The marine aquarium trade in South Africa:

A vector for alien invasive species?

by

Gitte Kirsten Wehr

Submitted in fulfilment of the academic

requirements for the degree of

Master of Science

in Biology

School of Life Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Westville

South Africa

June 2019

ABSTRACT

Biological invasions are increasingly recognized as a primary threat to biodiversity. Global transport and trade play an important role in the movement of alien species around the world, and as transport and trade have intensified over the decades, so too has the number of alien species introductions. As preventing the introduction of harmful species is a more cost-effective and efficient method to managing biological invasions, it is imperative that scientific studies are aimed at identifying the pathways responsible for introductions. The marine aquarium trade is an ever-growing business globally and only up until recently has been identified as a major pathway for the introduction of alien species. Except for the notorious cases of the invasive lionfish, *Pterois volitans*, and seaweed, *Caulerpa taxifolia*, the role of the aquarium trade towards the introduction of alien marine organisms has been largely unevaluated. With popularity rising for the marine aquarium hobby in South Africa, it is of concern that the trade remains predominantly unregulated. This study aims to investigate the risk posed by the marine aquarium trade as a pathway for the introduction of alien invasive species in South Africa. One such vector examined in this study is live rock: any type of rock or dead coral skeleton encrusted with, and containing within its crevices, a wide variety of marine organisms, including colourful sessile invertebrates and encrusting algae. A combination of morphological and DNA barcoding molecular identifications, based on the phylogenetic inference of mitochondrial cytochrome *c* oxidase subunit 1 (COI) and internal transcribed spacer region (ITS) sequence data, was used to assign identifications to 174 taxa harvested from imported Indonesian and Kenyan live rock. Of the 6 diverse phyla identified, one alien species was flagged as harmful due to its successful invasion of other ecosystems in the world: the glass sea anemone *Aiptasia pulchella*. Non-target DNA amplification of live rock associated taxa exposed the coral pathogenic bacteria *Vibrio* spp., further suggesting that the marine aquarium trade is also a pathway for the introduction of pathogens. A second component of the study involved the assessment, via formal survey, of the role that pet stores and hobbyists play in the introduction and transmission of marine organisms around South Africa. Irresponsible aquarium pest disposal methods and informal trading pathways were revealed highlighting the need for an education intervention to promote responsible aquarium ownership skills. The third part of the study entailed a national stock inventory of marine aquarium traded fish and revealed that the number of species traded is vast (n = 228) and that 60 % are alien to South African waters. Although the strength of this vector was not defined, this study confirms that the marine aquarium trade is a pathway for the introduction of alien and potentially invasive organisms and serves as the foundation for future research into marine aquarium trade vectors in South Africa. The findings and conclusions presented here should be considered by biosecurity monitoring and management initiatives.

Keywords: alien invasive species; DNA barcoding; live rock; marine aquarium trade; vector.

PREFACE

The research contained in this thesis was completed by the candidate (January 2015 - December 2015 and January 2017 - June 2019) while based at the School of Life Sciences at the University of KwaZulu-Natal, Westville Campus, South Africa. The research was financially supported by the South African National Biodiversity Institute (SANBI).

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

As the candidate's supervisor, I agree with the above, and have approved this dissertation for submission:

Signed: [redacted]

Dr Angus H.H. MacDonald, Supervisor; 27 June 2019

Signed: [redacted]

Dr Kerry Sink, Co-Supervisor; June 2019

DECLARATION – PLAGIARISM

I, Gitte Kirsten Wehr, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

    a. Their words have been re-written but the general information attributed to them has been referenced

    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

[redacted]

Signed: ___________________________________

Gitte Kirsten Wehr; 26 June 2019

DECLARATION – RESEARCH OUTPUTS

Oral presentation:

Wehr, GK; Macdonald, AHH; Sink, K; Porter, S (2017). Alien invasive risk assessment of the marine aquarium trade in South Africa. The 7th International Barcode of Life conference, Skukuza, Kurger National Park, South Africa. 20 – 24 November 2017.

[redacted]

Signed: ___________________________________

Gitte Kirsten Wehr; 26 June 2019

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

First and foremost, I wish to thank Dr Angus Macdonald for your patience and understanding as you helped me persevere through the many set-backs I have faced over the past years. Thank you for believing in me and for not letting me give up, and for the final boost of confidence in my abilities that got me over the last hurdle.

I would like to take this opportunity to express my gratitude to Dr Kerry Sink for securing the funding for this research through the South African National Biodiversity Institute (SANBI). I will always cherish the memories from our Stellenbosch tour with Dr Phil Alderslade after the Soft Coral Taxonomy Workshop. Thank you to my SANBI project leader, Ms. Prideel Majiedt – your patience through my many ups and downs is much appreciated. Finally, many thanks go to Dr Sean Porter for the ingenious experimental design and concept.

With much appreciation to the South African National Biodiversity Institute (SANBI) for my personal funding.

Thank you to the members of the Marine Evolution Laboratory (MAREVOL). Kerry Etsebeth - for always checking in on me over the years and especially for caring for me after my injury at the IBOL conference. Ruven Pillay - you made the long nights of lab work that extended over the weekends a lot more bearable!

Thank you to Mr Roy Jackson for your physical and technical assistance with setting up the aquarium holding tanks for my live rock.

With much gratitude owed to Dr Jyothi Kara of the Carol-Simon Polychaete Laboratory for the many days you sacrificed to teach me the art of polychaete taxonomy. Your passion is contagious and I have a newly-found admiration for them! Also, thank you to the following taxonomy experts: Dr Reza Naderloo (the University of Tehran) and Dr Nasreen Peer (Nelson Mandela Metropolitan University) for assigning identifications to the two crab specimens; Dr Usha V. Parameswaran (Centre for Marine Living Resources and Ecology, Cochin, India) who assisted in the morphological identification of the brittle stars and starfish through ResearchGate collaboration; and to Dr Phil Alderslade, thank you for teaching me the wonders of soft coral taxonomy.

My dear cousin, Dr Nina Hunter: thank you for your ongoing spiritual support and upliftment over the years. It has been wonderful to have a family member that I can relate to who is also in the postgraduate research field - with the added bonus that you joined the same department towards the end of my degree! Also, I am very grateful for your editing assistance - you are the only one I trust when it comes to perfection! I will forever be indebted to you for the time you sacrificed during your busy schedule.

To my schatzi, Thomas Hikins: I hope to be at least half as good of a support to you when in need as you were to me over these past two years. I look forward to turning over a new leaf and starting afresh with you by my side.

Most importantly, I would like to express my sincere gratitude to my family: Erika Wehr, Alfred Wehr, and Lisa Wehr. The obstacles we faced as a family over the past four and a half years weren't easy, but we have all come out stronger. Thank you all for your love and support during the good times, as well as the bad. Mom, you carried the weight of this work and its many challenges as if it were your own. Thank you for your enduring support, encouragement, and care through the many times I doubted myself and couldn't physically carry on. I will be forever grateful. Lili, thank you for your many gentle words of wisdom and for being my number one cheerleader!

Finally, with much gratitude and appreciation to the examiners and reviewers of this dissertation.

CHAPTER 1: INTRODUCTION

Biological invasions of the marine realm have become more frequent in recent decades (Pagenkopp Lohan et al., 2015; Chan and Briski, 2017; Mantelatto et al., 2018; Ellul et al., 2019). Rapid globalisation and increasing trends in transport, trade, and travel have resulted in increased introductions of alien species (Katsanevakis and Crocetta, 2014). Invasions of alien species can be economically costly and ecologically destructive, and are considered a major driver of and primary threat to global environmental change (Simberloff et al. 2013; Anton et al., 2019). The pervasive, devastating, and irreversible impacts of invasive species on marine ecosystems are well recognized and documented (Brunel et al., 2013; Katsanevakis et al., 2014; Barreiros, 2017; Anton et al., 2019). Regardless of the fact that extensive studies have been conducted to predict and gain a better understanding of biological invasions, there is some inconsistency in the amount of research devoted across ecosystems in ecological studies, with the majority focusing on terrestrial rather than aquatic ecosystems (Thomaz et al., 2014; Chan and Briski, 2017).

The aquarium trade has been identified as a pathway for the dispersal of alien invasive species and the need for further research and management actions have been emphasized (Mendoza et al., 2010; Patoka et al., 2018; Vranken et al., 2018). The fish-keeping hobby is a multi-billion dollar industry and its popularity has resulted in a steady trade expansion in more than 125 countries (Dey, 2016). The trade in marine aquarium fish, invertebrates, and corals has led to the introduction of several alien invasive species, both intentionally with the release of unwanted organisms (Holmberg et al., 2015), and unintentionally in the case of non-target organisms that can be unintentionally introduced to aquariums (Wabnitz et al., 2003; Calado and Narciso, 2005). Live rock (i.e. rock or dead coral skeletons that support a variety of organisms) is a potentially important vector for the introduction of non-target organisms, referred to as 'live rock associated taxa' hereafter. Marine aquarium hobbyists refer to these taxa associated with live rock as 'hitch-hikers'. This trade is responsible for the introduction of some of the highest profile marine invaders such as *Caulerpa taxifolia*, a green alga in the Mediterranean Sea, and the Indo-Pacific lionfish, *Pterois volitans* and *Pterois miles*, in the Caribbean and on the east coast of North America (Minchin et al., 2005; Mantelatto et al., 2018).

Although the knowledge of biological invasions in South Africa has increased significantly over the last two decades (Clusella-Trullas and Garcia, 2017), there has been very little focus on introduction pathways and vectors, with most scientific research aimed at the establishment and spread of alien marine species (Mead et al., 2011; Alexander et al., 2016). For an alien species to become invasive, it must be transported and introduced to a novel location. These are debatably the most important steps on which to prioritize management initiatives, however, this importance is not reflected in the resulting marine bioinvasion research (Alexander et al., 2016). The completeness and accuracy of introduction vectors, spread, and impact of marine alien fish, invertebrates and plants in South Africa has either not been assessed, or is associated with low confidence (van Wilgen et al., 2018). While the number of introductions of alien species through the marine aquarium trade is considerably lower than introductions through shipping and aquaculture (Zenetos et al., 2012), the trade and hobby has seen rapid growth in recent decades and therefore should be noted as a serious pathway with destructive consequences (Padilla and Williams, 2004; Williams et al., 2015). In a country where alien invasive species are considered a significant threat to native biodiversity and ecosystem function (Griffiths et al., 2010; Kimberg et al., 2014; Alexander et al., 2016; Egoh et al., 2020), it is imperative that scientific studies are aimed at quantifying the risk of the marine aquarium trade as a pathway for the introduction of alien invasive species into South Africa.

Despite the high diversity and volume of marine organisms traded globally, there are few regulations and laws enforced for the monitoring and control thereof (Ojaveer et al., 2018). Overall, there is a lack of knowledge regarding species, and their respective quantities, associated with the marine aquarium trade in South Africa. Legislation does not require pet shop owners to keep record of species imported, making it impossible to gain insight into the species traded (van der Walt et al., 2017). Furthermore, there are no existing quarantine or inspection points in place, leaving it the sole responsibility of the importer to treat disease and discard the water used to transport fish and other marine organisms (Mouton et al., 2001). As a signatory of the Convention on Biological Diversity, South Africa is obliged to identify, prioritise, and control alien invasive species and their various vectors of introduction and dispersal (Marr et al., 2017). It is important to bear in mind that focusing research and control strategies on preventing the introduction of high-risk alien taxa is more cost-effective than dealing with established and destructive invasions (Simberloff et al. 2013; Ojaveer et al., 2015; Faulkner et al., 2016).

1.1 Aims and objectives

Against this background, the thesis of this study is to determine whether the marine aquarium trade is a pathway for the introduction of alien invasive species in South Africa. The aims of this study are threefold:

(1) to determine if live rock is a vector for marine alien invasive species into South Africa;
(2) to determine the role that pet stores and hobbyists play in the introduction, movement, and trade of marine fish, invertebrates, and live rock around South Africa and to evaluate their current awareness and knowledge of marine aquarium pest species; and
(3) to determine the identities and quantities of marine fish traded in South Africa.

Respectively, this will be achieved by:

(1) harvesting marine invertebrates from imported Kenyan and Indonesian live rock and, thereafter, utilizing morphological identifications combined with molecular identifications inferred from the phylogenetic reconstructions of mitochondrial (COI) and nuclear (ITS) sequence data;
(2) distributing a questionnaire to marine pet stores and marine aquarium hobbyists in South Africa which assesses their hobby habits and basic current knowledge of marine aquarium pest species South Africa; and
(3) compiling an inventory of all marine fish sold from major pet stores in Durban, Cape Town, and Pretoria/Johannesburg by photographic identification.

1.2 Limitations

This study did not identify and quantify macroalgae associated with live rock as additional DNA extraction kits, barcoding primers, and further sequencing would be required and the study budget was limited. Macroalgae, corals, and other marine invertebrates were not identified and quantified during the assessment of marine pet store stock inventories as characteristics used to assign species-level identifications cannot be accurately inferred from photographs alone.

1.3 Arrangement of dissertation

This dissertation consists of six chapters: an introductory chapter (Chapter 1); a detailed literature review (Chapter 2); three individual studies which present the identification assessment of taxa associated with live rock (Chapter 3), the marine aquarium hobbyist survey (Chapter 4), and the pet store stock inventories (Chapter 5); and a concluding chapter (Chapter 6). The methods and materials, results, and discussion are presented within each study.

CHAPTER 2: LITERATURE REVIEW

Every day, every hour, tens of thousands of unnoticed species are introduced into new environments (Hulme, 2009; Ricciardi, 2016). Coinciding with a more globalized world, increasing trends of travel, trade, and transport have accelerated marine invasions by increasing the rate at which new introductions occur into previously inaccessible habitats (Katsanevakis et al., 2014). Both intentional and unintentional human-mediated physical conveyances of species is the sole reason for biological invasions (Minchin et al., 2005; Webber and Scott, 2011; Lins et al., 2018).

Biological invasions are one of the most pervasive, irreversible, and devastating impacts of human activity on biodiversity, natural ecosystems, and natural resources (Anton et al., 2019). According to the International Union for the Conservation of Nature (IUCN) (IUCN, 2000), such invasions may be as damaging to native species and ecosystems on a global scale as the loss and degradation of habitat. The spread of non-native species, together with the extinction of rare species, is homogenizing the global biota, in other words, causing ecosystems worldwide to become more similar (Vander Zanden, 2005).

Even though only a small proportion of introduced alien species become invasive in the recipient habitat, their impacts can be disastrous (Chan and Briski, 2017) and can lead to a number of ecological, social, and economic consequences (Holland, 2000). In marine ecosystems, alien invasive species may result in declines or even extinctions of native species, cause the loss of native genotypes, enhance the transmission of pathogens, disrupt food web properties and ecosystem functions, alter community structure, affect human health, and cause extensive destruction to ecosystem services and natural resources (Molnar et al., 2008; Katsanevakis et al., 2014; Early et al., 2016; Chan and Briski, 2017).

Due to the increased prevalence of introductions of alien invasive species into coastal waters, a considerable amount of research has been undertaken into both the mechanisms of anthropogenic dispersal of marine organisms, and the economic and ecological impacts of the resulting invasions (Johnston et al., 2017; Bourne et al., 2018; Robertson et al., 2018; Anton et al., 2019). The overwhelming majority of this research, however, corresponds to the United States of America, Australia, New Zealand, Canada, and European countries (Dias et al., 2017), in comparison to the limited data published on marine invasions in other areas, particularly

Africa (Alexander et al., 2016). Ultimately, a better understanding of how invasive species are able to survive and thrive in novel environments needs to be achieved in order to predict the spread and persistence of biological invasions (Tepolt, 2015).

