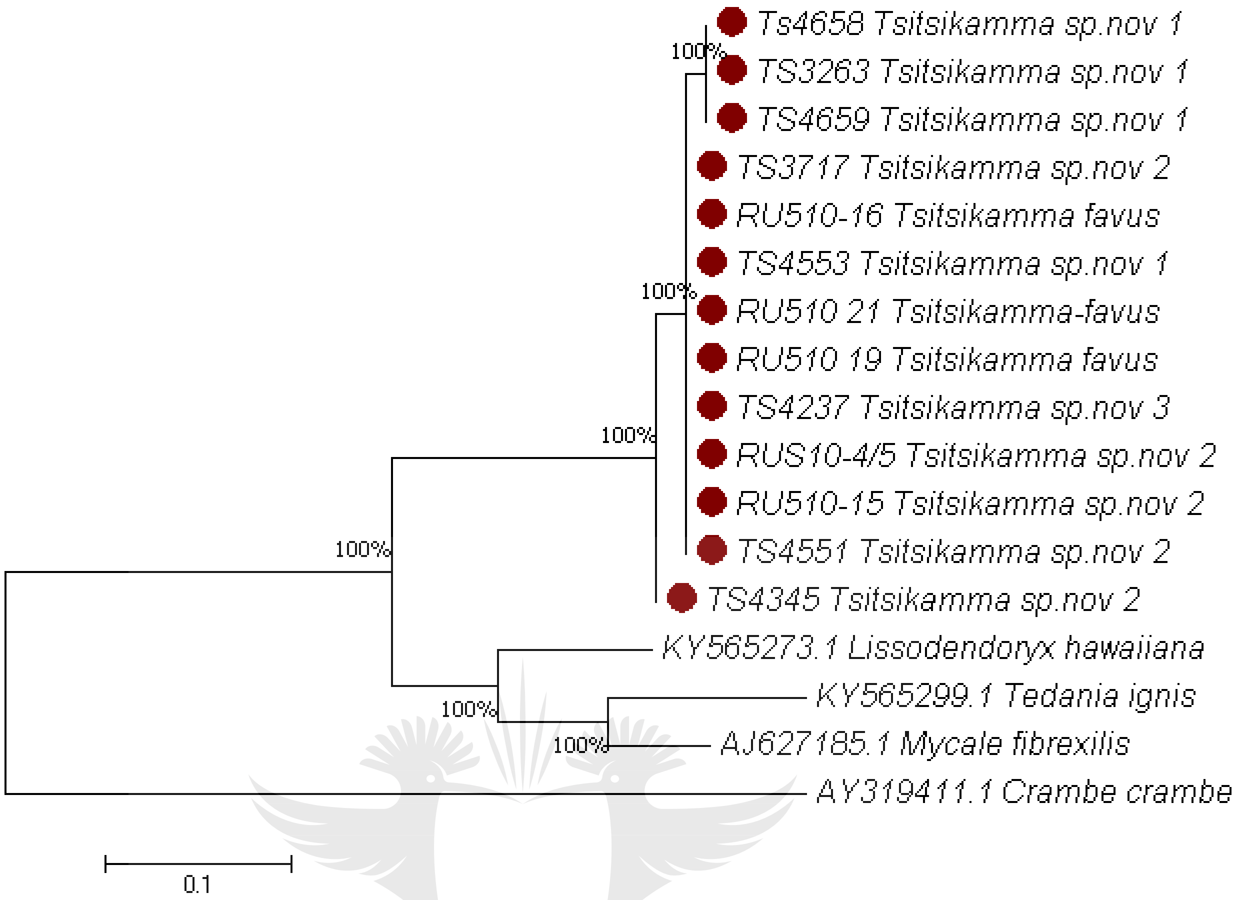

Fig 29b: The Phylogeny of the *Tsitsikamma* complex species based on sequences generated from Internal Transcribe spacers. It shows a single lineage though with some genetic variations but includes four morphospecies.

4.2.27. Higginsia spp.

The phylogeny of *Higginsia sp.* was reconstructed based on the cox1 sequences generated from the sponge samples collected from the south- east coast. It forms three different lineages consisting of eight different morphospecies (Fig. 30).

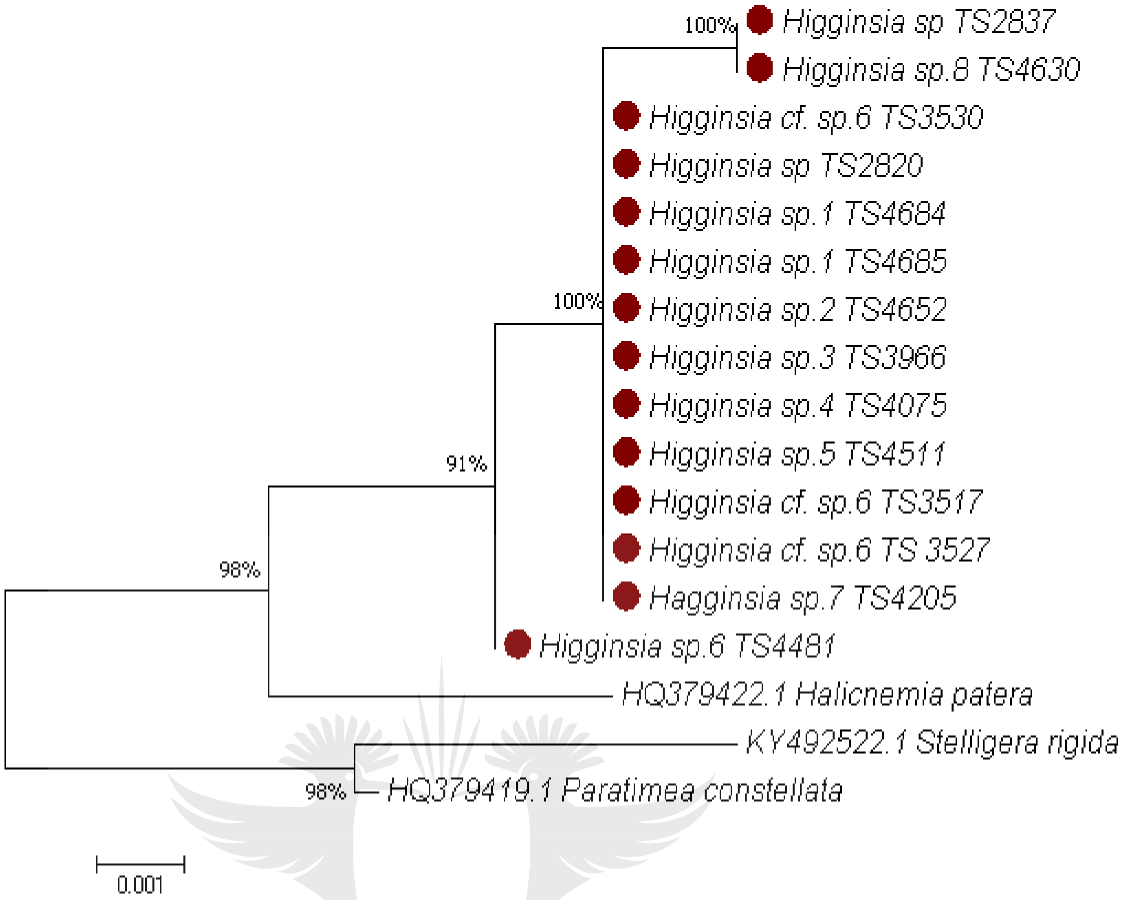

Fig 30: Phylogeny of the *Higginsia* species from the south-east coast (red) South Africa based on the neighbour joining method with three lineages including eight morphospecies.

4.2.28. The order Haplosclerida and the genus *Haliclona*

The molecular evolution of the order Haplosclerida has been described as 'enigmatic' because they evolve very slowly in different a manner to other demosponges and their mitochondrial genome has a number of features separating it from the mitochondrial genome of other demosponges (Redmond *et al*., 2015). In the phylogenetic tree of all haplosclerid sequences generated from this study, there were well supported lineages, but containing multiple morphologically distinct species (Niphatidae spp., *Haliclona* spp., *Petrosia* spp., Petrosidae spp. and Phloeodictydae spp.), confirming the findings that the order Haplosclerida is polyphyletic (e.g McCormack *et al*., 2002; Borchiellini *et al*., 2004; Nichols, 2005). The haplosclerids are also very diverse along the South African coast (Fig. 31a).