2.1 Definitions

As early as six decades ago, Charles Elton described invasive species as "the mingling of thousands of kinds of organisms from different parts of the world that set up terrific dislocations in nature" and refers to biological invasions as "ecological explosions" (Elton, 1958). The terms native, indigenous, alien, exotic, or introduced, are used interchangeably throughout the literature (Colautti and MacIsaac, 2004; Webber and Scott, 2011; Mineur et al., 2012) and has led to much confusion defining the nature of biological invasions, and in turn has hindered progress in invasion biology (Robinson et al., 2016). The inconsistent use of terminology can also hinder the flow of vital information from researchers to managers and policy-makers, who rely on scientific literature that has been consistently and clearly articulated to further inform management initiatives and regulatory frameworks (Robinson et al., 2016).

For the purpose of this review, 'species that are not indigenous or indigenous species that are translocated or are intended to be translocated outside their natural distribution range in nature' will be referred to as an alien species (Robinson et al., 2016), and those specifically related to the marine environment will be referred to as marine alien species. Traditionally, alien species that impose a negative impact on a native species, the environment, or economy, are termed invasive (IUCN 2000). A unified framework for definitons in invasion biology proposed by Blackburn et al. (2011) deems this as outdated and inappropriate as it does not consider 'spread beyond the point of introduction' as a defining characteristic. This led to the re-definition of an invasive species as a 'species whose establishment and spread outside of their natural distribution range, threaten ecosystems, habitats or other species or have a demonstrable potential to threaten ecosystems, habitats or other species, or may result in economic or environmental harm or harm to human health' (Robinson et al., 2016).

2.2 The invasion process

To establish successfully, an introduced alien species has to pass through a number of different barriers in the receiving environment, such as: geographic, environmental, reproductive, and dispersal barriers (Theoharides et al., 2007; Acharya, 2014). Even if the organism enters into similar abiotic conditions as in its native environment, it may still have to compete with a variety of new biotic conditions (Wong and Candolin, 2015). Only a small proportion of introduced alien species are able to overcome those barriers and establish themselves in the recipient environment, as can the proportion of established species that spread and successfully invade (Blackburn et al., 2011; Chan and Briski, 2017). These proportions are dependent on the species in question, and the environment to which they are introduced (Keller et al., 2011).

Williamson and Fitter (1996) proposed the 'tens rule' as a rule of thumb which suggests that one in ten species of those imported will escape and appear in the wild, one in ten of those escapees will become established, and one in ten of those established will become successful invaders (Keller et al., 2011; Acharya, 2014). Theoretically, this means that one in every one thousand species introduced to a new area outside of its native range will have an economical or ecological impact on the native biota, and thereby be defined as an invasive species (Klinck, 2009).

The invasion process is described by three major stages: introduction, establishment, and invasion (Theoharides et al., 2007) with different barriers impacting on each (Acharya, 2014). First, propagules must be transported beyond their native range and released from the vector (a human-mediated physical conveyance from one geographical region to another) (Blackburn et al., 2011). The combination of these two processes is regarded as the species' introduction. The process of establishment requires that the species must survive and reproduce, producing viable offspring in the new range until it achieves a self-sustaining population. Before further expansion of the population, the introduced species' passes through a lag phase where it may go undetected for many years until an invasive behaviour is observed (Sakai et al., 2001). This phase may correspond to a lack of genetic diversity and is generally considered a period where the species undergoes adaptive evolution. Gene flow and migration between populations in the invaded region, together with further introductions of propagules from source populations, may shorten the lag phase time, allowing the adaptive evolution period to take place more rapidly (Kawecki and Ebert, 2004; Klinck, 2009; Acharya, 2014).

Once established, the species may extend its distribution in the new habitat, by expanding the existing population, and further dispersal and establishment of new populations. This is recognized as the species' spread, or invasion (Gravuer, 2004). A fourth stage is defined by some authors, termed integration (Vermeij, 1996) or impact (Kolar and Lodge, 2002). In this stage, the species becomes more fully incorporated into the recipient regions' local food webs and ecosystem processes, which often has negative consequences for native occurring species and ecosystem conditions and processes (Gravuer, 2004).

2.3 Characteristics of marine invasive species

Understanding how invasive species are able to survive and thrive in a novel habitat is one of the fundamental questions investigated in invasion ecology (Tepolt, 2015; Geburzi and McCarthy, 2018). This is crucial to predicting the spread and persistence of biological invasions, and thus is vital for the development of effective conservation and management strategies (Tepolt, 2015; Sherman et al., 2016). Over five decades of research have been devoted to addressing this question, and have shown that a number of biological characteristics and other life-history traits promote the success of an invasive species (Kelley, 2014; Whitney and Gering, 2014).

2.3.1 Biological life-history characteristics

Studies have demonstrated that alien species that are most likely to establish are those introduced in the highest numbers and most often (McMahon, 2002). Aquatic invasive species tend to have a higher abundance and reproduce more frequently (higher propagule pressure), larger body size and longer life span, shorter generation times, a variety of reproduction strategies which enable colonization by a single organism, feeding habits that allow the exploitation of food resources more efficiently, and are more dominant in their communities (Sakai et al., 2001; Grabowski et al., 2007; Statzner et al., 2007; Leuven et al., 2009). Species that are able to tolerate broader abiotic conditions are more likely to have a chance of surviving transport, establishing in new environments, and expanding beyond the area into which they were introduced (Bates et al., 2013; Chan and Briski, 2017). The local conditions, such as predators, food availability, and diversity and level of perturbation of the local community will also have an impact on the establishment of introduced alien species (Neves and da Rocha, 2008). In addition to this, the breadth of ecological niches and similarity of environmental conditions in the donor and the receiving region can also be important (Keller et al., 2011). All in all, the impact of an alien species tends to be much greater when it establishes itself in high abundance and has a strong functional distinctiveness from a native occurring species (Strayer et al., 2006).

2.3.2 Genetic characteristics and evolutionary potential

An alien species' transition from introduced to invasive in a novel environment can be strongly influenced by a host of genetic and evolutionary processes, such as genetic drift, genetic bottleneck, natural selection, adaptation, and admixture (Chan and Briski, 2017). It may evolve both during its initial establishment, and during subsequent expansion of its geographic range, sparked by selection pressures and other stressors generated by the new environment (Sakai et al., 2001; Novak, 2007; Zenni et al., 2014). Considering this, it is surprising to note that there is a lack of knowledge regarding evolutionary aspects of biological invasions and how they may contribute to the success thereof (Allendorf and Lundquist, 2003; Lambrinos, 2004; Novak, 2007).

Genetic variation determines a population's evolutionary potential to adapt to new or changing abiotic conditions (Bernatchez, 2016). This may be especially important since traditionally, alien invasive populations are thought to have significantly reduced levels of genetic variation (Lawson Handley et al., 2011). Introduced populations generally suffer from genetic bottlenecks after introduction due to the number of initial founders often being small (Nei et al., 1975). Genetic population bottlenecks are stochastic events that arise when a large number of individuals in the introduced population die, or are prevented from breeding, causing a drastic decline in population numbers. Genetic drift is one of the most powerful forces acting on small founding populations (i.e. after a population has experienced a bottleneck) and can cause a significant decline in genetic variation at the population level (Holland, 2000).

Furthermore, successive bottlenecks during introduction and establishment would further reduce genetic diversity (Clegg et al., 2002; Estoup et al., 2004). As a consequence, introduced populations are likely to be less genetically diverse than the populations from which they are derived (Allendorf and Lundquist, 2003; Estoup et al., 2004; Acharya, 2014). Theoretically, low population genetic variation should constrain a species from successfully invading a new habitat by reducing the population's capacity to handle selection stressors (Lawson Handley et al., 2011). Additionally, chances of inbreeding are drastically increased due to small population sizes, which can expose homozygous individuals to deleterious recessive mutations (Keller and Waller, 2002). Overall, the growth of the population and the ability of the introduced species to adapt to the new environment are limited (Allendorf and Lundquist, 2003; Kinziger et al., 2011; Acharya, 2014).

Propagule pressure (the number of individuals dispersing into a recipient environment) has emerged as a key factor for predicting whether an alien species will become established, directly correlating with invasion success (Kolar and Lodge, 2001; Bock et al., 2015). Propagule pressure includes both the number of release events and the number of individuals introduced (Allendorf and Lundquist, 2003). The genetics of introduced alien populations are influenced in two ways: (1) multiple introductions may originate from genetically divergent native populations and may lead to the formation of populations which are genetic admixtures from a number of source populations; and (2) larger founder populations of alien species would preserve a greater proportion of genetic variation of the species' native range (Novak, 2007).

Phenotypic plasticity, a characteristic of one genotype to produce different phenotypes under different environmental conditions (Fusco and Minelli, 2010), has been proposed as an important trait that contributes to the successful invasion of an introduced species in a novel environment (Davidson et al., 2011; Acharya, 2014). Phenotypic plasticity research has mainly focused on genotype-environment interactions (DeWitt and Scheiner, 2004). The link between phenotypic plasticity and invasive species is important for better understanding its role in successful establishment, geographical range expansion, and high abundance in the invasive range (Hulme, 2008; Acharya, 2014). Higher plasticity of traits in an invading species compared to native species has been widely predicted to be a key characteristic for the explanation of why an introduced species can successfully invade a new habitat (Acharya, 2014; Chan and Briski, 2017). This trait is also considered essential in order for a colonisation and establishment event to be successful (Ghalambor et al., 2007).

Integrating genetics with biological invasions is important and can reveal characteristics that make it possible for a species to establish and become invasive (Tepolt, 2015). If genetic variation during and after establishment are characteristic of invasive species, it is vital to gain a sound understanding of the role that genetic variation plays during the invasion process. Research on genetic variation may also help predict the potential that an invasive population will evolve to increase resistance to agents of biological control (Sakai et al., 2001). Also, a better understanding of expansion, introduction patterns, and gene flow during a biological invasion event can be achieved which is key for the making of comprehensive conservation and management decisions (Sakai et al., 2001; Acharya, 2014).

2.4 DNA barcoding: a tool for invasive species identification

In order to reduce the introduction of invasive species into a given environment, policy makers, government officials, and retailers will need to know the identity of the species in question (Keller and Lodge, 2007). Often, a taxonomic expert is not available to accurately describe the specimen to the species level. Furthermore, the detection procedure becomes complicated and tedious due to the existence of morphologically indistinguishable species (Nagarajan et al., 2018), and because many marine organisms have larval stages that are challenging to identify based on morphology alone (Radulovici et al., 2010). This demand for a rapid, reliable, and accurate identification technique has led to the pursuit of a ground-breaking molecular-based method for the identification of an unknown species.

The DNA barcoding concept proposed by Hebert et al. (2003a) analyses sequence diversity in a standardized region of DNA to aid in the molecular identification of known species, and to assist in the discovery of those yet to be described (Ratnasingham and Hebert, 2007; Chen et al., 2011). The primary goal of DNA barcoding is to identify an unknown specimen by comparison with a known classification (Miller, 2007), and it therefore does not intend to replace or invalidate traditional taxonomic practices, but rather facilitate, complement, and enhance them (Stoeckle et al., 2003; Bucklin et al., 2011).

In the animal kingdom, a 648 base pair (bp) region of the mitochondrial cytochrome *c* oxidase 1 (COI) gene is considered to be the standard barcode for identification (Hebert et al., 2003b). There are however some exceptions to the resolving power of COI, which include the Ctenophora (comb jellies) and Porifera (sponges) phyla, and members of the cnidarian class Anthozoa (sea anemones and corals) (Bucklin et al., 2011). The mitochondrial genome evolutionary rates of these organisms is 10 to 20 times lower than that of other metazoan animals (Shearer et al., 2002), and therefore cannot provide reliable discrimination between species of close descent (Bucklin et al., 2011). There is considerable interest in the amplification of nuclear regions to rectify this issue (Hellberg, 2006; Frézal and Leblois, 2008), indicating potential for the rRNA internal transcribed spacer (ITS), 18S ribosomal DNA, the signal recognition particle 54kDA (SRP54), and the 28S ribosomal RNA regions (Stemmer et al., 2012; Fuller and Hughey, 2013; Haverkort-Yeh, 2013; Ma et al., 2016; Benayahu et al., 2018). The barcoding of plants has proven to be more challenging and currently there is no standard barcode that has been successful at identifying species across the plant kingdom (Chase et al., 2007). Several gene loci (e.g. ITS, *rbc*L, and *tuf*A), together with a combination thereof, have been proposed as promising barcodes for algae and seaweed (Famà et al., 2002; Stam et al., 2006; Hu et al., 2009; Zou et al., 2016; Vranken et al., 2018).

Traditional DNA barcoding is performed by amplification of the marker region after which the resulting sequence is analysed by comparison to an online database of known species. There are two databases that are most commonly used to search for matches to the queried sequence: the National Center for Biotechnology Information (NCBI) GenBank online search tool BLAST (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov) (Altschul et al., 1990), and the Barcode of Life Data System (BOLD) database (http://v3.boldsystems.org/) (Ratnasingham and Hebert, 2007). Thereafter, phylogenetic tree-construction methods (Neighbour-Joining, Maximum Likelihood, or Bayesian) calculate the genetic distance between species and assign a cut-off value (known as the 'barcode gap') to divide operational taxonomic units (OTUs) into species. Ideally, distinct clades will appear on phylogenetic trees and, depending on the node support of that clade (i.e. bootstrap value), are interpreted as species (Zou et al., 2016).

Species identification using DNA barcoding requires reliable, comprehensive, and accurate sequence reference libraries of known taxa for it to be effective (Weigand et al., 2019). However, as this method relies on the previous identification of species which were most likely assigned using morphological methods, it is common that DNA sequences are stored in databases that have been incorrectly assigned to a specific species (Meyer and Paulay, 2005; Mioduchowska et al., 2018). Ultimately, for DNA barcoding to be a successful and reliable means of species identification, global community efforts are required to establish comprehensive large-scale public reference libraries (Radulovici et al., 2010; Coissac et al., 2016).

2.5 Vectors

Pathways of introduction are defined as the physical processes that lead to the movement of alien taxa from one geographic region to another (Richardson et al. 2011), and includes both the vector that carries an organism and the route along which it travels (Essl et al. 2015; Faulkner et al., 2020). With the expansion of human populations globally, opportunities for species to breach biogeographical dispersal barriers has increased, allowing them to colonise regions beyond their natural habitats (Thomaz et al., 2014). Intentional (also known as deliberate) introductions of alien species occur when a non-native organism is introduced into the natural environment for a specific reason, such as for aquaculture production or the releasing of pets and research subjects. Unintentional, or accidental, introductions occur when non-native organisms escape from captivity or when they are introduced associated with products, and people, and disperse into the environment (Molnar et al., 2008).

There are a diverse number of vectors for the introduction of alien marine species (Ojaveer et al., 2018). Carlton (2001) identified 15 broad marine vector categories, Williamson et al. (2002) identified eight, and Minchin and Gollasch (2002) five. The number of these major vector categories is dependent on how they are arranged as they all include a number of dispersal methods. The most recent work proposed in a document for the Convention on Biological Diversity (CBD) (CBD, 2014), presents a unified system that categorises introduction pathways of alien invasive species. This categorisation scheme is referred to as the 'CBD pathways' and distinguishes intentional and/or unintentional introductions, and the introduction mechanism as either the establishment of an anthropogenic dispersal corridor, the importation of a commodity, arrival via a transport vector, or the natural spread from a region where the species is itself alien (Harrower et al., 2018). These introduction mechanisms are further divided into six main groups: release; escape; transport (contaminants); transport (stowaway); corridors; and unaided (Harrower et al., 2018). Forty-four pathway subcategories have been described that relate to these six main pathway categories (see Harrower et al., 2018).

Vectors particularly susceptible to aiding marine invasions include ballast water from ships, hull fouling (biofouling), floating structures (drilling platforms, dry docks), canals, aquaculture activities, the ornamental pet industry, research laboratories, snorkelling and SCUBA equipment, and recreational fishing activities (Robinson et al., 2020). Vectors associated with global shipping (mainly ballast water and biofouling) are widely considered to play the most significant role in human mediated dispersal of invasive species across the oceans (Minchin et al., 2005; Mineur et al., 2012; Ricciardi, 2016). Shipping is responsible for around 91% of the introductions of marine species to South Africa (Robinson et al., 2020). More recently, researchers have identified novel pathways for the introduction of non-native species: floating debris made up of anthropogenic litter (Carlton and Fowler, 2018; Miralles et al., 2018b; Rech et al., 2018), and scientific gear, such as human occupied vehicles and remote operated vehicles (Voight et al., 2012; Thaler et al., 2015). While natural mechanisms are important for the maintenance of natural populations, the increasing incidences of natural disasters in the form of hurricanes, floods, tsunamis, and landslides are also significantly increasing the rate of introduction of alien species (Walther et al., 2009).