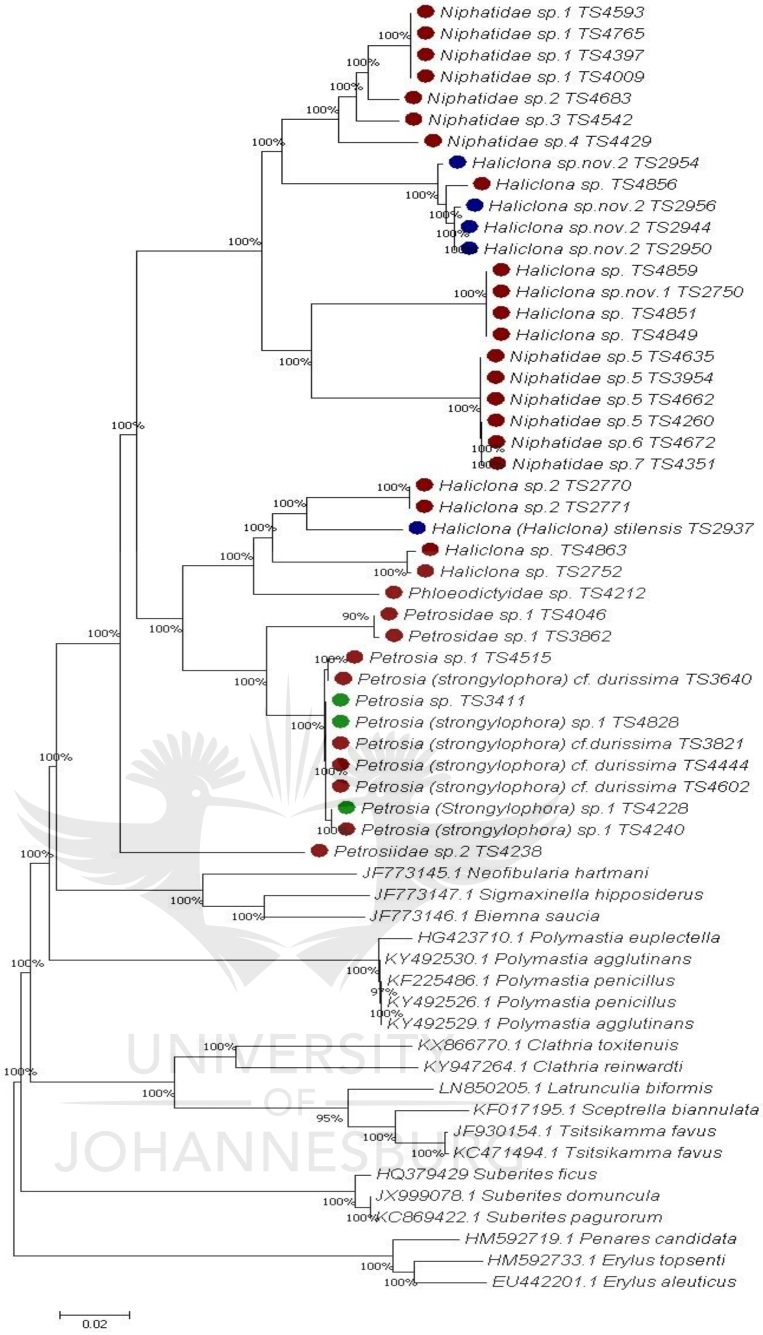

Fig 31a: Phylogeny of all haplosclerid species along the South African coast, based on the neighbour joining method showing it polyphyly status.

The phylogeny of the *Haliclona* species was reconstructed based on the cox1 sequences generated from specimens collected around the west coast and the south-east coast of South Africa. This result disagrees with morphological identification as distinct lineages include different morphological species. It also showed evidence that the genus Haliclona is polyphyletic, as outgroups which are sister species and also sis genus clusters within the Haliclona ingroup lineages (Fig. 31b).

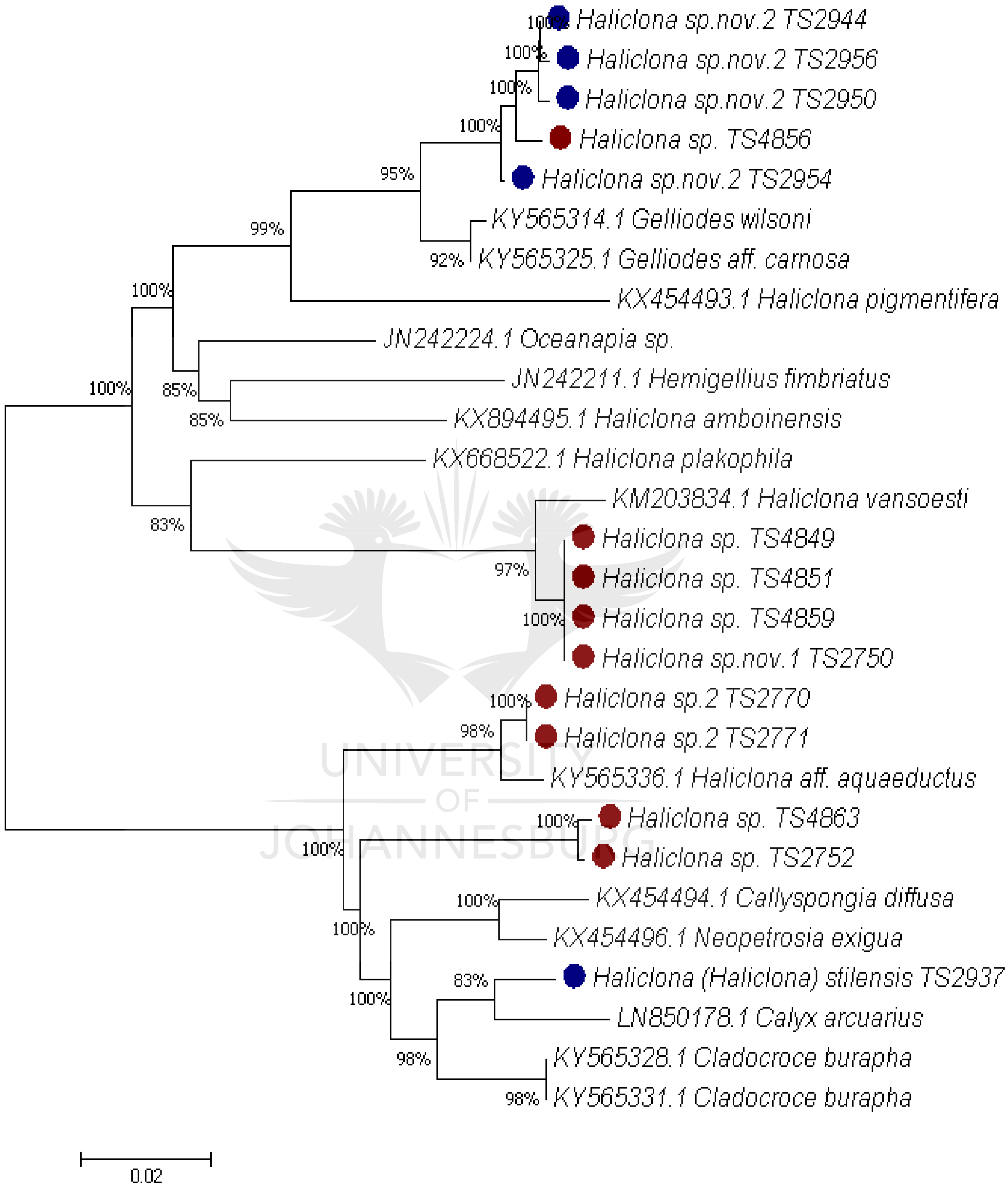

Fig 31b: Phylogeny of the Haliclona species from the west (blue) and south-east coast (red) ofSouth Africa base based on the neighbour joining method showing its polyphyly status.

4.3. Taxonomic and phylogenetic implications

A total of 56 (47%) sequences from 317 distinct sequences were identified to Family level. These include the families Microcionidae, Raspailiidae, Niphatidae, Scopalinidae, Microcionidae, Suberitidae, Phloeodictyidae, Phymaraphiniidae, Crambeidae, Corallistidae, Petrosidae, Tetillidae and Axinellidae. Only two (0.6%) sequences could only be identified to the level of Order (Order Bubarida), and the remaining sequences were all identified to genus or even species level.

Seventy-five (23%) of 317 sequences were assigned to the Class Demosponge, Order Poecilosclerida. Most of the Poecilosclerida sequences were identified as species belonging to the following genera: *Cyclacathia, Tsitsikamma, Coelosphaera, Clathria, Antho*, *Tedania, Fibulia, Echinostylinos, Isodictya, Myxilla, Phorbas, Ectyonopsis* and *Iophon.*

Seventy-nine (26%) sequences were assigned to the Order Tetractinellida, belonging to the genera *Characella, Penares, Callipelta, Erylus, Geodia*, *Rhabdastrella*, *Tetilla*, , *Pachastrella, Stelleta, Theonella* and *Fangophilina*

Fourty (13%) of the sequences obtained in this study were assigned to the demosponge order Haplosclerida. This is one of the most successful demosponge orders worldwide in terms of biodiversity (Van Soest and Hooper 2001, Van Soest et al., 2012), but also the most disputed in terms of composition of its families. Major discrepancies between morphological and molecular data challenge any plausible characters re-interpretation (Raleigh et al., 2007, Redmond et al., 2007, 2013), unlike other demosponge orders that could be readily reclassified based on molecular data (see Morrow and cárdenas, 2015). As a consequence, Haplosclerida are currently a neglected major group of Demospongiae in terms of diversity (Dervisty et al., 2012). Most of the Haplosclerida sequences found in this study were assigned to *Haliclona, Petrosia*, Niphatidae, and Petrosidae.

Fourty sequences (14%) were be assigned to genera in the Order Suberitida (*Suberites, Hymeniacidon, Rhixazinella* and *Homaxinella*), 12% to the Order Axinellida (Raspailiidae, *Higginsia, Didiscus, Axinella*, Axinellidae and *Lothoplocamia*), 6% to the Order Biemnida (*Biemna and Sigmaxinella*), 4% to the Order Polymastiida (*Polymastia and Sphaerotylus*), 2% to the Order Scopalinida

(Scopalinidae) and 0.6% each to the Orders Clioniaida (*Cliona*), Spongillida (*Macandrewi*) and Bubarida. Finally, one sequence obtained in this study could be assigned to the subclass Hexasterophora of the class Hexactinellida.

Chapter 5: Discussion

In recent years, studies on marine sponges have developed rapidly in the light of new studies combining molecular and morphological analysis i.e. integrative taxonomy (Cárdenas *et al*. 2012; Hill *et al*. 2013, Willenz *et al*., 2016, Cruz-Barraza *et al*., 2017, Dohrmann *et al*., 2017, Carballo *et al*., 2018, Setiawan *et al*., 2018, Vicente *et al*., 2019). In an attempt to better understand South African sponge biodiversity; we used both morphological and molecular data to accelerate progress in identifying and classifying sponges in South Africa. This is the first DNA barcoding study directed towards sequencing sponge specimens collected along the temperate coastline of this region, and the combined approach yielded a good overview of new information about South Africa’s sponge community. Although genetic differences between morphospecies were often minimal, a large percentage of genetic results conformed with the morphological results. Comparing morphological characters against phylogenetic information thus proves to be a fruitful approach for integrating the strengths of morphological data with those of DNA sequences, and confirms the usefulness of integrated taxonomy (Dayrat, 2005).

Finding distinct lineages in our widespread and genetically diverse species further indicates the usefulness of the *cox*1 partitions for the molecular distinction of species, although there was clear evidence for different levels of genetic differentiation between morphospecies, with the cox1 markers likely being insufficient in cases where discrepancies were found. In some taxa such as *Hymeniacidon stylifera*, the relation between morphological taxonomy and evolutionary lineages was incongruent because genetically cryptic species are present. Cryptic species are prevalent in sponges, particularly in what were previously considered ‘cosmopolitan’ species, which often reveals genetic divergence among distinct species that are morphologically indistinguishable (Reveillaud *et al*., 2010).

Many studies on sponges have found incongruence between morphological taxonomy and genetic lineages, with taxa subsequently being either split or lumped (DeBiasse & Hellberg, 2015). The scarcity of informative taxonomic characters in the Porifera makes morphological species delimitation difficult because long generation times and large effective population sizes can lead to incomplete lineage sorting and gene tree/species tree discordance. While we identified divergent lineages within morphologically identical specimens, we also found the opposite pattern in *Sigmaxinella* sp., *Geodia* sp., *Biemna* sp., *Haliclona* sp., *Tsitsikamma* sp,, *Higginsia* sp. and Niphatidae sp. as they included same haplotype sharing different morphological distinct species, potentially due to incomplete lineages sorting.

Although there was often a good match between lineages and marine ecoregions, some lineages were present in more than one ecoregions. Examples are *Geodia* sp., *Tedania* sp., *Biemna sp*, *Antho* sp., *Penares* sp., *Hymeniacidon* sp. and *Poecillastra* sp.. Although it cannot be ruled out that some species tolerate a wide variety of environmental conditions, genetical research from the region suggests that this is more likely a result of either incomplete lineage sorting, or migration of a few individuals in to the habitat of their sister lineage (Teske et al., 2011)

Phylogenetic results have shown the order Haplosclerida and in particular genus *Haliclona* to be polyphyletic based on mitochondrial DNA. The order Haplosclerida as a group has been well described morphologically (e.g Van Soest, 1980; Van Soest & Hooper, 2002a), however some higher level definitions appear to be largely groupings of convenience (Van Soest & Hooper, 2002a) containing a number of diverse sponges, but this existing classification may represent true evolutionary relationships. Such phylogenetic patterns may be explained by slow evolution of the mitochondrial DNA in sponges (DeBiasse & Hellberg, 2015), misidentification of specimens, phenotypically plasticity and/or DNA contamination based on comparison of molecular and morphological data. However, there is need for morphological re-examination of this particular taxon (*Haliclona*).

In this study, Hypothesis 1 was supported. We proved that South Africa has a highly diverse sponge fauna. One hundred and thirty three species (133) were identified in this study, which far exceeds the convention that assemblies with 40 species are considered diverse.

Hypothesis 2 was supported. More than 80 new species and a number of species that had been previously described were discovered in this study. A number of species were also endemic to South Africa.

Hypothesis 3 was also supported. There was evidence that different ecoregions had different sponge assemblages, suggesting that these are uniquely adapted to the region's diverse environmental conditions.

5.1. Implications for distribution of South African sponge taxa

In terms of coverage reached by barcoding these sponges, about 45% of the sponge specimens used for this study were successfully barcoded. In this regard, DNA barcoding was successful in gathering information about South African sponge communities that can be used for the phylogenetic inference and phylodiversity comparisons, or for sorting large collections and complement classical taxonomic work.

It was also found that sponges along the South African coastline are phylogenetically diverse. Genetic differentiation among samples of what were assumed to be the same species but collected from different locations indicates that levels of cryptic diversity are high among South African sponges. This follows a general trend in South African marine invertebrates, in which most species that are present in more than one ecoregion are actually at least two distinct cryptic species (Teske et al. 2011). This supports the growing evidence that South African sponges are still very underexplored and that their biodiversity has been greatly underestimated (Samaai 2006, Metobole *et al*., 2017). It was further observed that some species have evolve quite recently, and they are not yet genetically distinct even though they may already show adaptations to envoronmental conditions unique to the ecoregions (Teske *et al*., 2018). However, lack of differentiation between ecoregions may also be a result of gene flow (Maldonado & Riesgo 2008). Fragments of some sponge species during asexual reproduction contain developing embryos of sexual origin, which can successfully complete development and leave fragments as free-swimming larvae. The dispersal of these embryo bearing fragments maximizes the chance that several distinct genotypes will reach a new area, increases the chance of establishing a new population and increase variation between similar species (Maldonado & Riesgo, 2008).

5.2. Considerations for molecular taxonomic approaches on sponges

We here explore the molecular diversity of collection of sponges along the South African coast using mitochondrial DNA marker and, in one case, Internal transcribe Spacer. Such molecular biodiversity surveys provide a suitable framework for subsequent in-depth taxonomic studies and represent a particularly useful addition to studying phenotypically character-poor and plastic taxa.

Chapter 6: Conclusion

South Africa's marine regions are inhabited by a wide variety of sponge species. The discovery of large number of new species shows that there are probably more undescribed species in South African coastline and that more taxonomic and molecular research is needed.