A substantial amount of research activity and management attention has focused on the unintentional introduction vectors of alien invasive species through ballast and bilge water transfer in ships (Verna et al., 2016; Fletcher et al., 2017; David et al., 2019; Gollasch et al., 2019), transport via recreational fishing and SCUBA-diving gear (Acosta and Forrest, 2009; Bacela-Spychalska et al., 2013; Smith et al., 2020), hull fouling (Ashton et al., 2006; Neves and da Rocha, 2008; Clarke Murray et al., 2011; Sylvester et al., 2011), and escapes from aquaculture operations (Naylor et al., 2001; de Silva, 2012; Grosholz et al., 2015; Lin et al., 2015; Xiong et al., 2017). By contrast, the marine aquarium trade has received far less attention and has only recently been recognized as a major pathway for the introduction of marine alien invasive fish, invertebrates, and algae (Strecker et al., 2011).

2.6 The marine aquarium trade

The global trade of live tropical marine organisms is a rapidly expanding, multi-million dollar industry, driven by the demand for traditional medicinal products, live food fish markets, the research and pharmaceutical industries, and the aquarium, curio and jewellery trades (Bruckner, 2005; Rhyne et al., 2009; Dey, 2016). The hobby of collecting live fish and invertebrates for public and home aquaria spread from a small cottage industry in Sri Lanka in the early 1920s and 1930s, through to Hawaii and the Philippines in the 1950s (Wijesekara and Yakupitiyage, 2001; Herath and Wijewardene, 2014). The marine aquarium trade had established itself as a major industry by the 1980s with collectors throughout the Pacific Islands, Southeast Asia, Brazil, Hawaii, the Red Sea, the Caribbean and Florida (Wabnitz et al., 2003). The marine aquarium trade, as a low volume but high value market, has the potential to provide financial stability for low-income, rural coastal communities that collect and supply the trade (King, 2019).

Since 1990, marine aquarium hobbyists have shifted their preference from fish-only aquariums to that of miniature ecosystems, which mimic those of coral reefs (Rhyne and Tlusty, 2011). Aquarists are now able to supply their marine aquariums with aesthetic and life-support services by drawing upon the full suite of coral reef diversity (Rhyne et al., 2012). This is due to the rapid advances in husbandry technologies which allow aquarists to replicate living ecosystem processes with the use of high-tech equipment, particularly UV lighting systems, commercial sea salt mixes, and protein skimmers (Ogawa and Brown, 2001; Wabnitz et al., 2003; Rhyne and Tlusty, 2011). These technological improvements have allowed for a much wider diversity of fish, corals, and invertebrates to be kept that were otherwise too fragile before (Ogawa and Brown, 2001).

At present, an estimated 30 million tropical reef fishes are supplied by 76 countries annually in a worldwide trade (Bruckner, 2005; Rhyne et al., 2017). The global import market demand is dominated by the United States of America (60%), while the remainder is largely imported to Western Europe, Japan and Australia (Murray et al., 2012). A total of 2,300 fish and 725 invertebrates are currently being traded globally (Rhyne et al., 2017), which has increased substantially compared to the 1,200 species of fish and 300 species of invertebrates that were reported by Green (2003). The majority (90-99%) of these marine organisms are harvested from biodiverse coral reefs within the Coral Triangle Region, which includes the waters off the Pacific countries of Malaysia, Papua New Guinea, Indonesia, the Philippines, Timor-Leste and the Solomon Islands (Wood, 2001; Hoeksema, 2007; Murray et al., 2012).

2.6.1 Monitoring, quantification, and challenges

So far, only a handful of studies have attempted to record quantity and species-specific data of fish, corals, and invertebrates provided from trade invoices (Parks et al., 2003; Wabnitz et al., 2003; Smith et al., 2008; Monticini, 2010; Rhyne et al., 2012; Wabnitz and Nahacky, 2014; Prakash et al., 2017; Rhyne et al., 2017). These records, however, are largely based on import data from the USA, but still provide a valuable insight into the species being traded globally. It is estimated that the trade targets 50 taxonomic families of marine fish (Monticini, 2010), 140 species of hard coral (almost entirely Scleractinia), 61 species of soft coral, and 516 invertebrate species (mostly shrimps, anemones, and molluscs) (Wabnitz et al., 2003). However, the current poor standard of taxonomy makes arriving at a precise figure challenging (Murray et al., 2012).

A number of data sources have been used to gain insight into the marine aquarium trade even though these data systems are not sufficient, nor even intended for, the monitoring thereof (Wood, 2001; Green, 2003; Wabnitz et al., 2003; Smith et al., 2008; Rhyne et al., 2017). For example, it is compulsory to submit trade data to the Convention on International Trade in Endangered Species (CITES). A number of ornamental clams, sea horses, and hard corals are protected by CITES, but for the majority of marine aquarium species it is not known whether they are at risk of exploitation. These taxa groups only account for a small fraction of the total number of marine fish, corals, and invertebrates being traded (Rhyne et al., 2017). Studies have also shown discrepancies in CITES records (Blundell and Mascia, 2005; Rhyne et al., 2012) and therefore, CITES monitoring alone has been suggested to be an ineffective management tool for the marine trade industry as a whole (Green and Hendry, 1999).

The Marine Aquarium Biodiversity and Trade Flow online database (https://www.aquariumtradedata.org/) was developed by Rhyne et al. (2015) to assess the number of marine aquarium species imported into the USA, and to document the diversity of marine organisms imported from around the world (Rhyne et al., 2017). Invoices pertaining to imports of live marine fish and invertebrates were analysed over 2008, 2009, and 2011. This database provides a comprehensible understanding of marine aquarium trade data for the USA, the country of greatest import demand globally, and gives great insight into the countries which supply marine organisms to the marine aquarium trade (Figure 2.1).

Although the monitoring and reporting of the marine aquarium trade industry is fundamentally important to its management, it is not sufficiently developed (Tissot et al., 2010; Murray et al., 2012). Trade numbers are frequently underreported as a result of the misclassification or exclusion of shipment invoices, and where invoices are present, they are generally classified in value or weight instead of number of individual organisms (Wood, 2001; Murray et al., 2012). The majority of shipment invoices do not record the scientific names of the traded species (Smith et al., 2008; Allen et al., 2017), and mostly report according to common names. This presents even greater challenges as traditional common names can differ depending on the region from where the species have been sourced, resulting in a host of synonyms for the common names (Allen et al., 2017; Prakash et al., 2017). Harvested organisms that die, or are rejected by buyers before a point of sale, often are not reported (Militz et al., 2016). The volume of species vary significantly during the different phases of the supply chain, as they pass through stages of quarantine, handling, and shipping before reaching the pet stores (Prakash et al., 2017). These discrepancies are of immediate concern as importation for the ornamental aquarium trade is listed among the top five pathways of non-native introductions of species, and account for more than one-third of the aquatic species on the IUCN 100 Worst Invaders list (Lowe et al., 2000; Padilla and Williams, 2004; Semmens et al., 2004; Smith et al., 2008; Strecker et al., 2011).

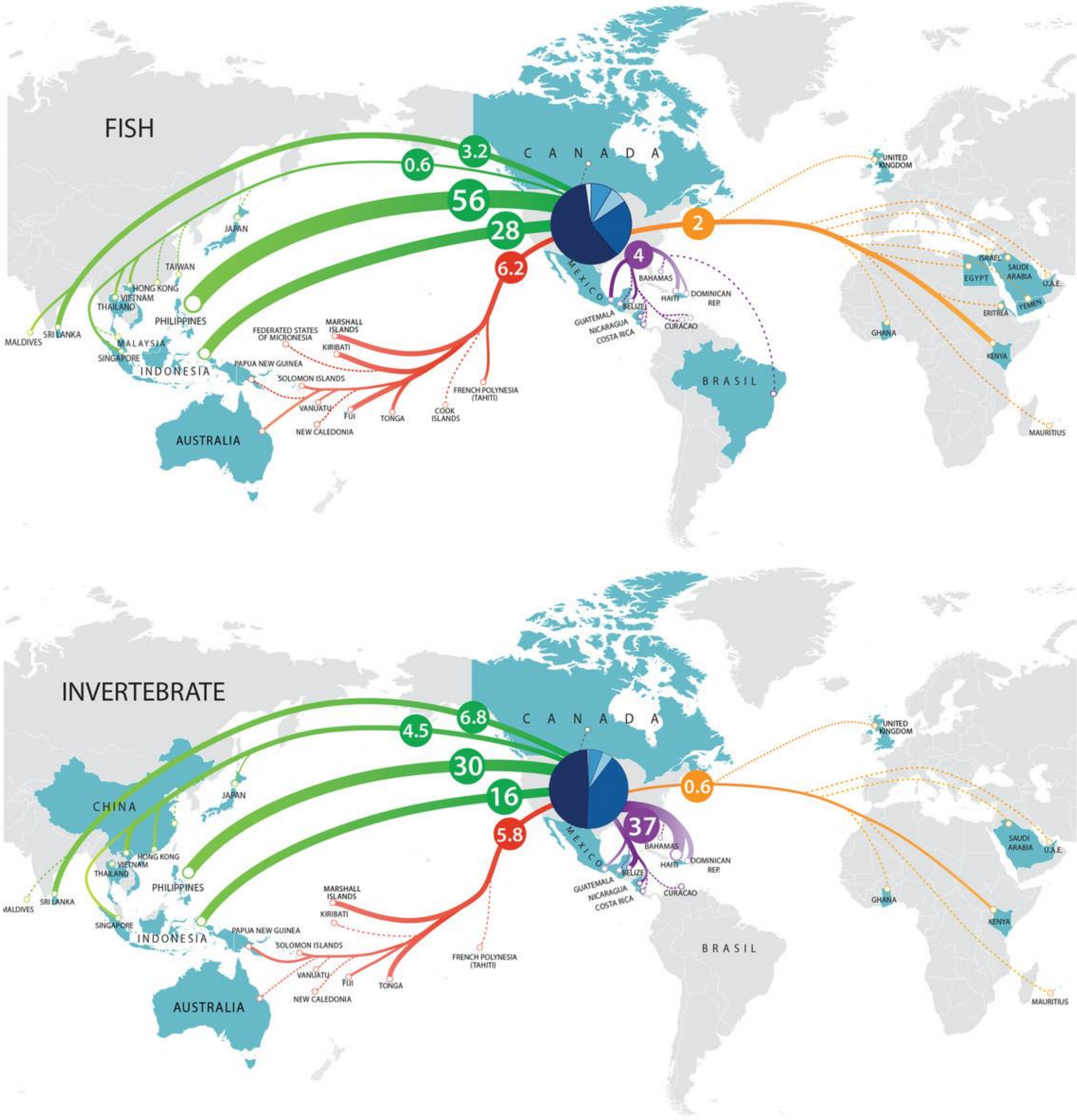


Figure 2.1: Trade flow of marine aquarium fish (top) and invertebrates (bottom) into the United States of America from importing countries over 2008, 2009, and 2011. *Numbers on lines indicate the percentage of trade supplied by the source. The pie chart illustrates ports of entry into the USA (with the Midwest starting at 0 degrees, and clockwise, North East, South East, South West, and North West). Image taken from The Marine Aquarium Biodiversity and Trade Flow online database (<u>https://www.aquariumtradedata.org/</u>) (Rhyne et al. 2017).*

2.6.2 A vector for impactful marine alien species

Several examples exist around the world of species introduced by marine hobbyists which have become invasive. This is primarily due to the release of unwanted organisms by good-intentioned but uneducated hobbyists for reasons such as high reproduction rates, aggressiveness towards other species in the aquarium, large size, and an act of humane treatment (Padilla and Williams, 2004; Gertzen et al., 2008; Strecker et al., 2011). Marine fish that are purchased as juveniles and outgrow the marine aquarium in which they are kept may be released at a large size, and are therefore more likely to survive over juveniles of the same species (Holmberg et al., 2015). Padilla and Williams (2004) also suggest that releases may take place by the draining of water from marine aquaria (e.g. during maintenance) which contains adult organisms or their sexual (i.e. larvae or eggs) or asexual propagules.

One of the most notorious invasions is that of the lionfishes, *Pterois volitans* and *Pterois miles*, after their accidental release from marine aquaria into the ocean in the mid 1980's (Morris and Akins, 2009). Since then, it has successfully spread across the eastern coast of the USA, the Gulf of Mexico, and the entire Caribbean region, including Mexico, Belize, Colombia, Puerto Rico, Cayman Islands, Honduras, Venezuela, and Brazil (González et al., 2009; Ferreira et al., 2015; Sandel et al., 2015; Trégarot et al., 2015; Arellano-Méndez et al., 2017). Even though this is not the first incidence where releases from an aquarium have been documented as a probable source of marine fish introductions (Randall, 1987), Semmens et al. (2004) suggest that it may be the first aquarium introduction that has caused the successful establishment and proliferation of a non-native marine fish.

The green seaweed *Caulerpa taxifolia*, known as the killer algae, together with *Caulerpa racemosa*, the sea grape, have invaded many parts of the Mediterranean Sea (Occhipinti-Ambrogi, 2007; Ellul et al., 2019). Even though both of these species have caused wide ecological and economic damage, members of the same genus can still be found for sale in various pet shops and online retailers in Europe and the USA (Walters et al., 2006; Klein and Verlaque, 2008; Vranken et al., 2018). *Caulerpa taxifolia* was also introduced to the Californian coast (Jousson et al., 2000), but has since been eradicated (Anderson, 2005).

More recently, two soft coral species (*Clavularia cf. viridis* termed "Green Star Polyps", and *Sansibia* sp. known as "Xenia Blue"), originally introduced to the Brazilian coastal waters by a marine hobbyist, were found to be well established and out-competing native species during a monitoring project (Mantelatto et al., 2018). Despite this, both of these species are available to Brazilian aquarists for purchase online (Mantelatto et al., 2018) and are popular amongst beginner aquarium hobbyists for their hardy nature and fast growth rate (Ellis and Sharron, 1999; Mantelatto et al., 2018). Another member of the xeniid soft coral family, with close genetic affinities to *Xenia membranacea*, was illegally released into Venezuelan waters for propagation and farming purposes (Ruiz Allais et al., 2014). This soft coral species grows over native stony corals at a rapid pace (Benayahu and Loya, 1985).

2.6.3 Live rock

Driven by the advances in filtration and lighting technologies, marine aquarists are moving from fish-only tanks to reef aquariums that replicate conditions found in coral reef ecosystems (Rhyne and Tlusty, 2011). In order to achieve optimum biological filtration and recreate natural conditions, 'live rock' and deep beds of coralline sand are often used (Nilsen and Fossa, 2003). Live rock is a broad taxonomic term used for any type of rock or dead coral skeleton encrusted with, and containing within its crevices, a wide variety of marine organisms, including colourful sessile invertebrates and encrusting algae (see Figure2.2) (Yuen et al., 2009). Many hobbyists consider it to be an essential component of marine aquariums as it acts as a refuge for mobile invertebrates and fish, a substrate for sessile organisms (Walters et al., 2006), and as a biological filtration system due to it containing a host of nitrifying and denitrifying bacteria (Yuen et al., 2009; Li et al., 2017). Additionally, live rock plays a crucial role in providing a substrate for coral spat and other recruiting juvenile microbenthic organisms (Muhammad Hamizan et al., 2015). As it serves as the ecological foundation within living marine aquaria, it is frequently traded within the ornamental marine market (Parks et al., 2003).

There are several types of live rock available for importation, named in the trade according to where it is harvested. Caribbean/Florida, Fiji, Bali/Java/Indonesia, Kenya, Tonga, Manado, Vanuatu, Irian Jaya and Marshall Islands live rock are examples (from 'Live Rock Overview' at http://www.livestockusa.org/LIVEROCK.html). Due to increasing conservation concerns over reef degradation as a result of the extraction methods, cultured and artificial live rock is often used as an alternative to rock that is harvested from the wild (Parks et al., 2003; Lecaillon, 2004).

Cultured live rock is sourced from within the earth (e.g. quarried limestone) and then placed within cage-like structures on the seafloor to allow invertebrates to recruit and become established as live rock that can then be harvested over time (Antozzi, 1997; Herndon, 1998;

Parks et al., 2003). As a result of this production process, a more natural-looking live rock is created. Artificial live rock is made by forming rocks with cement and then adding it to an already established aquarium system to be seeded by existing live rock. It is, however, a lot less popular with aquarium hobbyists due to its synthetic appearance, reduced biological effectiveness, and altered water parameters due to the leeching of chemicals.

Harvested live rock is known to support a variety of invertebrates and coralline algae. Common inhabitants are crabs, mussels, snails, bristle worms, soft and hard corals, sponges, zooanthids, anemones, and even small star fish (Parks et al., 2003). During preparation for exportation, the objective is to kill and wash out these organisms and keep the coralline algae. This process is known as 'curing' and is usually done by spraying the rock with high salinity water and removing organisms that can be seen by hand. Curing processes are not standardized and are unmonitored, therefore many hardier organisms remain on the rock and 'hitch-hike' to many places around the world (Wabnitz et al., 2003).

Due to its high demand by marine hobbyists, together with its ability to house a variety of diverse organisms, live rock has the potential to transport alien species around the globe (Wabnitz et al., 2003). A study conducted by Bolton and Graham (2006) recognized the invasion threat posed by organisms associated with live rock. It was emphasized that the major downfall in regulation and control of importing live rock is that it does not have to undergo quarantine when entering a country. This study discovered polyps and juvenile medusae of the upside-down jellyfish (*Cassiopea* sp.) in a home aquarium developing from live rock imported from the Indo-Pacific to the USA. Live rock was suggested to be a possible vector of *Carijoa riisei*, an invasive soft coral, which has successfully established and invaded areas of Hawaii (Concepcion et al., 2010). As a preventative measure, Stam et al. (2006) recommended that the sale of live rock be banned in the USA after identifying *Caulerpa* sp.

Well-known marine aquarium pests amongst hobbyists are the glass sea anemones of the genus *Aiptasia*. These are highly resistant organisms that are often accidentally introduced into marine aquaria on live rock (Calado and Narciso, 2005; Ram, 2013). A review of the literature revealed that studies often refer to *Aiptasia* sp. as being introduced via live rock into marine aquaria, with no formal studies verifying these observations. Natural predators of *Aiptasia* sp. are often used as a form of biological control by marine hobbyists as they are extremely difficult to eradicate (Ram, 2013). Some of these are the Monaco shrimp (*Lysmata seticaudata*), the peppermint shrimp (*Lysmata wurdemanni*), the aeolid nudibranch (*Berghia verrucicornis*), and the copperband butterflyfish (*Chelmon roslralus*) (Carroll and Kempf, 1990; Calado and Narciso, 2005; Leal et al., 2013; Ram, 2013).

Figure 2.2: A piece of live rock displaying a colourful variety of encrusting coralline algae and seaweed. *Image: KP Aquatics © (https://www.kpaquatics.com).*

2.6.4 The marine aquarium trade in South Africa

In South Africa, the freshwater aquarium trade has seen far more attention as a pathway for the introduction of alien invasive species compared to that of the marine trade (Martin and Coetzee, 2011; Ellender and Weyl, 2014; Appleton and Miranda, 2015; Hoveka et al., 2016; Marr et al., 2017; Nunes et al., 2017; van der Walt et al., 2017). Currently, only one study attempted to gain an insight into the marine fish, coral, and invertebrate species being traded, and numbers thereof, in South Africa. Everett and van der Elst (2008) inspected pet shops in KwaZulu-Natal and reported that a total of 56 marine fish species (from 17 families) were being kept for sale. Of the 56 species recorded, 44.6% are found in South African waters, but are not strictly endemic thereto. Pomacentridae (damselfishes) was the most prominent family of fish being sold (67.9 %), Gobiidae gobies (7.2%) and Serranidae groupers (6.3%). Everett and van der Elst (2008) also gave valuable insight into the marine aquarium fish species collected by hobbyists in KwaZulu-Natal by examining catch return data from the Ezemvelo KZN Wildlife Congella Office for the period 1994 to 1998. There are no existing databases or monitoring programmes in place for the monitoring and quantification of the marine aquarium trade in South Africa.

An important aspect of national biosecurity in any country is the management of introductions of aquatic fish, plants/algae, and invertebrates (Padilla and Williams, 2004; van der Walt et al., 2017). South Africa is a signatory of the Convention on Biological Diversity and its Strategic Plan for Biodiversity 2011-2020 (UNEP, 2011), and therefore is required to implement the Aichi Target 9 of the Strategic Plan for Biodiversity 2011–2020 . This obligation to the international community requires that alien and invasive species, and their respective vectors of introduction, are identified, prioritised, and managed (UNEP, 2011; Marr et al., 2017). South Africa fulfilled this obligation by adopting a 'blacklist' approach, and passed the National Environmental Management: Biodiversity Act (NEMBA, Act No. 10 of 2004). A 'blacklist' documents a smaller group of high-risk taxa that are subject to stricter regulations (van der Walt et al., 2017). NEMBA intends to promote the conservation and protection of South Africa's rich biodiversity. In 2014, new regulations were announced in terms of this Act, by which biological invasions are to be managed. These regulations address the import of non-native species, specify how non-native species are to be managed or controlled, and place existing invasive species into a number of categories (van Wilgen et al., 2018).

The Act also states that those wishing to import non-native species need to obtain a permit. However, the permit may only be issued after a risk assessment on potential impacts on biodiversity has been conducted. In order to apply for a permit to import marine ornamental organisms, one has to provide, amongst other documents, the following: invoices of origin and health certificates reflecting the details of the seller/exporter; the species and quantity of organisms to be sold; an indication of a single point of entry into South Africa; and a CITES certificate for all CITES listed species (DAFF, 2015).

Even though the South African government spends millions of rands annually on the control and management of invasive species (de Lange and van Wilgen, 2010), there are no existing quarantine establishments or inspection points in place for the monitoring of the aquarium trade. The quarantine of imported fish and other aquatic organisms is the responsibility of the importer and not of the government regulatory authorities. The importer is also held accountable for the treatment of fish for diseases and parasites, and chlorination and disposal of the accompanying transport water (Mouton et al., 2001).

The internet is often turned to by aquarium hobbyists to gain safe trade practices and scientific knowledge in a variety of areas (Livengood and Chapman, 2007). Marine Aquariums South Africa (MASA) (https://www.marineaquariumsa.com/) is the largest marine aquarium forum and reefkeeping resource in South Africa. This free forum is used for providing information on aquarium setup, husbandry, and filtration techniques. Hobbyists also discuss problems encountered with saltwater fish, invertebrates, and corals , where species associated with live rock are a common topic. MASA is also turned to by hobbyists for the identification of unknown species, where they can upload photos and discuss possible classifications amongst themselves on the forum. Many pet shops advertise stock for sale, which has brought about the trade and exchange of species amongst the hobbyists. Based on MASA statistics, there are an estimated 10,500 marine aquarium hobbyists in South Africa.

CHAPTER 3: IDENTIFICATION OF TAXA ASSOCIATED WITH LIVE ROCK

3.1 Methods and materials

3.1.1 Specimen collection and preservation

After visiting the pet stores described in Chapter 5, it was concluded that Indonesian and Kenyan live rock were the only live rock types available for purchase across South Africa. Therefore, these live rock types were chosen for the current analysis. Three kilograms of Indonesian live rock (equating to one replicate) were purchased from a marine aquarium pet store in Cape Town, Durban, and Pretoria/Johannesburg. This was replicated for the Kenyan live rock, and led to a total of 18 kilograms (6 replicates) of live rock being purchased. A replicate did not necessarily have to comprise one 3 kilogram piece of live rock (i.e. it could be made up of several pieces). It must be noted that it was assumed that the live rock was of a specific type according to the best knowledge of the pet store employee. Prices ranged from R100 to R390 per kilogram of live rock. Where possible, contamination of the live rock from the pet store aquaria was avoided by contacting the pet store in advance and enquiring when new live rock stock would arrive. Requests were made to the pet stores to not quarantine, dip, or remove any of the associated organisms.

Each replicate of live rock was held within a separate holding aquarium, filled with temperature controlled (27°C) artificial sea water. The main water supply was pumped using a Bubble Magus WP400 water pump and circulated through a UV light system to purify the water of any pathogens, algae, or other invertebrates that could potentially contaminate the live rock. Thereafter, the water was skimmed of any remaining organic matter using a Bubble Magus Curve 5 Protein Skimmer, before returning to the holding aquaria.

Any visible live rock associated taxa, or those that had fallen to the bottom of the holding aquarium, were sampled. Every sample was rinsed of any salt water with distilled water before preserving it with 70 % molecular grade ethanol for subsequent morphological identification and DNA extraction. The live rock was monitored on a daily basis for any visible taxa which were then removed and preserved. After one month, the live rock was submerged in solutions of diluted magnesium chloride and fresh water in a separate container to gradually relax and drive out any remaining invertebrates within the live rock in line with Williams and Van Syoc (2007). This was necessary to ensure that those organisms (i.e. polychaetes) dwelling deep within the live rocks' crevices, were also sampled.

3.1.2 Morphological identification

Preliminary morphological identifications were assigned to all samples to the lowest taxonomic rank possible. Quantities of organisms belonging to major taxa groups were then recorded for each live rock type, per replicate. Soft coral tissue fragments were excised from the polyps and subsequently dissolved in 12% sodium hypochlorite. A compound microscope (Eclispe 80i; Nikon) was used to examine the sclerites at 40 and 100x magnification. Identifications were inferred according to Fabricius and Alderslade (2001). Polychaete's were examined under a compound microscope and identified according to Fauchald's (1977) and Day's (1967) monographs with the assistance of an expert polychaete taxonomist (Dr J. Kara, pers. comm., April 2017). Bivalve shell colour, hinge type, and characteristics (i.e. external features, growth lines, shell shape, umbo position, ribs) were observed and used to infer identifications according to Huber's (2010) Compendium of Bivalves. Taxonomic experts were consulted for their assistance in the identification of the crabs (Dr R. Naderloo and Dr N. Peer, pers. comm., June 2017), brittle stars and starfish (Dr U. Parameswaran, pers. comm., March 2019).

3.1.3 DNA extraction and amplification

Genomic DNA was extracted from invertebrate tissue using the ZR Genomic DNA™ Tissue MiniPrep kit (Zymo Research Corporation, California, United States of America). Approximately 25mg of tissue was cut from each invertebrate and rinsed with distilled water to remove excess ethanol that could inhibit the enzymatic activity of proteinase-k. Thereafter, tissue samples were cut up finely or ground using a pestle and mortar. The tissue was then added to a microcentrifuge tube containing a solution of 95 µl of 2X digestion buffer, 10 µl of proteinase-k, and 95µl of molecular biology grade water. Samples were vortexed and incubated overnight in a dry bath incubator on a gyro-rocker at 55° C.

After incubation, samples were removed and 700 µl of genomic lysis buffer was added to each microcentrifuge tube. Samples were mixed thoroughly by vortexing and then centrifuged for one minute at 10,000 x *g* using an Eppendorf Centrifuge 5418. The supernatant was transferred to a Zymo-Spin™ IIC Column in a collection tube and centrifuged for one minute at 10,000 x *g*. In a new collection tube, 200 µl of DNA pre-wash buffer was added to the spin column and thereafter centrifuged at 10,000 x *g* for one minute. Subsequently, 400 µl of g-DNA wash buffer was added to the spin column and centrifuged for one minute at 10,000 x *g*. The spin column was then transferred to a clean microcentrifuge tube and 50 µl DNA elution buffer was added to each sample and left to incubate for 10 minutes at room temperature. Finally, samples were centrifuged at top speed for 30 seconds to elute the DNA. The eluted DNA was checked for quantity and quality using a NanoDrop 3000 spectrophotometer and thereafter run on a 1% agarose gel (1 g agarose powder into 100 ml of 1X TBE buffer), using 5 µl of DNA sample and 1 µl of loading dye. DNA was stored at -20° C until PCR amplification.

The isolated DNA was amplified via polymerase chain reaction (PCR) using primers designed to amplify the mitochondrial cytochrome *c* oxidase I (mtCOI) and the nuclear ribosomal internal transcribed spacer (ITS) (Table 1). The targeted internal transcribed spacer region, which lies between the nuclear ribosomal 18S and 28S regions, is made up of the internal transcribed spacer 1 (ITS1), 5.8 S, and internal transcribed spacer 2 (ITS2) ribosomal subunits. PCR reactions for both markers contained 12,5 µl of *OneTaq® 2X Master Mix* with Standard Buffer (New England BioLabs), 8,5 µl of molecular biology grade water, 1 µl of bovine serum albumin (BSA) (10 mM), 1 µl of forward and reverse primer, and 1 µl of template DNA to make up a total reaction volume of 25 µl. The thermal cycler conditions used for mtCOI were as follows: [95 °C for 3 min], 35x [(95 °C for 30 s) (47 °C for 45 s) (72 °C for 1 min)], [72 °C for 10 min], and [4 °C for ∞]; and for ITS: [94 °C for 5 min], 30x [(94 °C for 45 s) (47 °C for 1 min) (72 °C for 1 min 20 s)], [72 °C for 7 min], and [4 °C for ∞]. In the case of multiple bands being amplified, the targeted band of DNA was cut out of the agarose gel and recovered using a Zymoclean™ Gel DNA Recovery Kit, according to the manufacturer’s instruction manual.

PCR amplicons, along with a negative control and 5 µl of GeneRuler 100bp DNA Ladder (Thermo Scientific™), were run on a 1,3 % agarose gel (1,3 g agarose powder into 100 ml of 1X TBE buffer) for 1 hour at 100 volts. Gels were imaged using a Bio-Rad ChemiDoc™ MP System 170-8280 imaging system and photographed with ImageLab™ Version 2.0.1© (Bio-Rad Laboratories). The PCR amplicons were sequenced in the forward direction with an ABI 3730 Capillary sequencer at the DNA Sequencing Unit, Central Analytical Facility, Stellenbosch.

Table 3.1: Primers used for amplification of the mitochondrial cytochrome *c* oxidase I (mtCOI) and the nuclear ribosomal internal transcribed spacer (ITS) regions of DNA from live rock associated taxa.

Gene region	Primer name	Sequence (5'-3')	Size of target sequence	Reference
mtCOI	LCO1490	TGCTAGCCGCAGGCATTA	710 bp	Folmer et al. (1994)
	HCO2198	GGGTGCCCAAAGAATCAGAAC		
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	900 bp	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC		

3.1.4 Sequence editing and alignment

Raw sequences were individually edited and aligned in BioEdit version 7.2.5 (Hall, 1999). Nucleotide ambiguities at different loci were identified by searching for the strongest signal peak on the chromatograms of the respective sequences and corrected using the IUPAC ambiguity codes. The resulting COI and ITS barcode sequences were compared to the GenBank online search tool BLAST (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov) (Altschul et al., 1990). Also, COI barcode sequences were compared to the Barcode of Life Data System (BOLD) database using the BOLD identification search engine (http://v3.boldsystems.org/) (Ratnasingham and Hebert, 2007). Initial comparisons were restricted to the published records, as BOLD contains many sequences with uncertain identifications.