This study presents results for the first DNA barcoding initiative directed towards sequencing sponges from the South African coastal region, and thus most of the species sequenced here will be represented in the barcoding database for the first time. It serves as a starting point in developing a reference library for South African sponges and this will be used to advance the systematic and evolutionary research of the region's sponges. Further, it provides an opportunity for the rapid taxonomic identification and sponge collection for ecological research along the South African coastline. It has undoubtedly contributed towards a better understanding of South African sponge species diversity, and the barcode data has provided first insights into the evolutionary processes that have produced both high diversity and endemism, which will reveal areas of taxonomic uncertainty in need of further research. Identifying sponges collected in coastal waters of South Africa correctly will provide an understanding on a broad range of subjects such as sponge ecology, biodiversity, dispersal, speciation evolution and discovery of pharmaceutically and biotechnologically valuable taxa within species found. Creating a reference library with DNA barcodes for South African sponges for as many ofthe region's sponge species as possible, will provide every researcher with the possibility to test their hypotheses immediately and without the need of collecting comparative materials (with uncertain taxonomy) and time consuming data generation. It is expected to open up a new dimension and quality in biodiversity research, and provided that the research started here is carried on in the future, will put South Africa on the map as a sponge biodiversity hotspot over the coming decades.

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Table 1: A Table of appendix showing all identified sponges from this study (indicating their specimen number, location, coordinates, depth, Genus and species names, and authors) collected along the South African coast

Specimen number	Location	Latitude	Longitude	Depth (M)	Ecoregion	Genus name	Species name	Species Status	Author
TS1525	West coast	-30.466667	15.73333263	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1584	West coast	-31.800833	17.68333244	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1585	West coast	-31.800833	17.68333244	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1592	West coast	-29.333333	15.98333359	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1611	West coast	-31.851944	17.96666718	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1614	West coast	-29.135556	16.30000114	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1633	West coast	-30.000	16.34999847	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1640	West coast	-32.101944	17.41666603	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1798	West coast	-31.151944	16.8696700	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1807	West coast	-31.050833	17.58567000	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1810	West coast	-30.71617	16.88883000	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1815	West coast	-31.79967	17.90817000	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray,

									2017
TS1816	West coast	-31.234444	17.69483000	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS1819	West coast	-29.74983	16.48833000	_	Benguela	*Suberites*	*dandelenae*	New species	Samaai & Maduray, 2017
TS4185	South coast	-33.256944	25.22100000	_	Agulhas	*Suberites*	Sp.	New species	To be describe
TS4186	South coast	-33.256944	25.22100000	_	Agulhas	*Suberites*	Sp.	New species	To be describe
TS4187	South coast	-33.256944	25.22100000	_	Agulhas	*Suberites*	Sp.	New species	To be describe
TS4188	South coast	-33.256944	25.22100000	_	Agulhas	*Suberites*	Sp.	New species	To be describe
TS4189	South coast	-33.256944	25.22100000	_	Agulhas	*Suberites*	Sp.	New species	To be describe
TS4190	South coast	-33.256944	25.22100000	_	Agulhas	*Suberites*	Sp.	New species	To be describe
TS4191	South coast	-33.256944	25.22100000	_	Agulhas	*Suberites*	Sp.	New species	To be describe
TS3603	Eastern Cape	-32,75173667	28,41591333	_	Agulhas	*Cyclacanthia*	sp.nov.1	New species	To be describe
TS4324	Eastern Cape	-32° 45.5828	28° 24.7149 E	_	Agulhas	*Cyclacanthia*	sp. nov. 1	New species	To be describe
TS4342	Eastern Cape	-32° 45.5828	28° 24.7149	_	Agulhas	*Cyclacanthia*	sp. nov. 1	New species	To be describe
TS4552	Eastern Cape	-32° 57.0033	28° 04.1875	_	Agulhas	*Cyclacanthia*	sp. nov. 1	New species	To be describe
RU510 - 4/5	Algoa Bay	-33°50.578S	25°48.988 E	30 m	Agulhas	*Tsitsikamma*	Sp.nov.2	Old species	To be describe
RU510 - 12	Algoa Bay	-33°50.578S	25°48.988 E	30 m	Agulhas	*Tsitsikamma*	*favus*	Old species	_
RU510 - 14	Algoa Bay	-33°50.578S	25°48.988 E	30 m	Agulhas	*Tsitsikamma*	*favus*	Old species	_
RU510 -	Algoa	-33°50.578S	25°48.988	30 m	Agulhas	*Tsitsikamma*	Sp.nov.2	Old	_

15	Bay		E					species	
RU510 - 16	Algoa Bay	-33°58.4245S	25°40.869 E	30 m	Agulhas	*Tsitsikamma*	*favus*	Old species	_
RU510 - 18	Algoa Bay	-33°50.578S	25°48.988 E	30 m	Agulhas	*Tsitsikamma*	*favus*	Old species	_
RU510 - 19	Algoa Bay	-33°50.578S	25°48.988 E	30 m	Agulhas	*Tsitsikamma*	*favus*	Old species	_
RU510 - 21	Algoa Bay	-33°50.578S	25°48.988 E	30 m	Agulhas	*Tsitsikamma*	*favus*	Old species	_
RU510 - 22	Algoa Bay	-33°50.578S	25°48.988 E	30 m	Agulhas	*Tsitsikamma*	*favus*	Old species	_
Sp02	Algoa Bay	-33°50.578S	25°48.988 E	30 m	Agulhas	*Tsitsikamma*	*favus*	Old species	_
Sp03	Algoa Bay	-33°50.578S	25°48.988 E	30 m	Agulhas	*Tsitsikamma*	*favus*	Old species	_
TS2804	Eastern Cape	-32 56.0 S	38 04.8 E	_	Agulhas	*Tsitsikamma*	Sp.nov.2	New species	_
TS3717	Eastern Cape	- 33,134665	27,7689	33m	Agulhas	*Tsitsikamma*	Sp.nov.2	New species	_
TS3852	Eastern Cape_	- 33,134665	27,7689	33m	Agulhas	*Tsitsikamma*	Sp.nov.2	New species	_
TS4237	Eastern Cape	-32° 40.8934 S	- 28° 27.7000 E	52 - 54,6m	Agulhas	*Tsitsikamma*	Sp.nov.3	New species	_
TS4267	Eastern Cape	-32° 40.8934 S	28° 27.7000 E	52 - 54,6m	Agulhas	*Tsitsikamma*	Sp.nov.2	New species	_
TS4551	Eastern Cape	-32° 57.0033 S	28° 04.1875 E	40.5m	Agulhas	*Tsitsikamma*	Sp.nov.2	new species	_
TS4553	Eastern Cape	-32° 57.0033 S	28° 04.1875	40.5m	Agulhas	*Tsitsikamma*	Sp.nov.1	Old species	_
0CDN7241 -G	Eastern Cape	-34 00.46S	023 29.79E	_	Agulhas	*Tsitsikamma*	*favus*	Old species	_
0CDN7414	Eastern	-34 03.14S	025 41.36E	17m	Agulhas	*Tsitsikamma*	Sp.nov 1	New	To be

-S	Cape							species	describe
TS4263	Eastern Cape	-32° 40.8934 S	28° 27.7000 E	52 - 54,6m	Agulhas	*Tsitsikamma*	sp. nov. 1	New species	To be describe
TS4345	Eastern Cape	-32° 45.5828 S	28° 24.7149 E	45.1m	Agulhas	*Tsitsikamma*	sp. nov. 2	New species	To be describe
TS4655	Eastern Cape	-33° 09.7909 S	27° 46.5201 E	35,9 - 36,1m	Agulhas	*Tsitsikamma*	sp. nov. 1	New species	To be describe
TS4658	Eastern Cape	-33° 09.7909 S	27°46.4774 E	35,9 - 36,1m	Agulhas	*Tsitsikamma*	sp. nov. 1	New species	To be describe
TS4659	Eastern Cape	-33° 09.7909 S	27° 46.5201 E	35,9 - 36,1m	Agulhas	*Tsitsikamma*	sp. nov. 1	New species	To be describe
TS2748	East coast	-31.65083333	29.51733333	_	Agulhas	*Coelosphaera (Coelosphaera)*	*navicelligera*	Old species	_
TS2759	East coast	-31.98533333	29.15166667	_	Agulhas	*Coelosphaera (Coelosphaera)*	*navicelligera*	Old species	_
TS2762	East coast	-31.98533333	29.15166667	_	Agulhas	*Coelosphaera (Coelosphaera)*	*navicelligera*	Old species	_
TS2835	South coast	_	_	_	Agulhas	*Coelosphaera (Coelosphaera)*	*navicelligera*	Old species	_
TS3516	East coast	-32.83003667	28.45927	_	Agulhas	*Biemna*	sp. 1	New species	To be describe
TS3723	East coast	-33.134665	27.7689	_	Agulhas	*Biemna*	*megalosigma* var. *sigmodragma*	Old species	Lévi, 1963
TS3818	East coast	-32.82361833	28.52109833	_	Agulhas	*Biemna*	sp.1	New species	To be describe
TS3955	East coast	-33.159735	27.77769167	_	Agulhas	*Biemna*	*megalosigma* var. *sigmodragma*	Old species	Lévi, 1963
TS3992	East coast	-32.66736833	28.42686	_	Agulhas	*Biemna*	Sp. 2	New species	To be describe
TS4003	East coast	-33.01367333	28.31424667	_	Agulhas	*Biemna*	sp. 1	New species	To be describe

TS4032	East coast	-33.01367333	28.31424667	_	Agulhas	*Biemna*	Sp. 1	New species	To be describe
TS4208	South coast	-34.67116667	21.366667	_	Agulhas	*Biemna*	cf. sp. 2	New species	To be describe
TS4245	East coast	-32.68155667	28.45845667	_	Agulhas	*Biemna*	sp. 1	New species	To be describe
TS4299	East coast	-32.65112667	28.46087833	_	Agulhas	*Biemna*	cf. sp. 2	New species	To be describe
TS4850	East coast	-32.230935	28.9277075	_	Agulhas	*Biemna*	*fistulosa*	Old species	_
TS4862	East coast	-32.230935	28.9277075	_	Agulhas	*Biemna*	*fistulosa*	Old species	_
TS4864	East coast	-32.76407028	28.25149806	_	Agulhas	*Biemna*	*fistulosa*	Old species	_
TS4689	East coast	-33.39101	27.53744	_	Agulhas	*Biemna*	Sp .1	New species	To be describe
TS3999	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	sp. 3	New species	To be describe
TS4024	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	sp. 3	New species	To be describe
TS4025	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	sp. 1	New species	To be describe
TS4026	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	cf. sp.1	New species	To be describe
TS4029	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	cf. sp.1	New species	To be describe
TS4030	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	cf. sp. 1	New species	To be describe
TS4035	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	cf. sp.1	New species	To be describe
TS4036	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	cf. sp. 1	New species	To be describe
TS4047	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	cf. sp. 1	New species	To be describe

TS4049	East coast	-33.01367333	28.31424667	_	Agulhas	*Characella*	cf. sp.1	New species	To be describe
TS4377	East coast	-32.75803667	28.45889	_	Agulhas	*Characella*	sp. 2	New species	To be describe
TS4469	East coast	-32.939515	28.26853333	_	Agulhas	*Characella*	sp. 2	New species	To be describe
TS2766	East coast	-32.47433333	28.65133333	_	Benguela	*Hymeniacidon*	*stylifera*	Old species	_
TS2935	West coast	-30.94685	17.63510000	_	Benguela	*Hymeniacidon*	*stylifera*	Old species	_
TS2943	West coast	-30.81065	17.55391667	_	Benguela	*Hymeniacidon*	*stylifera*	Old species	_
TS2946	West coast	-30.81466667	17.55416667	_	Benguela	*Hymeniacidon*	*stylifera*	Old species	_
TS2957	West coast	-30.8107	17.55436667	_	Benguela	*Hymeniacidon*	*stylifera*	Old species	_
TS2962	West coast	-29.133611	17.08000000	_	Benguela	*Hymeniacidon*	*stylifera*	Old species	_
TS2963	West coast	_	_	_	Benguela	*Hymeniacidon*	*stylifera*	Old species	_
TS4841	East coast	-32.31082389	28.82852333	_	Agulhas	*Hymeniacidon*	*stylifera*	Old species	_
TS4855	East coast	-32.230935	28.9277075	_	Agulhas	*Hymeniacidon*	*stylifera*	Old species	_
TS4860	East coast	-32.76407028	28.25149806	_	Agulhas	*Hymeniacidon*	*stylifera*	Old species	_
TS4861	East coast	-32.76407028	28.25149806	_	Agulhas	*Hymeniacidon*	*stylifera*	Old species	_
TS2767	East coast	-32.47433333	28.65133333	_	Agulhas	*Lissodendoryx (Lissodendoryx)*	*ternatensis*	Old species	_
TS4791	Deep secret	-36.04155	19.69017	_	Agulhas	*Pseudosuberites*	sp.		
TS4397	Amathole; Eastern Cape	-32.75408	28.45525333	_	Agulhas	Niphatidae	sp. 1	New species	To be described

TS4593	Amathole; Eastern Cape	-33.28556667	27.90989333	_	Agulhas l	Niphatidae	sp. 1	New species	To be described
TS4009	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	Niphatidae	sp. 1	New species	To be described
TS4765	Amathole; Eastern Cape	-32.96286167	28.31934833	_	Agulhas	Niphatidae	sp. 1	New species	To be described
TS4683	Amathole; Eastern Cape	-33.39101	27.53744	_	Agulhas	Niphatidae	sp. 2	New species	To be described
TS4542	Amathole; Eastern Cape	-32.950055	28.06651	_	Agulhas	Niphatidae	Sp .3	New species	To be described
TS4429	Amathole; Eastern Cape	-32.84186667	28.51148333	_	Agulhas	Niphatidae	sp. 4	New species	To be described
TS4635	Amathole; Eastern Cape	-33.15962833	27.97420833	_	Agulhas	Niphatidae	sp. 5	New species	To be described
TS4662	Amathole; Eastern Cape	-33.16318167	27.77462333	_	Agulhas	Niphatidae	sp. 5	New species	To be described
TS4260	Amathole; Eastern Cape	-32.68155667	28.45845667	_	Agulhas	Niphatidae	sp. 5	New species	To be described
TS3954	Amathole; Eastern Cape	-33.159735	27.77769167	_	Agulhas	Niphatidae	sp. 5	New species	To be described
TS4672	Amathole; Eastern Cape	-33.16318167	27.77462333	_	Agulhas	Niphatidae	sp. 6	New species	To be described
TS4351	Amathole; Eastern Cape	-32.75971333	28.41097667	_	Agulhas	Niphatidae	sp. 7	New species	To be described

TS2954	West coast	-30.133611	17.55391667	_	Benguela	*Haliclona*	sp. nov. 2	New species	To be described
TS2944	West coast	-30.81065	17.55391667	_	Benguela	*Haliclona*	sp. nov. 2	New species	To be described
TS2950	West coast	-30.81466667	17.55416667	_	Benguela	*Haliclona*	sp. nov. 2	New species	To be described
TS4856	Eastern cape	-32.230935	28.9277075	_	Agulhas	*Haliclona*	sp.	New species	To be described
TS2956	West coast	-30.81466667	17.55416667	_		*Haliclona*	sp. nov. 2	New species	To be described
TS4851	Eastern cape	-32.230935	28.9277075	_	Agulhas	*Haliclona*	sp.	New species	To be described
TS4849	Eastern cape	-32.230935	28.9277075	_	Agulhas	*Haliclona*	sp.	New species	To be described
TS4859	Eastern cape	-32.76407028	28.25149806	_	Agulhas	*Haliclona*	sp.	New species	To be described
TS2750	East coast	-31.98533333	29.15166667	_	Agulhas	*Haliclona*	sp. nov. 1	New species	To be described
TS4863	East coast	-32.76407028	28.25149806	_	Agulhas	*Haliclona*	sp.	New species	To be described
TS2752	East coast	-31.98533333	29.15166667	_	Agulhas	*Haliclona*	sp.	New species	To be described
TS2937	West coast	17.63510000	-30.94685	_	Benguela	*Haliclona (Haliclona)*	*stilensis*	old	-
TS2771	East coast	-32.47433333	28.65133333	_	Agulhas	*Haliclona*	sp. 2	New species	To be described
TS2770	East coast	-32.47433333	28.65133333	_	Agulhas	*Haliclona*	sp. 2	New species	To be described
TS3953	East coast	-33.159735	27.77769167	_	Agulhas	*Sigmaxinella*	sp. 1	New species	To be described
TS3525	East coast	-32.83003667	28.45927	_	Agulhas	*Sigmaxinella*	sp. 2	New species	To be described
TS4610	East coast	-33.28556667	27.90989333	_	Agulhas	*Sigmaxinella*	sp. 3	New species	To be described
TS4372	East coast	-32.75803667	28.45889	_	Agulhas	*Sigmaxinella*	sp. 1	New species	To be described