COI and ITS sequence identifications were validated by constructing phylogenetic trees. This was done for every order of the queried species [soft coral (Alcyonacea), star fish (Valvatida), sea anemones (Actiniaria), isopods (Isopoda), amphipods (Amphipoda), and polychaetes], and only where there were sufficient barcodes to generate informative trees. Separate trees were constructed for each polychaete order (i.e. Phyllodocida, Eunicida, Amphinomida, and Sabellida) due to the abundance of barcodes available. Species belonging to the same taxon as the query species were searched for using the World Register of Marine Species (WORMS) database (http://www.marinespecies.org/). Where possible, the barcodes of these species were downloaded from NCBI's GenBank and BOLD for both COI and ITS (see Appendix A). Some of the query sequences belonged to taxa groups that were under-represented or not available in the barcode reference libraries, and therefore it was not possible to phylogenetically analyse them. If a barcode was generated for the COI and ITS marker for the same species, then a tree was generated for the marker that had a better matching score. This was done to avoid duplication of results, and due to time restrictions.

The edited sequences were aligned, with their respective downloaded barcode sequences, using the ClustalW multiple alignment method in BioEdit version 7.2.5 (Hall, 1999). COI alignments were trimmed to approximately 630 bp, and the ITS alignment to 830 bp.

3.1.5 Phylogenetic analyses

For each data set, neighbour-joining (NJ) and maximum likelihood (ML) trees were constructed by installing and implementing the *ape* (Paradis et al., 2004), *seqinr* (Charif and Lobry, 2007), and *phangorn* (Schliep, 2011) packages in R (R Core Team, 2013). Models of evolution (Table 3.2) were determined using the *ape* package and selected from the Akaike information criterion (AIC) (Akaike, 1973) for COI and ITS, and implemented in subsequent phylogenetic analyses. A distance matrix was created and used to generate the NJ and ML trees, assuming a Jukes-Cantor (JC69) distance between sequences.

Using *phangorn*, maximum log likelihood estimates were calculated with the relevant NJ trees as a starting point. Finally, a bootstrap algorithm was run using 1000 bootstrap samples to construct each ML tree based on their respective models of evolution. Bootstrap support values were categorized as: strong (85-100 %), moderate (70-85 %), and low (50-69 %) (Kress et al., 2002). Support values below 50% were not displayed. All trees were illustrated using the *phytools* package (Revell, 2011) in R, and edited using FigTree version 1.4.0 (Rambaut, 2012).

Table 3.2: Models of evolution determined for the nuclear ribosomal internal transcribed spacer (ITS) and the mitochondrial cytochrome *c* oxidase I (mtCOI) markers. *Abbreviations: number (no.); base pairs (bp); General Time Reversible (GTR); Hasegawa-Kishino-Yano (HKY); gamma distribution (G); proportion of invariable sites (I); Akaike information criterion (AIC).*

Taxon	Marker	No. of sequences	Length (bp)	Model of evolution	Log-likelihood	AIC
Alcyonacea	ITS	59	830	GTR+G	-3930.563	8109.125
Actiniaria	COI	49	620	HKY+G+I	-2612.452	5426.904
Amphinomida	COI	63	640	GTR+G+I	-6865.491	13996.98
Amphipoda	COI	37	650	GTR+G+I	-5638.797	11439.59
Eunicida	COI	79	650	HKY+G+I	-19072.83	38463.66
Isopoda	COI	58	590	GTR+G+I	-8222.219	16690.44
Phyllodocida	COI	63	620	HKY+G+I	-12088.60	24435.20
Sabellida	COI	70	620	GTR+G	-12251.94	24795.88
Valvatida	COI	55	755	GTR+G+I	-3103.471	6376.943

3.1.6 Species assignment and invasion status

Species assignments were finalized by taking the best-scoring BLAST and BOLD hits, morphological identifications, and the phylogenetic inferred identifications into account. For a species to be positively assigned, a sequence divergence of 2% or less was used as the cut-off point (identity/similarity >98%, E-value of zero) (Ratnasingham and Hebert, 2007; van der Walt et al., 2017). For a genus-level identification, the best hit as well as the next most similar match had to belong to the same genus. This was also a constraint for higher taxonomic ranks (e.g. family) to be assigned where positive species matches did not occur. Identifications were listed as 'inconclusive' where no hit was found, or where more than one species match occurred within the 99-100% identity range. Phylogenetic trees were used to infer species identifications based on the evolutionary relationships among the given taxa.

Biogeographic statuses were assigned for each species according to the framework proposed by Robinson et al. (2016), which is an interpretation of the Blackburn et al. (2011) categorisation scheme but for marine systems. The invasion status of each alien species was checked with reference to a number of databases, such as the World Register of Introduced Marine Species (WRiMS) (http://www.marinespecies.org/introduced/), the Invasive Species Specialist Group (ISSG) global database (http://www.iucngisd.org/gisd/) of the International Union for Conservation of Nature (IUCN), the Global Register of Introduced and Invasive Species (GRIIS) (http://www.griis.org/), the Delivering Alien Invasive Species In Europe (DAISIE) project (http://www.europe-aliens.org/), and the AquaNIS database (Information system on aquatic non-indigenous and cryptogenic species database) (http://www.corpi.ku.lt/databases/index.php/aquanis). This could only be performed for identifications that were assigned to the species level. Where molecular identifications could not be assigned, literature on the invasion status was reviewed according to the morphological identification made. For those specimens identified to the genus-level, the literature was reviewed for relations that have been flagged as introduced, pest, or invasive species. The World Register of Marine Species (WORMS) (http://www.marinespecies.org/) and SeaLifeBase (https://www.sealifebase.ca/) databases were used to verify the taxonomic nomenclature and native ranges of the identified species.

3.2 Results

In total, 174 marine invertebrates spanning 6 phyla were harvested from the 6 replicates of live rock (Kenya = 85; Indonesia = 89). Polychaetes were the most diverse (i.e. number of species) and abundant live rock associated species (Table 3.3). The species richness is estimated at 24 species. However, this number should be considered an underestimation of the true diversity as identification to species level was not always possible.

It can be confidently said that all live rock replicates (except for Indonesia 3) were not dipped into a curing agent or manually rid of any live rock associated taxa as they were received in the original shipping styrofoam container. The third Indonesian live rock replicate was purchased from a pet store in the trust that it had only been placed into their holding aquarium for one day, and had also not been stripped of any live rock associated taxa. A variety of seaweeds and coralline algae were growing on some of the live rock (Figure 3.1).

Table 3.3: Total species richness and abundance of taxa harvested from Indonesian and Kenyan live rock. *Species richness is indicated by the number (no.) of species identified per taxon and abundance by the total number of organisms per taxon. The minimum number of species is presented where identifications could not be assigned below family level. Arthropods listed under 'other' represent the isopod and amphipod taxa.*

Phylum	Taxon	No. of species	Total no. of organisms per taxon
Annelida	Polychaetes	10	62
Arthropoda	Crabs	2	2
	Other	2	7
Cnidaria	Sea anemone	1	17
	Soft coral	1	14
Echinodermata	Brittle stars	2	35
	Starfish	2	15
Mollusca	Bivalves	2	11
	Gastropods	1	5
Platyhelminthes	Flatworms	1	6

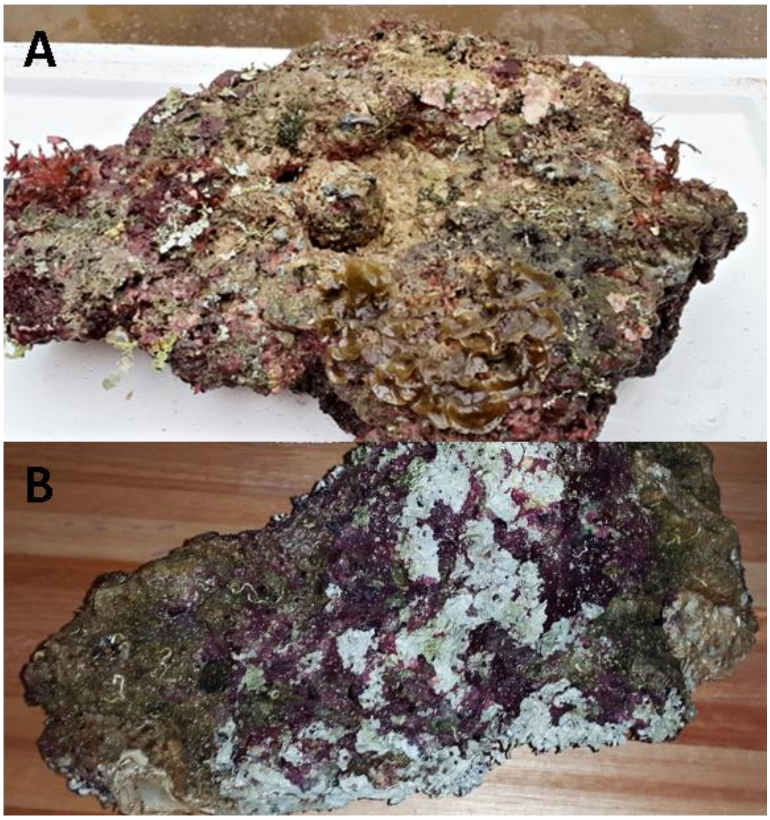


Figure 3.1: Kenyan (A) and Indonesian live rock (B) displaying a variety of green, red, and brown macroalgae (A) and corraline algae (B). *Images: G. Wehr.*

3.2.1 Morphological identifications

Of the 174 taxa sampled, 107 genus-level and 6 species-level identifications based on morphology were assigned. The remaining samples were left at the family, order, subclass, and class rankings due to uncertainty and the lack of taxonomic skills required to make positive identifications. Morphological identification of some polychaetes was not possible due to decomposition (samples 69, 94, 98 – 102). To avoid repetition, the morphological identifications of all 174 samples are listed with the molecular identifications in Table 6.10 of Appendix B. Where possible, photographs were taken and are presented below (Figure 3.2 – 3.9).

Figure 3.2: Microscopic view of *Xenia* sp. sclerites (Family: Xeniidae) (40X). *The minute, iridescent spheroids are characteristic of sclerites from Xenia sp. polyps.*

Figure 3.3: The live rock associated glass sea anemone *Aiptasia* sp. (Family: Aiptasiidae) (Sample 54). *Scale bar represents 1 cm.*

Figure 3.4: The live rock associated bivalve *Septifer bilocularis* (Family: Mytilidae). *Left: internal view of sample 144. Right: external and internal view of sample 145. Scale bars represent 5 mm.*

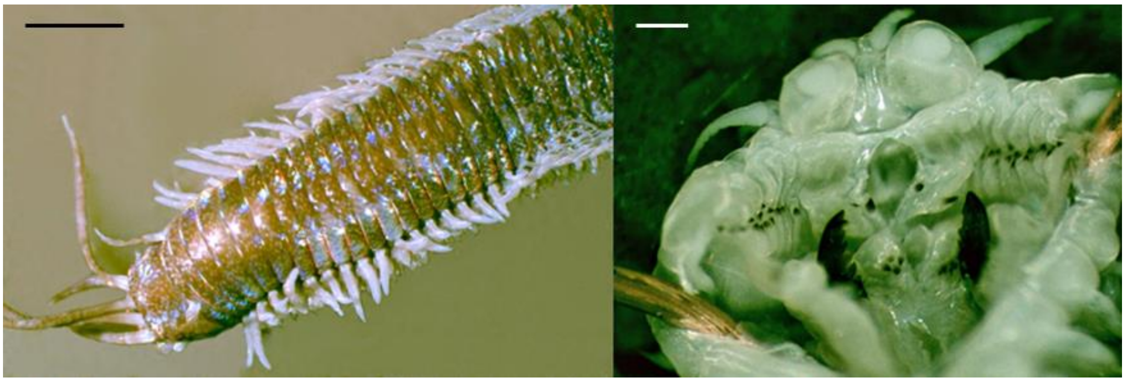


Figure 3.5: The opalescent live rock associated *Eunice* sp. (Family: Eunicidae) (Sample 71). *Left: anterior view of the head featuring five antennae without ringed ceratophores, and a pair of tentacular cirri on the second apodus segment. Scale bar represents 5 mm. Right: mouth displaying the jaws and teeth. Maxillary formula: Mx. I = I + I; Mx. II = 4 + 5; Mx. Ill =7 +0; Mx. IV = 4 + 7; Mk. V = I + I. Scale bar represents 0.4 mm.*

Figure 3.6: The live rock associated *Eurythoe* sp. (Family: Amphinomidae) (Sample 72). *Left: general view of live specimen. Scale bar represents 5 mm. Right: anterior end in dorsal view featuring the long and narrow caruncle and two pairs of eyes. Scale bar represents 0.5 mm.*

Figure 3.7: Dorsal (left) and ventral (right) view of the live rock associated crab *Heteropanope* sp. (Family: Pilumindae) (Sample 168). *Scale bars represent 5mm.*

Figure 3.8: Dorsal (left) and ventral (right) view of the live rock associated crab *Petrolisthes* sp. (Family: Porcellanidae) (Sample 167). *Scale bars represent 5 mm.*

Figure 3.9: The live rock associated starfish *Asterina* sp. (Family: Asterinidae). *Left: aboral view of the specimens sampled. Right: oral view of specimen 44. Scale bars represent 1 cm.*

3.2.2 Molecular phylogenetic identifications

Of the 174 taxa sampled, 105 COI and 103 ITS sequences were generated. The remaining unsequenced samples were amplified but were not sequenced due to financial constraints. Species-level assignments were generated for 54 of the COI sequences generated after BLAST and BOLD searches (identity >98%, E-value of zero) (Table 6.11 – Appendix B). When coupled with phylogenetic analyses, an extra 18 family-level and 2 subfamily identifications were assigned. Only 14 species-level assignments were generated out of the 103 ITS sequences generated (Table 6.12 – Appendix B). Eighty-two percent of the ITS sequences produced uninformative BLAST hits (identity >90%) and could not be identified further by means of phylogenetic analysis. Fortunately, 70% of these uninformative ITS sequences had already been identified by amplification of the COI barcoding region. Twelve ITS sequences produced no BLAST or BOLD hits, nine of which were not sequenced for the COI barcoding region (sample ID's 14-17, 22, 24, 137, 139, and 145). Overall, based on the number of informative species assignments produced through BLAST and BOLD searches and further phylogenetic inference, 90% of the COI barcodes generated species assignments which is significantly higher in comparison to the 13.5% success rate achieved by that of the ITS region. A detailed list of the sequencing success per sample for the COI and ITS coding regions can be found in Table 6.10 of Appendix B.

Twenty-four COI and seven ITS sequences were generated by amplifying DNA from non-target species which are listed in Table 6.13 of Appendix B. Although not part of the aim of this study, additional valuable insight was gained as some of these non-target organisms were identified as species of the coral disease causative agent genus, *Vibrio* (Olivotto et al., 2009; Arboleda and Reichardt, 2010) (Table 6.13 - Appendix B).

All phylogenetic analyses produced highly similar tree topologies for both maximum likelihood (ML) and neighbour-joining (NJ) reconstructions. The forthcoming ML and NJ bootstrap support values, respectively, are listed in parentheses and are categorized as: strong (85-100 %), moderate (70-85 %), and low (50-69 %) (Kress et al., 2002).

(i) phylum Cnidaria

ITS sequence identifications were validated by constructing a phylogenetic tree using ITS sequence data for the soft coral family Xeniidae (Figure 3.10). The reconstruction shows samples 146-159 as incorporated within the *Xenia* genus with strong support (100/100). Samples 146-159 however form a further strongly supported monophyletic clade within *Xenia* (100/100). *Xenia* is inferred as a polyphyletic genus in which several species (i.e. *X. ainex*, *X. actuosa*, and *Xenia* sp.) were dispersed amongst the *Heteroxenia* and *Ovabunda* genera within Figure 3.10. This observation has been verified by previous studies (Janes and Mary, 2012; Stemmer et al., 2012; Haverkort-Yeh, 2013; Janes et al., 2014; Mcfadden et al., 2014; Benayahu et al., 2018). There clearly is confusion surrounding the many taxonomic ambiguities within the Xeniidae family and this emphasizes the importance of morphological analysis by the examination of sclerite microstructure. Fortunately, sclerite analysis of samples 146-159 supports the genus-level identification as *Xenia* sp. (Figure 3.2).