TS4396	East coast	-32.75408	28.45525333	_	Agulhas	*Sigmaxinella*	sp. 2	New species	To be described
TS2685	South Coast	_	_	_	Agulhas	*Polymatia*	*littoralis*	Old species	-
TS2938	West coast	17.60281667	-30.91543333	_	Benguela	*Polymastia*	*atlantica*	Old species	-
TS2947	West coast	17.55416667	-30.81466667	_	Benguela	*Polymastia*	*atlantica*	Old species	-
TS4154	Cape Canyon; West Coast	-32.85645	17.53886667	_	Benguela	*Sphaerotylus* cf.	*strobilis*	Old species	Plotkin, Morrow, Gerasimova & Rapp, 2017
TS4697	East coast	-33.39101	27.53744	_	Agulhas	*Polymastia*	sp. 1	New species	To be described
TS4699	East coast	-33.39101	27.53744	_	Agulhas	*Polymastia*	sp. 1	New species	To be described
TS4700	East coast	-33.39101	27.53744	_	Agulhas	*Polymastia*	sp. 1	New species	To be described
TS3628	East coast	-32.98308	28.31967333	_	Agulhas	*Polymastia*	sp. 1	New species	To be described
TS4254	East coast	-32.68155667	28.45845667	_	Agulhas	*Polymastia*	sp. 2	New species	To be described
TS4442	East coast	-32.703535	28.43105167	_	Agulhas	*Polymastia*	sp. 3	New species	To be described
TS3976	Amathole; Eastern Cape	-33.159735	27.77769167	_	Agulhas	*Polymastia*	sp. 4	New species	To be described
TS4146	Cape Canyon; West Coast	-33.07333333	17.57166667	_		*Polymastia*	sp. 5	New species	To be described
TS4698	East coast	-33.39101	27.53744	_	Agulhas	*Polymastia*	sp. 6	New species	To be described
TS4240	Amathole; Eastern	-32.68155667	28.45845667	_	Agulhas	*Petrosia (Strongylophora)*	sp. 1	New species	To be described

	Cape								
TS4828	South Coast	-35.16333333	23.01066667	_	Agulhas	*Petrosia (Strongylophora)*	sp. 1	New species	To be described
TS4046	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	Petrosiidae	sp. 1	New species	To be described
TS3821	East coast	-32.82361833	28.52109833	_	Agulhas	*Petrosia (Strongylophora)*	cf. *durissima*	Old species	(Dendy, 1905)
TS3640	East coast	-32.98308	28.31967333	_	Agulhas	*Petrosia (Strongylophora)*	cf. *durissima*	Old species	Dendy, 1905
TS3862	East coast	-32.75628167	28.43847	_	Agulhas	Petrosiidae	sp. 1		
TS4444	East coast	-32.939515	28.26853333	_	Agulhas	*Petrosia (Strongylophora)*	cf. *durissima*	Old species	Dendy, 1905
TS4602	East coast	-33.28556667	27.90989333	_	Agulhas	*Petrosia (Strongylophora)*	cf. *durissima*	Old species	Dendy, 1905
TS4515	East coast	-32.939515	28.26853333	_	Agulhas	*Petrosia*	sp. 1		
TS3411	South coast	_	_	_	Agulhas	*Petrosia*	sp. 1	New species	To be described
TS4228	South Coast	-35.16333333	23.01066667	_	Agulhas	*Petrosia (Strongylophora)*	sp. 1	New species	To be described
TS4013	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	Raspailiidae	sp. 1	New species	To be described
TS3627	East coast	-32.98308	28.31967333	_	Agulhas	Raspailiidae	sp. 1	New species	To be described
TS4425	East coast	-32.839185	28.51261833	_	Agulhas	Raspailiidae	sp. 1	New species	To be described
TS4426	East coast	-32.839185	28.51261833	_	Agulhas	Raspailiidae	sp. 1	New species	To be described
TS4594	East coast	-33.28556667	27.90989333	_	Agulhas	Raspailiidae	sp. 1	New species	To be described
TS4262	East	-32.68155667	28.4584566	_	Agulhas	Raspailiidae	sp. 3	New	To be

	coast		7					species	described
TS4624	East coast	-33.28556667	27.90989333	_	Agulhas	Raspailiidae	sp. 3	New species	To be described
TS4642	East coast	-33.15962833	27.97420833	_	Agulhas	Raspailiidae	sp. 4	New species	To be described
TS4638	East coast	-33.15962833	27.97420833	_	Agulhas	Raspailiidae	sp. 4	New species	To be described
TS4243	East coast	-32.68155667	28.45845667	_	Agulhas	Raspailiidae	sp. 5	New species	To be described
TS4294	East coast	-32.65112667	28.46087833	_	Agulhas	Raspailiidae	sp. 6	New species	To be described
TS4296	East coast	-32.65112667	28.46087833	_	Agulhas	Raspailiidae	sp. 6	New species	To be described
TS4246	East coast	-32.68155667	28.45845667	_	Agulhas	Raspailiidae	sp. 6	New species	To be described
TS4694	East coast	-33.39101	27.53744	_	Agulhas	Raspailiidae	sp. 7	New species	To be described
TS4695	East coast	-33.39101	27.53744	_	Agulhas	Raspailiidae	sp. 7	New species	To be described
TS4295	East coast	-32.65112667	28.46087833	_	Agulhas	Raspailiidae	sp. 7	New species	To be described
TS4204	South Coast	-34.67116667	21.366667	_	Agulhas	Raspailiidae	sp. 8	New species	To be described
TS4759	Amathole; Eastern Cape	-32.96286167	28.31934833	_	Agulhas	*Penares*	cf. *orthotriaena*	Old species	Burton, 1931
TS4480	East coast	-32.939515	28.26853333	_	Agulhas	*Penares*	cf. *orthotriaena*	Old species	Burton, 1931
TS4578	East coast	-33.28556667	27.90989333	_	Agulhas	*Penares*	cf. *orthotriaena*	Old species	Burton, 1931
TS4772	Deep Secrets	-34.93321833	18.22474667	_	Agulhas	*Penares*	cf. *orthotriaena*	Old species	Burton, 1931
TS4510	East coast	-32.939515	28.26853333	_	Agulhas	*Penares*	sp. 1	New species	To be described

TS4261	East coast	-32.68155667	28.45845667	_	Agulhas	*Penares*	sp. 1	New species	To be described
TS4518	East coast	-32.939515	28.26853333	_	Agulhas	*Penares*	sp. 1	New species	To be described
TS4571	East coast	-33.28556667	27.90989333	_	Agulhas	*Penares*	sp. 1	New species	To be described
TS4595	East coast	-33.28556667	27.90989333	_	Agulhas	*Penares*	sp. 1	New species	To be described
TS4391	East coast	-32.75408	28.45525333	_	Agulhas	*Penares*	sp. 2	New species	To be described
TS4583	East coast	-33.28556667	27.90989333	_	Agulhas	*Penares*	sp. 3	New species	To be described
TS4217	South Coast	-36.03566667	21.35583333	_	Agulhas	*Penares*	*sphaera*	Old species	(Lendenfeld, 1907)
TS4685	East coast	-33.39101	27.53744	_	Agulhas	*Higginsia*	sp. 1	New species	To be described
TS4684	East coast	-33.39101	27.53744	_	Agulhas	*Higginsia*	sp. 1	New species	To be described
TS4652	East coast	-33.16318167	27.77462333	_	Agulhas	*Higginsia*	sp. 2	New species	To be described
TS3966	Amathole; Eastern Cape	-33.159735	27.77769167	_	Agulhas	*Higginsia*	sp. 3	New species	To be described
TS4075	East coast	-32.83003667	28.45927	_	Agulhas	*Higginsia*	sp. 4	New species	To be described
TS4511	East coast	-32.939515	28.26853333	_	Agulhas	*Higginsia*	sp. 5	New species	To be described
TS3517	East coast	-32.83003667	28.45927	_	Agulhas	*Higginsia*	cf. sp. 6	New species	To be described
TS3527	East coast	-32.83003667	28.45927	_	Agulhas	*Higginsia*	cf. sp. 6	New species	To be described
TS3530	East coast	-32.83003667	28.45927	_	Agulhas	*Higginsia*	cf. sp. 6	New species	To be described
TS4481	East coast	-32.939515	28.26853333	_	Agulhas	*Higginsia*	sp. 6	New species	To be described
TS4205	South	-34.67116667	21.366667	_	Agulhas	*Higginsia*	sp. 7	New	To be