ML and NJ analyses yielded identical tree topologies that both strongly support (100/99) the placement of samples COI 51-61 and COI 63-67 within a monophyletic clade exclusively consisting of *Aiptasia pulchella* reference sequences (Figure 3.11). Two distinct clades arise within Aiptasiidae, placing COI 66 sister to the rest of the COI sequences, but grouped with strong support (91/98) with one *A. pulchella* reference sequence (CNIDC587-17). These six *A. pulchella* reference sequences were the only available COI sequences belonging to the

Aiptasiidae family found on GenBank and BOLD and produced matches to *A. pulchella* (HG423148.1) with an e-value of 0 and identities of 99 and 100%, respectively (Table 6.11 - Appendix B). Non-target amplification of the ITS region of samples 63-67 occurred, resulting in the identification of *A. pulchella*'s symbiotic association *Symbiodinium* sp. (Table 6.13 - Appendix B). ITS amplification of samples 51-61 was successful, however there were no informative hits in the GenBank and BOLD databases. The closest ITS sequence hits for each database belonged to species of the Metridioidea families Sagartiidae (identity <80%) and Metridiidae (similarity <80%), respectively (Table 6.12 – Appendix B).

(ii) phylum Annelida

Originally, the phylogeny of all polychaete orders (Phyllodocida, Eunicida, Amphinomida, and Sabellida) were analysed together. Each order formed a monophyletic clade and had strong support for both ML and NJ analyses. Thereafter, each order was analysed separately to allow for better resolution and to reduce the large-scale complexity of inferring species identifications from one tree.

The phylogenetic reconstruction of the fanworm family Sabellidae based on COI sequences grouped the 108-121 samples into an exclusive and strongly supported clade (100/100) and is positioned within a monophyletic clade consisting of individuals belonging to the genus *Bispira* (Figure 3.12). These 14 samples are sister to *Bispira monroi* (YCHAE298-08). The greater *Bispira* clade has strong ML (90) and moderate NJ (77) support. BLAST searches of the ITS sequences generated for the same samples also resulted in hits for the species *Bispira manicata* (KX894910.1; e-value: 0.0; identity: 89%) (Table 6.12 - Appendix B). GenBank did not have sufficient ITS sequence coverage of the Sabellidae family to produce an informative phylogenetic tree.

Figure 3.13 illustrates a reconstruction of Phyllodocida COI sequence data. COI samples 68 and 70 formed a strongly supported clade (100/100) within the moderately supported (78/69) Nereididae clade. These queried sequences are sister to *Pseudonereis gallapagensis* with weak ML and NJ support. *Pseudonereis* is inferred as a polyphyletic genus in which several species (i.e. *P. anomala, P. gallapagensis*, and *P. variegata*) were dispersed amongst the *Nectoneanthes*, *Perinereis*, *Alitta*, and S*implisetia* genera within Figure 3.13. Morphologically identified as *Perinereis* sp., the COI 68 and 70 clade is sister to a weakly supported (50/-) polyphyletic clade that consists of *Perinereis*, *Nereis*, and *Neanthes* genera.

The Chrysopetalidae family of phyllodocids is sister and basally situated to the Nereididae clade (Figure 3.13). Overall, it has strong ML (88) but weak NJ (50) support. The reconstruction shows that sample COI 89 forms a strongly supported (100/100) monophyletic clade with *Bhawania cryptocephala*. This grouping is sister to *Bhawania heteroseta* samples with strong ML (90) and moderate NJ (69) support. The placement of COI 89 within the *Bhawania* genus is supported by morphological analysis.

The COI 93 and 96 phyllodocids form a strongly supported (100/100) exclusive cluster within the monophyletic strongly supported (94/92) Syllinae subfamily clade of Syllidae (Figure 3.13). Further genus-level inference was not possible due to the limited sequencing coverage of Syllinae species in GenBank and BOLD.

Two Amphinomida families are represented by two major monophyletic clades within the phylogenetic reconstruction of COI sequence data: the moderately supported (70/76) Amphinominae, and the strongly supported (88/88) Archinominae (Figure 3.14). The 29 queried COI samples sequenced within this study (72, 74-85, 88, 90-92, 103-106, 122-128, and 161) formed a strongly supported (100/100) monophyletic clade within the Amphinominae family, grouping with four *Eurythoe complanata* and one *Eurythoe* sp. reference sequences. BLAST and GenBank searches of these queried COI samples produced matches to *E. complanata* (KY972418.1) with an e-value of 0 and identities ranging between 98-99% (Table 6.11 - Appendix B). The placement of the aforementioned COI samples within the *E. complanata* clade is supported by morphological analysis which assigned the samples to the genus *Eurythoe* (Figure 3.6). These inferred *E. complanata* specimens are sister taxa to members of the *Pherecardia* genus (Figure 3.14).

A phylogenetic reconstruction of species belonging to the order Eunicida using COI sequence data reveals five families within Figure 3.15. Specimens sequenced in this study (COI 71, 73, 86, 87, and 95) all fall within the moderately supported (79/80) Eunicidae family clade. COI 95 grouped with *Eunice harassii* (GQ497535) with strong support (99/99), and is nested within a greater monophyletic *Eunice* sp. clade with strong ML (100) and moderate NJ (80) support. GenBank and BOLD searches match COI 95 to *Eunice harassii* (GQ497535) with an e-value of 0 and identities of 99% and 99.54% respectively. Members of the genus *Marphysa* form a strongly supported (96/90) monophyletic clade that is sister to the previously mentioned *Eunice* clade. Overall, the *Eunice* genus is polyphyletic within the Eunicidae family clade. Strongly supported by ML analyses (96), members of the *Lysidice* genus form a sister clade to another monophyletic *Eunice* sp. clade. This clade groups COI 71 and 73 into an exclusive strongly supported cluster (100/99) that is sister to *E. mutilate* (GQ497540) and *E. amoureuxi* (GQ497538). When performing a BLAST search for COI 71 and 73, GenBank returns *Palola* sp. (DQ317813.1; e-value: 7,00E-121; identity: 80%) and *Palola* sp. (KT124732.1; e-value: 4,00E-98; identity: 78%) as the strongest hit, whereas BOLD searches return *Eunice* sp. (YCHAE114-08; similarity: 80.58%) and *Eunice* sp. (YCHAE097-08; similarity: 80.41%), respectively (Table 6.11 - Appendix B). Morphological identifications support the phylogenetic placement of COI within the *Eunice* genus, assigning sample 71 to *Eunice* sp. (Figure 3.5) and sample 73 to *Eunice petersi*.

COI 86 and 87 grouped closely with *Lysidice ninetta* with strong support (100/99) within a greater monophyletic clade of *Lysidice* spp. members with moderate support (74/62) (Figure 3.15). Both GenBank and BOLD searches resulted in an e-value of 0.0 and identities of 100% for *Lysidice ninetta* (KR916860.1) for samples COI 86 and 87 (Table 6.11 - Appendix B).

(iii) phylum Arthropoda

A phylogenetic reconstruction using COI sequence data for the family Idoteidae within the order Isopoda revealed that the queried COI 160 sample occupied a more basal position within the tree, and is the most basal sister to taxa of the genus *Synidotea* (Figure 3.16). COI 160 and *Synidotea* sp. form a monophyletic clade with weak ML but strong NJ (85) support. The reconstruction revealed no close evolutionary relationship to *Idotea urotoma* (MH242804.1) which was the strongest hit for both GenBank and BOLD searches (e-value: 0.0; identity/similarity: 85%) (Table 6.11 - Appendix B).

ML and NJ analyses yielded identical tree topologies that both place COI 162, 163, and 169 into a strongly supported exclusive cluster (100/100) within the Amphipoda family, Hyalidae (Figure 3.17). COI 162 and 163 form a further strongly supported exclusive clade (100/100). These samples are sister to a weakly supported clade that branches into a moderately supported (75/71) monophyletic clade consisting of members of the *Parhyale* genus, and a strongly supported (100/100) monophyletic clade of *Apohyale* spp. (Figure 3.17).

(iv) phylum Echinodermata

Phylogenetic analyses of the Asterinidae family placed COI 44-50 in a strongly supported monophyletic clade (100/100) with all the other reference *Asterina* sp. (Figure 3.18). These seven specimens formed a further strongly supported exclusive cluster (100/100) together with three reference sequences listed as *Asterina* sp., and is further validated by morphological analysis (Figure 3.9). These inferred *Asterina* sp. specimens are sister to *A. pancerii*, all within a clade with low branch support (58/52) (Figure 3.18). GenBank BLAST and BOLD searches produced *Asterina* sp. (KP768168.1; e-value: 0.0; identity: 99%) and *Asterina gibbosa* (GBEH7302-19; similarity: 93%) as best hits, respectively (Table 6.11 – Appendix B).

Phylogenetic analyses of ITS samples 9 - 13, 18-21, and 23 was not possible as GenBank BLAST searches were not informative, producing hits of species belonging to the order Valvatida (starfish) instead of Ophiurida (brittle stars) to which they were morphologically identified to belong (Appendix B - Table 6.12).

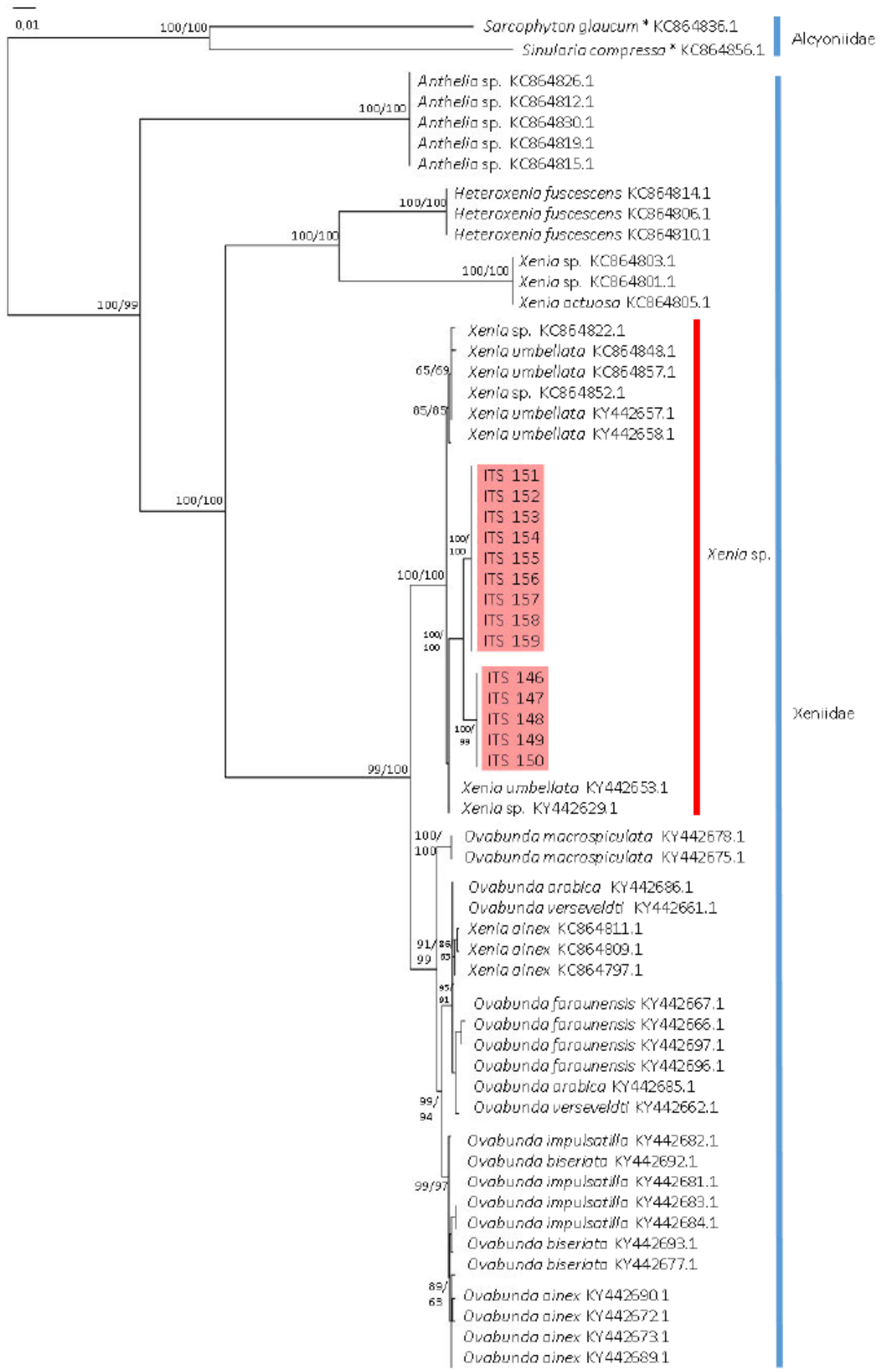


Figure 3.10: A neighbour-joining reconstruction of family Xeniidae based on nuclear ribosomal internal transcribed spacer (ITS) sequences. *Branch labels indicate maximum likelihood and neighbour joining bootstrap support values respectively (%). Only bootstrap values greater than 50% are indicated. A hyphen is used as a placeholder where a bootstrap is below 50%. Clade colours: blue = family; red = the taxa group to which the unidentified species most likely belongs. Taxa highlighted in light red were sequenced in the present study. GenBank accession numbers and BOLD ID's for sequence data are listed after the species name. Branch length scales are indicated. Sequences used to represent the outgroup are marked with an asterisk.*

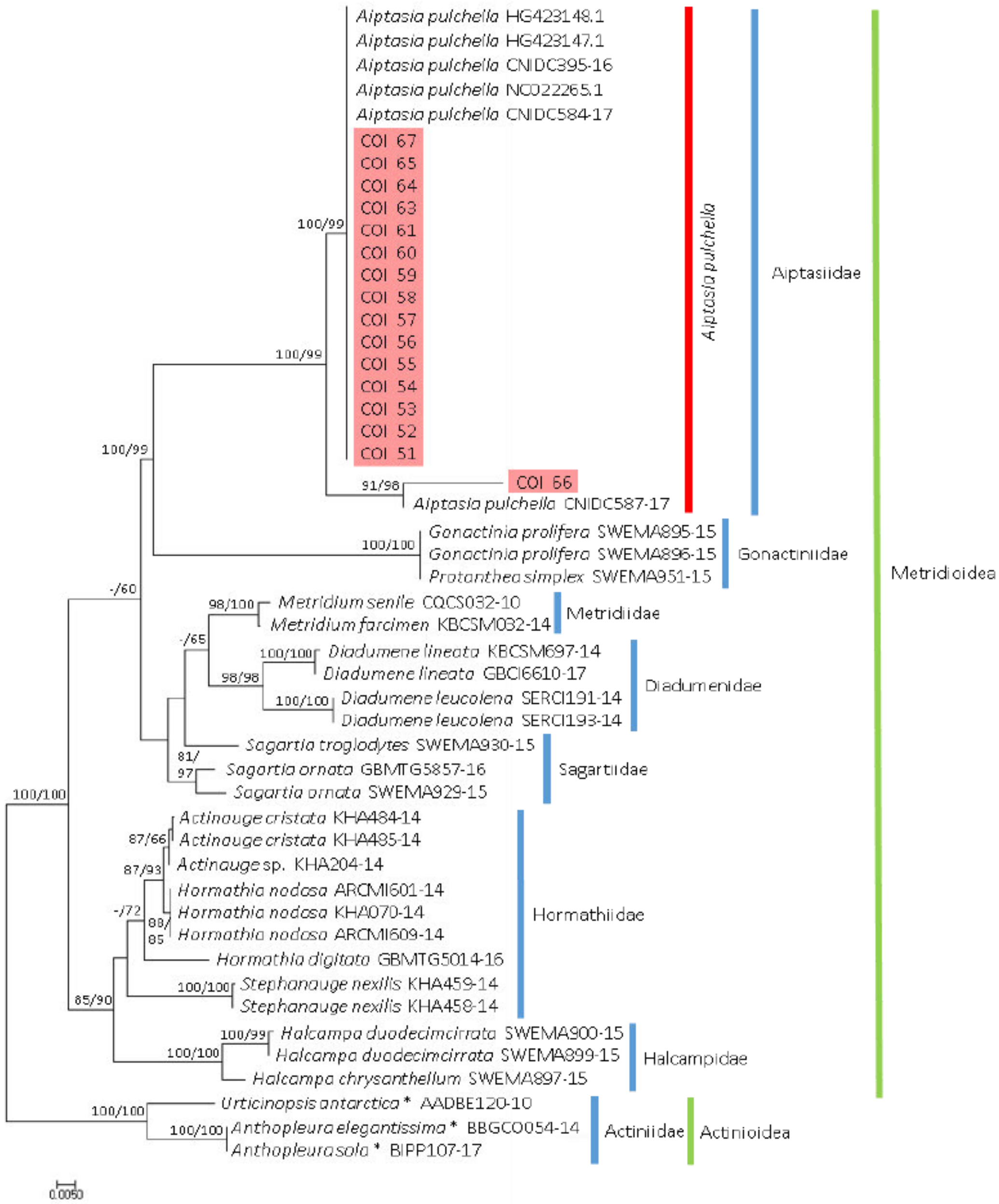


Figure 3.11: A neighbour-joining reconstruction of superfamily Metridioidea based on mitochondrial cytochrome *c* oxidase I (COI) sequences. *Branch labels indicate maximum likelihood and neighbour joining bootstrap support values respectively (%). Only bootstrap values greater than 50% are indicated. A hyphen is used as a placeholder where a bootstrap is below 50%. Clade colours: green = superfamily; blue = family; red = the taxa group to which the unidentified species most likely belongs. Taxa highlighted in light red were sequenced in the present study. GenBank accession numbers and BOLD ID's for sequence data are listed after the species name. Branch length scales are indicated. Sequences used to represent the outgroup are marked with an asterisk.*