	Coast							species	described
TS4630	East coast	-33.15962833	27.97420833	_	Agulhas	*Higginsia*	sp. 8	New species	To be described
TS2837	East coast	_	_	_	Agulhas	*Higginsia*	*sp*	New species	To be described
TS2820	East coast	_	_	_	Agulhas	*Higginsia*	*sp*	New species	To be described
TS2749	East coast	-31.98533333	29.15166667	_	Agulhas	*Aaptos*	*alphiensis*	old species	_
TS4440	East coast	-32.703535	28.43105167	_	Agulhas	Suberitidae	sp.	New species	To be described
TS3403	South Coast	-	-	_	Agulhas	*Aaptos*	*alphiensis*	old species	_
TS3922	East coast	-32.98828333	28.32851333	_	Agulhas	Microcionidae	sp. 1	New species	To be described
TS3920	East coast	-32.98828333	28.32851333	_	Agulhas	Microcionidae	sp. 1	New species	To be described
TS4306	East coast	-32.65112667	28.46087833	_	Agulhas	Microcionidae	sp. 2	New species	To be described
TS4315	East coast	-32.65112667	28.46087833	_	Agulhas	Microcionidae	sp. 3	New species	To be described
TS4409	East coast	-32.75408	28.45525333	_	Agulhas	Microcionidae	sp. 4	New species	To be described
TS4810	Deep Secrets	-35.11979833	23.04497667	_	Agulhas	*Clathria*	sp. 1	New species	To be described
TS4156	Cape Canyon; West Coast	-32.9411	17.51875	_	Benguela	*Clathria*	sp. 1	New species	To be described
TS4475	East coast	-32.939515	28.26853333	_	Agulhas	*Clathria*	sp. 2	New species	To be described
TS4445	East coast	-32.939515	28.26853333	_	Agulhas	*Clathria*	sp. 2	New species	To be described

TS3305	South Coast	_	_	_	Agulhas	*Clathria*	sp. 3	New species	To be described
TS2677	South Coast	_	_	_	Agulhas	*Clathria*	*Sp. 4*	New species	To be described
TS3405	South Coast	_	_	_	Agulhas	*Clathria*	*Sp. 3*	New species	To be described
TS3402	South Coast	_	_	_	Agulhas	*Clathria*	sp. 5	New species	To be described
TS4489	East coast	-32.939515	28.26853333	_	Agulhas	*Antho (Acarnia)*	cf. *prima*	old species	(Brøndsted, 1924)
TS4198	South Coast	-38.80283333	21.34033333	_	Agulhas	*Antho (Acarnia)*	cf. *prima*	Old species	(Brøndsted, 1924)
TS4202	South Coast	-34.67116667	21.366667	_	Agulhas	*Antho (Acarnia)*	cf. *prima*	Old species	(Brøndsted, 1924)
TS4200	South Coast	-34.67116667	21.366667	_	Agulhas	*Antho*	sp. 1	New species	To be described
TS4197	South Coast	-38.80283333	21.34033333	_	Agulhas	*Antho*	sp. 1	New species	To be described
TS4811	Deep Secrets	-35.11979833	23.04497667	_	Agulhas	*Clathria*	sp. 1	New species	To be described
TS2824	Grootbank, Plett	_	_	_	Agulhas	*Cliona*	sp. nov.	New species	To be described
TS2941	West coast	17.60281667	-30.91543333	_	Benguela	*Cliona*	sp. nov.	New species	To be described
TS4212	South Coast	-35.67883333	22.02416667	_	Agulhas	Phloeodictyidae	sp.	New species	To be described
TS3604	East coast	-32.98308	28.31967333	_	Agulhas	*Callipelta*	sp. 1	New species	To be described
TS4121	East coast	-32.73208167	28.51123667	_	Agulhas	*Callipelta*	sp. 2	New species	To be described
TS4021	Amathole; Eastern	-33.01367333	28.31424667	_	Agulhas	*Callipelta*	sp. 2	New species	To be described

	Cape								
TS4812	Deep Secrets	-35.11979833	23.04497667	_	Agulhas	*Erylus*	cf. *globulifer*	Old species	Pulitzer-Finali, 1993
TS4830	Deep Secrets	-34.78472667	24.76175167	_	Agulhas	*Erylus*	cf. *globulifer*	Old species	Pulitzer-Finali, 1993
TS4002	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	*Erylus*	sp. 1	New species	To be described
TS4004	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	*Erylus*	sp. 1	New species	To be described
TS4005	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	*Erylus*	sp. 1	New species	To be described
TS4006	Amathole; Eastern Cape _	-33.01367333	28.31424667	_	Agulhas	*Erylus*	sp. 1	New species	To be described
TS4833	Deep Secrets	-34.78472667	24.76175167	_	Agulhas	*Macandrewia*	sp. 1	New species	To be described
TS4834	Deep Secrets	-34.78472667	24.76175167	_	Agulhas	*Macandrewia*	cf. sp. 1	New species	To be described
TS4858	Haga Haga; Eastern Cape	-32.76407028	28.25149806	_	Agulhas	*Tedania (Tedania)*	*tubulifera*	Old species	_
TS4852	Dwessa (Mbanjana); Eastern Cape	-32.230935	28.9277075	_	Agulhas	*Tedania (Tedania)*	*tubulifera*	Old species	_
TS4854	Dwessa (Mbanjana); Eastern Cape	-32.230935	28.9277075	_	Agulhas	*Tedania (Tedania)*	*tubulifera*	Old species	_

TS2757	East coast	-31.98533333	29.15166667	_	Agulhas	*Tedania (Tedania)*	*tubulifera*	Old species	_
TS2746	East coast	-31.65083333	29.51733333	_	Agulhas	*Tedania (Tedania)*	*tubulifera*	Old spcies	_
TS2761	East coast	-31.98533333	29.15166667	_	Agulhas	*Tedania (Tedania)*	*tubulifera*	Old species	_
TS4074	East coast	-32.83003667	28.45927	_	Agulhas	*Tedania (Tedaniopsis)*	sp.	New species	To be described
TS2818	Cape St. Francis	_	_	_	Agulhas	*Tedania (Tedaniopsis)*	sp.	New species	To be described
TS4483	East coast	-32.939515	28.26853333	_	Agulhas	*Tedania (Tedaniopsis)*	sp.	New species	To be described
TS4216	South Coast	-36.03566667	21.35583333	_	Agulhas	*Tedania (Tedania)*	sp.	New species	To be described
TS4090	East coast	-32.83003667	28.45927	_	Agulhas	*Poecillastra*	sp. 1	New species	To be described
TS4082	East coast	-32.83003667	28.45927	_	Agulhas	*Poecillastra*	sp. 1	New species	To be described
TS4085	East coast	-32.83003667	28.45927	_	Agulhas	*Poecillastra*	sp. 1	New species	To be described
TS4533	East coast	-32.94846167	28.057705	_	Agulhas	*Poecillastra*	sp. 1	New species	To be described
TS4433	East coast	-32.84186667	28.51148333	_	Agulhas	*Poecillastra*	sp. 2	New species	To be described
TS4423	East coast	-32.839185	28.51261833	_	Agulhas	*Poecillastra*	sp. 3	New species	To be described
TS4080	East coast	-32.83003667	28.45927	_	Agulhas	*Poecillastra*	sp. 3	New species	To be described
TS4775	Deep Secrets_ West coas	-36.04155	19.69017	_	Benguela	*Poecillastra*	sp. 3	New species	To be described
TS4787	Deep Secrets(West coast)	-36.04155	19.69017	_	Benguela	*Poecillastra*	sp. 3	New species	To be described