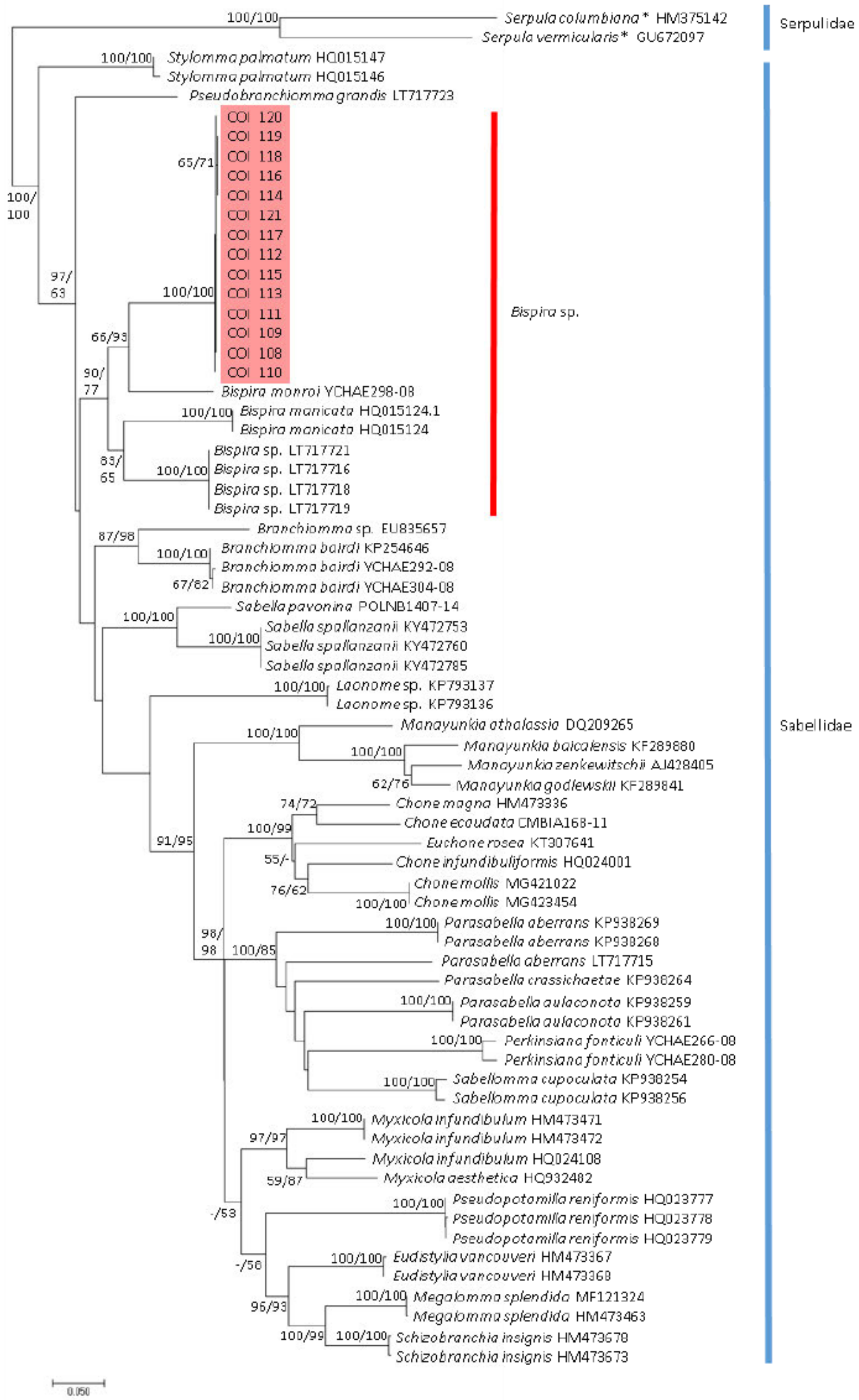


Figure 3.12: A neighbour-joining reconstruction of family Sabellidae based on mitochondrial cytochrome *c* oxidase I (COI) sequences. *Branch labels indicate maximum likelihood and neighbour joining bootstrap support values respectively (%). Only bootstrap values greater than 50% are indicated. A hyphen is used as a placeholder where a bootstrap is below 50%. Clade colours: blue = family; red = the taxa group to which the unidentified species most likely belongs. Taxa highlighted in light red were sequenced in the present study. GenBank accession numbers and BOLD ID's for sequence data are listed after the species name. Branch length scales are indicated. Sequences used to represent the outgroup are marked with an asterisk.*

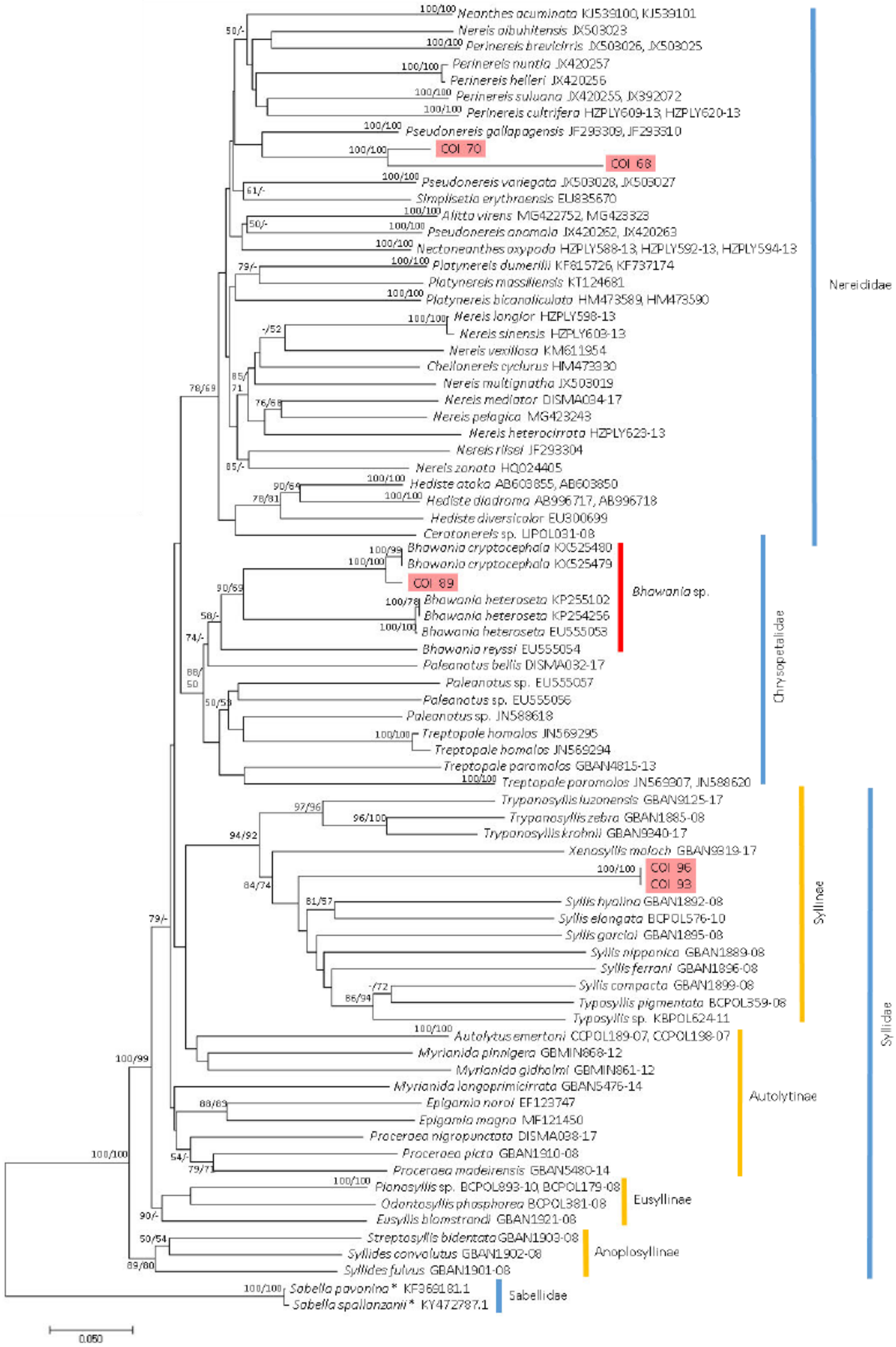


Figure 3.13: A neighbour-joining reconstruction of order Phyllodocida based on mitochondrial cytochrome *c* oxidase I (COI) sequences. *Branch labels indicate maximum likelihood and neighbour joining bootstrap support values respectively (%). Only bootstrap values greater than 50% are indicated. A hyphen is used as a placeholder where a bootstrap is below 50%. Clade colours: blue = family; orange = subfamily; red = the taxa group to which the unidentified species most likely belongs. Taxa highlighted in light red were sequenced in the present study. GenBank accession numbers and BOLD ID's for sequence data are listed after the species name. Branch length scales are indicated. Sequences used to represent the outgroup are marked with an asterisk.*

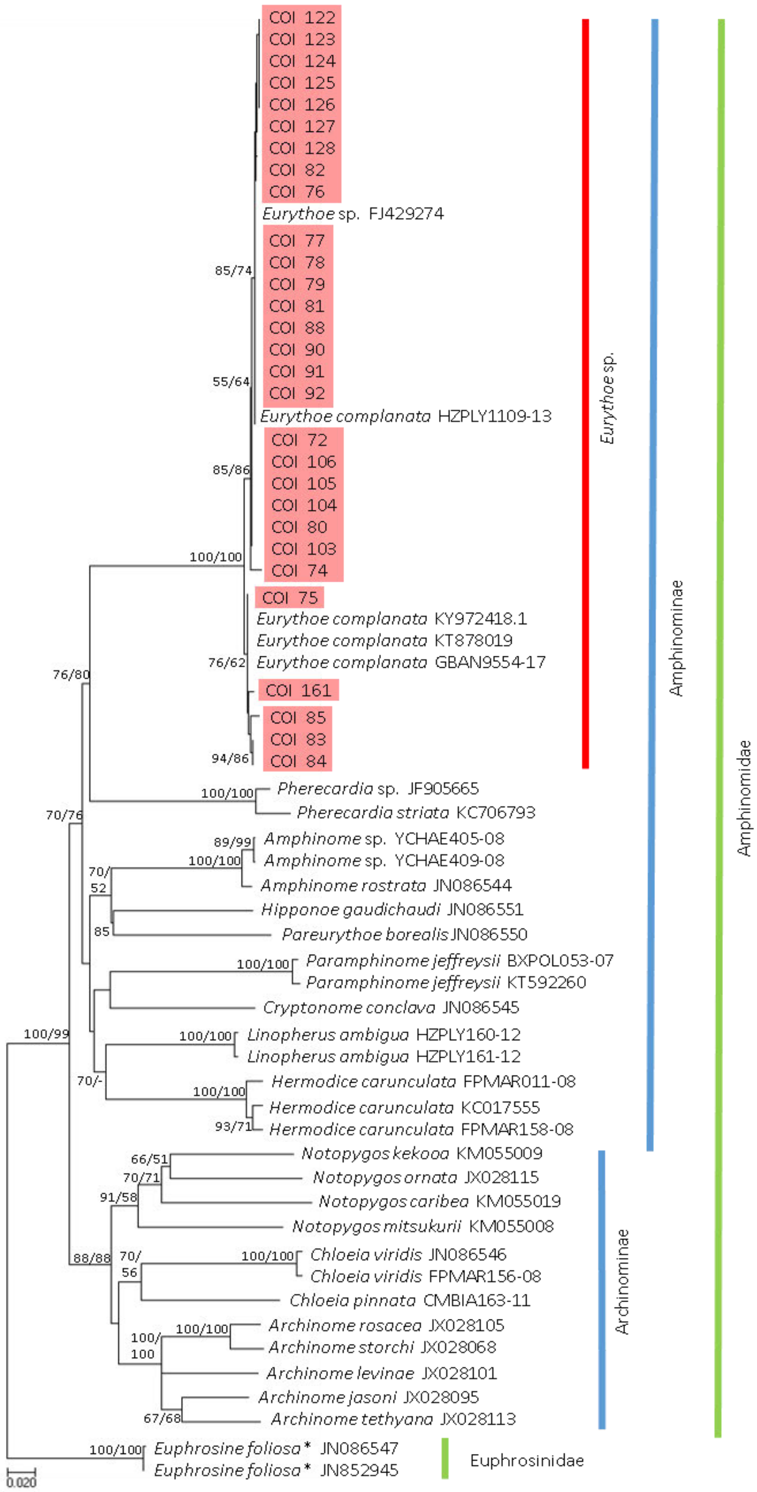


Figure 3.14: A neighbour-joining reconstruction of order Amphinomida based on mitochondrial cytochrome *c* oxidase I (COI) sequences. *Branch labels indicate maximum likelihood and neighbour joining bootstrap support values respectively (%). Only bootstrap values greater than 50% are indicated. A hyphen is used as a placeholder where a bootstrap is below 50%. Clade colours: green = order names; blue = family; red = the taxa group to which the unidentified species most likely belongs. Taxa highlighted in light red were sequenced in the present study. GenBank accession numbers and BOLD ID's for sequence data are listed after the species name. Branch length scales are indicated. Sequences used to represent the outgroup are marked with an asterisk.*