TS4780	Deep Secrets (West coast)	-36.04155	19.69017	_	Benguela	*Poecillastra*	sp. 3	New species	To be described
TS4795	Deep Secrets (West coast)	-36.04155	19.69017	_	Benguela	*Poecillastra*	sp. 3	New species	To be described
TS4799	Deep Secrets (West coast)	-36.04155	19.69017	_	Benguela	*Poecillastra*	sp. 3	New species	To be described
TS4248	East coast	-32.68155667	28.45845667	_	Agulhas	Geodia	Sp. 1	New species	To be described
TS4681	East coast	-33.39101	27.53744	_	Agulhas	*Geodia*	Sp. 2	New species	To be described
TS4688	East coast	-33.39101	27.53744	_	Agulhas	*Geodia*	Sp. 2	New species	To be described
TS4702	East coast	-33.39101	27.53744	_	Agulhas	*Geodia*	Sp. 2	New species	To be described
TS4703	East coast	-33.304265	27.86738	_	Agulhas	*Geodia*	Sp. 3	New species	To be described
TS4303	East coast	-32.65112667	28.46087833	_	Agulhas	*Geodia*	Sp. 4	New species	To be described
TS4825	Deep Secrets	-34.88942667	24.11568667	_	Agulhas	*Geodia*	cf. sp.2	New species	To be described
TS3906	East coast	-32.834525	28.46817833	_	Agulhas	Phymaraphiniidae	Sp. 1	New species	To be described
TS4400	East coast	-32.75408	28.45525333	_	Agulhas	Phymaraphiniidae	Sp. 2	New species	To be described
TS4007	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	Phymaraphiniidae	Sp. 3	New species	To be described
TS4053	East coast	-33.01367333	28.31424667	_	Agulhas	Phymaraphiniidae	cf.sp. 3	New species	To be described
TS4436	East coast	-32.703535	28.43105167	_	Agulhas	*Homaxinella*	Sp.	New species	To be described
TS4563	East coast	-33.28556667	27.90989333	_	Agulhas	*Homaxinella*	Sp.	New species	To be described

TS4601	East coast	-33.28556667	27.90989333	_	Agulhas	*Homaxinella*	Sp.	New species	To be described
TS4663	East coast	-33.16318167	27.77462333	_	Agulhas	*Homaxinella*	Sp.	New species	To be described
TS4285	East coast	-32.66633333	28.44124833	_	Agulhas	*Homaxinella*	Sp.	New species	To be described
TS4419	East coast	-32.66633333	28.44124833	_	Agulhas	*Homaxinella*	Sp.2	New species	To be described
TS4677	East coast	-33.16318167	27.77462333	_	Agulhas	*Didiscus*	cf. placospongioides	Old species	_
TS4220	South Coast	-36.2665	21.5355	_	Agulhas	*Fibulia*	ramosa	Old species	_
TS4233	South Coast	-36.2665	21.5355	_	Agulhas	*Fibulia*	Cf. ramosa	Old species	_
TS4141	Amathole; Eastern Cape	-32.75433	28.41878	_		*Rhabdastrella*	sp.1	New species	To be described
TS4842	Dwessa (Cottages); Eastern Cape	-32.31082389	28.82852333	_	Agulhas	*Rhabdastrella*	sp.2	New species	To be described
TS4857	Haga Haga; Eastern Cape	-32.76407028	28.25149806	_	Agulhas	*Rhabdastrella*	sp.2	New species	To be described
TS4676	East coast	-33.16318167	27.77462333	_	Agulhas	*Tetilla*	sp.1	New species	To be described
TS4679	East coast	-33.16318167	27.77462333	_	Agulhas	*Tetilla*	sp.1	New species	To be described
TS4687	East coast	-33.39101	27.53744	_	Agulhas	*Tetilla*	sp.1	New species	To be described
TS4196	South Coast	-38.80283333	21.34033333	_	Agulhas	*Tetilla*	*casula*	New species	To be described
TS4337	East coast	_	_	_	Agulhas	Crambeidae	Sp.	New species	To be described

TS4001	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	Corallistidae	sp.1	New species	To be described
TS4014	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	Corallistidae	sp.1	New species	To be described
TS4238	East coast	-32.68155667	28.45845667	_	Agulhas	Petrosiidae	sp.2	New species	To be described
TS3988	Amathole; Eastern Cape	-33.159735	27.77769167	_	Agulhas	*Pachastrella*	sp.1	New species	To be described
TS4249	East coast	-32.68155667	28.45845667	_	Agulhas	*Axinella*	sp.1	New species	To be described
TS3820	East coast	-32.82361833	28.52109833	_	Agulhas	*Bubarida*	sp.1	New species	To be described
TS4401	East coast	-32.75408	28.45525333	_	Agulhas	*Bubarida*	sp.1	New species	To be described
TS3606	East coast	-32.98308	28.31967333	_	Agulhas	*Stelleta*	sp.1	New species	To be described
TS4487	East coast	-32.939515	28.26853333	_	Agulhas	*Stelleta*	sp.1	New species	To be described
TS4495	East coast	-32.939515	28.26853333	_	Agulhas	*Stelleta*	sp.1	New species	To be described
TS4479	East coast	-32.939515	28.26853333	_	Agulhas	*Stelleta*	sp.2	New species	To be described
TS4199	South Coast	-38.80283333	21.34033333	_	Agulhas	*Stelleta*	*cf. agulhana*	Old species	To be described
TS4757	Amathole; Eastern Cape	-32.96286167	28.31934833	_	Agulhas	*Theonella*	Sp. 1	New species	To be described
TS4758	Amathole; Eastern Cape	-32.96286167	28.31934833	_	Agulhas	*Theonella*	Sp. 1	New species	To be described
TS3609	Amathole;	-32.96286167	28.3193483	_	Agulhas	*Theonella*	Sp. 1	New	To be

	Eastern Cape		3					species	described
TS4800	Deep Secrets	-36.04155	19.69017	_	Benguela	*Echinostylinos*	Sp.	New species	To be described
TS4798	Deep Secrets	-36.04155	19.69017	_	Benguela	*Tetillidae*	sp.2	New species	To be described
TS4474	East coast	-32.939515	28.26853333	_	Agulhas	*Tetillidae*	sp.1	New species	To be described
TS3823	East coast	-32.82361833	28.52109833	_	Agulhas	*Fangophilina*	*cf. gilchtiris*	Old species	To be described
TS3721				_		*Axinellidae*	sp.	New species	To be described
TS4432	East coast	-32.84186667	28.51148333	_	Agulhas	*Hexasterophora*	sp.	New species	To be described
TS4018	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	*Lithoplocamia*	sp.1	New species	To be described
TS4599	East coast	-33.28556667	27.90989333	_	Agulhas	Scopalinidae	sp.1	New species	To be described
TS4410	East coast	-32.75408	28.45525333	_	Agulhas	Scopalinidae	sp.1	New species	To be described
TS4712	East coast	-33.20203833	27.85864667	_	Agulhas	Scopalinidae	sp.1	New species	To be described
TS4008	Amathole; Eastern Cape	-33.01367333	28.31424667	_	Agulhas	Scopalinidae	sp.2	New species	To be described
TS4468	East coast	-32.939515	28.26853333	_	Agulhas	Scopalinidae	sp.2	New species	To be described
TS4340	East coast	_	_	_	Agulhas	*Isodictya*	sp.	New species	To be described
TS4773	Deep Secrets	-36.04155	19.69017	_	Agulhas	*Myxilla (Ectyomyxilla)*	sp.	New species	To be described
TS4783	Deep Secrets	-36.04155	19.69017	_	Agulhas	*Myxilla (Ectyomyxilla)*	sp.	New species	To be described

TS4387				_	Agulhas	*Phorbas*	sp.	New species	To be described
TS4211	South Coast	-35.67883333	22.02416667	_	Agulhas	*Ectyonopsis*	*pluridentata*	Old species	_
TS4215	South Coast	-35.90483333	20.118333	_	Agulhas	*Ectyonopsis*	*pluridentata*	Old species	_
TS4824	Deep Secrets	-34.88942667	24.11568667	_	Agulhas	*Ectyonopsis*	*pluridentata*	Old species	_
TS4579	East coast	-32.82361833	28.52109833	_	Agulhas	*Fangophilina*	*cf. gilchristi*	Old species	_
TS4079	East coast	_	_		Agulhas	*Lophon*	sp.1	New species	To be described
TS2936	West coast	-30.94685	17.63510000	_	Benguela	*Isodictya*	*ectofibrosa*	Old species	_
TS4154	Cape Canyon; West Coast	-32.85645	17.53886667	_	Benguela	*Sphaerotylus*	*cf. strobilis*	Old species	_