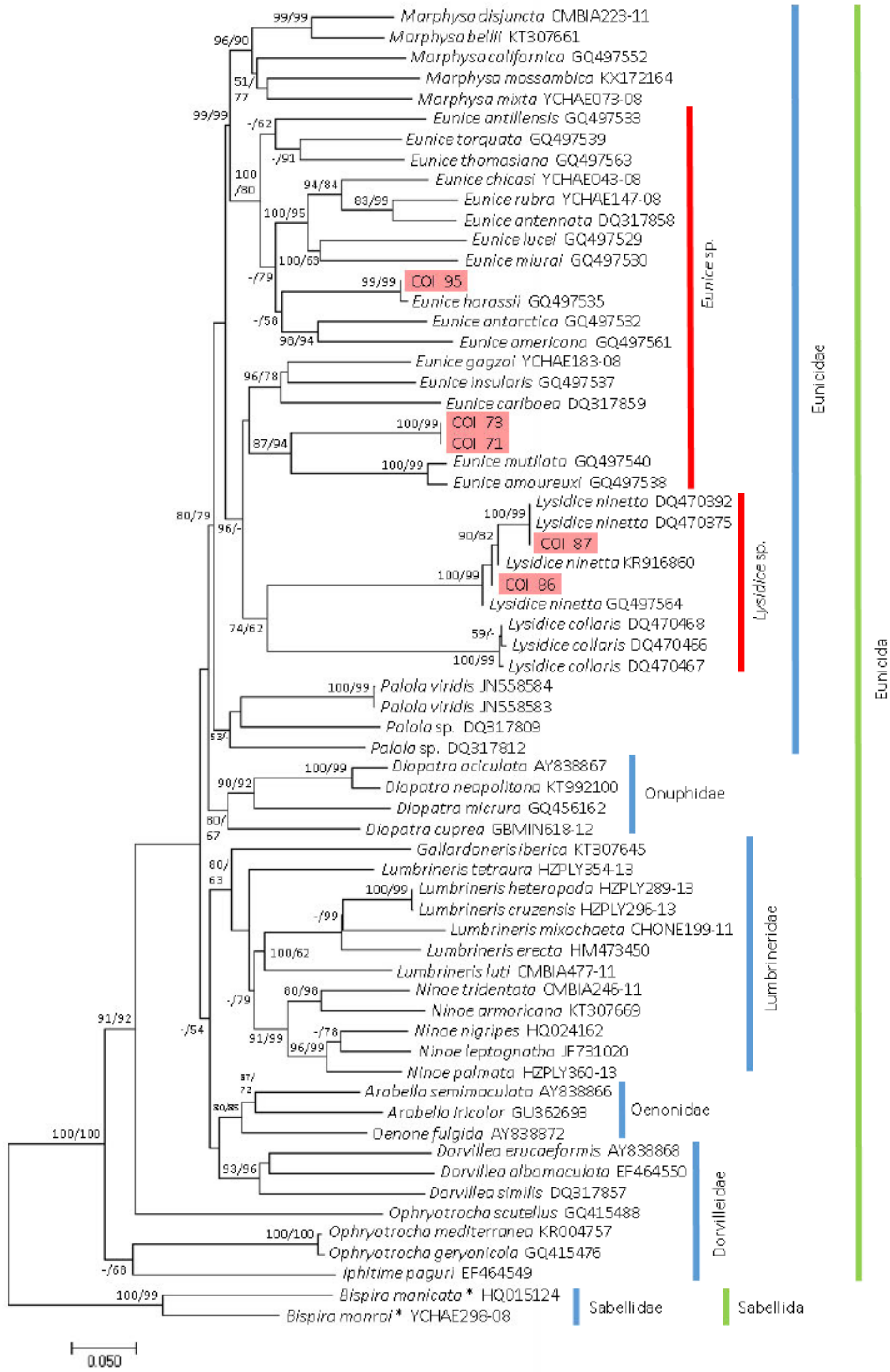


Figure 3.15: A neighbour-joining reconstruction of order Eunicida based on mitochondrial cytochrome *c* oxidase I (COI) sequences. *Branch labels indicate maximum likelihood and neighbour joining bootstrap support values respectively (%). Only bootstrap values greater than 50% are indicated. Clade colours: green = family; blue = subfamily; red = the taxa group to which the unidentified species most likely belongs. Taxa highlighted in light red were sequenced in the present study. GenBank accession numbers and BOLD ID's for sequence data are listed after the species name. Branch length scales are indicated. Sequences used to represent the outgroup are marked with an asterisk.*

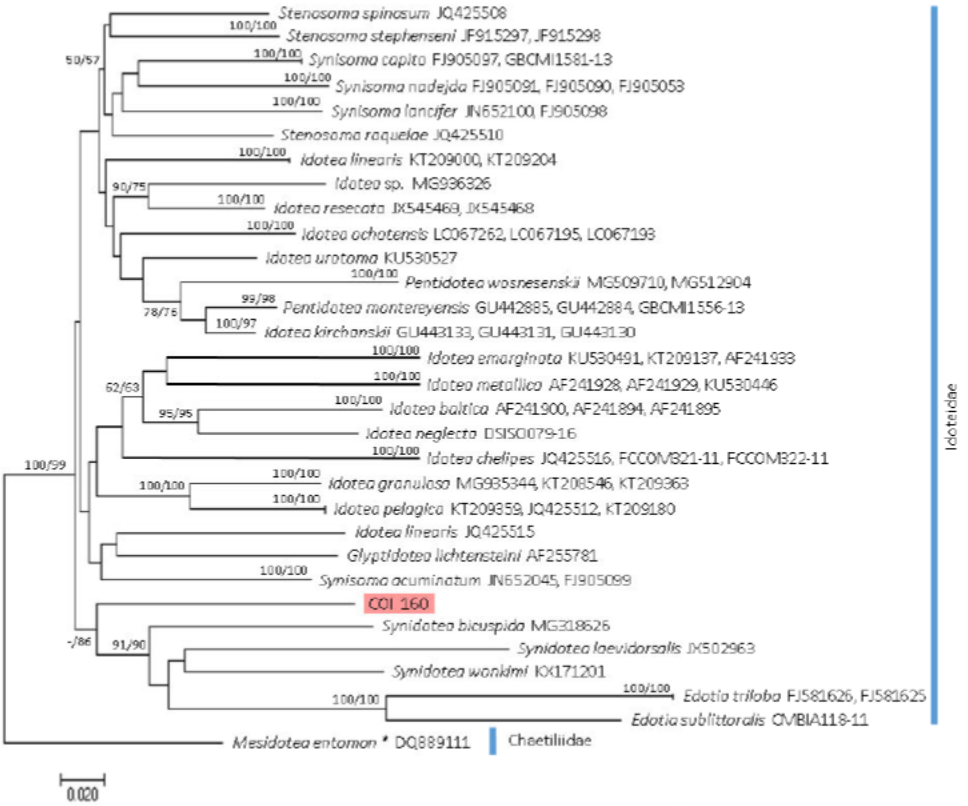


Figure 3.16: A neighbour-joining reconstruction of order Isopoda based on mitochondrial cytochrome *c* oxidase I (COI) sequences. *Branch labels indicate maximum likelihood and neighbour joining bootstrap support values respectively (%). Only bootstrap values greater than 50% are indicated. Clade colour: blue = family. Taxa highlighted in light red were sequenced in the present study. GenBank accession numbers and BOLD ID's for sequence data are listed after the species name. Branch length scales are indicated. Sequences used to represent the outgroup are marked with an asterisk.*

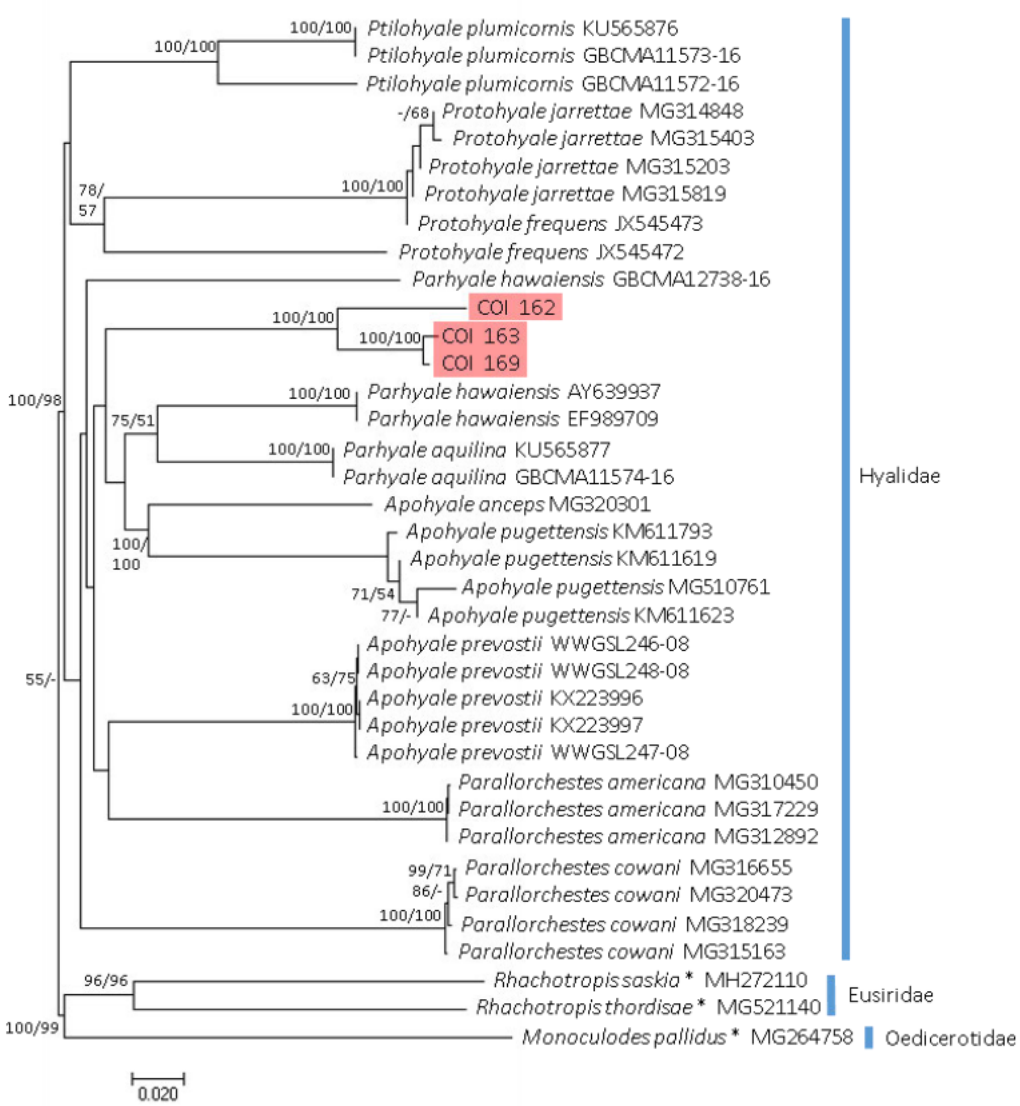


Figure 3.17: A neighbour-joining reconstruction of order Amphipoda based on mitochondrial cytochrome *c* oxidase I (COI) sequences. *Branch labels indicate maximum likelihood and neighbour joining bootstrap support values respectively (%). Only bootstrap values greater than 50% are indicated. Clade colour: blue = family. Taxa highlighted in light red were sequenced in the present study. GenBank accession numbers and BOLD ID's for sequence data are listed after the species name. Branch length scales are indicated. Sequences used to represent the outgroup are marked with an asterisk.*

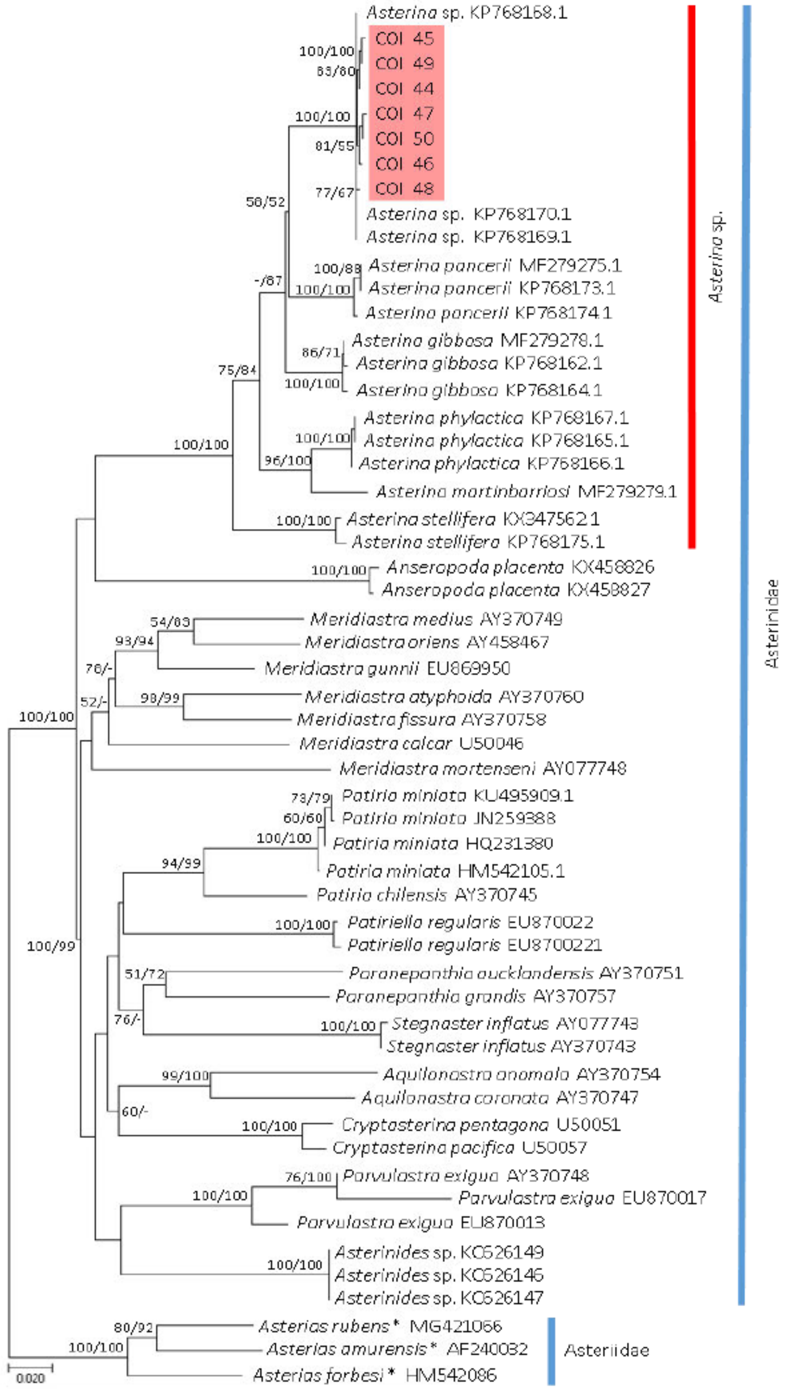


Figure 3.18: A neighbour-joining reconstruction of family Asterinidae based on mitochondrial cytochrome *c* oxidase I (COI) sequences. *Branch labels indicate maximum likelihood and neighbour joining bootstrap support values respectively (%). Only bootstrap values greater than 50% are indicated. A hyphen is used as a placeholder where a bootstrap is below 50%. Clade colours: blue = family; red = the taxa group to which the unidentified species most likely belongs. Taxa highlighted in light red were sequenced in the present study. GenBank accession numbers and BOLD ID's for sequence data are listed after the species name. Branch length scales are indicated. Sequences used to represent the outgroup are marked with an asterisk.*

3.2.3 Final species and invasion status assignments

Of the 174 taxa harvested from the live rock in this study, 67 specimens could be assigned to the species-level, 53 to the genus-level, and 20 to the family-level. 97% of the species-level, 47% of the genus-level, and 20% of the family-level assignments were inferred by a combination of both morphological and molecular phylogenetic identifications, where the remainder were identified based on morphology alone. The remaining taxa (20 order-level, 5 subclass-level, and 7 class-level) could only be identified by gross morphological characteristics. The documented global distribution ranges and biogeographic statuses (i.e. native or alien) with respect to South African waters is summarized for the eight species identified in this study (Table 3.4).

Aiptasia pulchella sampled in this study is listed as invasive on the Delivering Alien Invasive Species In Europe (DAISIE) project database. Both *Eunice harassii* and *Eunice petersi* have distributions within the Western Indian Ocean, however, there are no official records that state that they are native to South African waters. Upon review, the alien eunicids *E. harassii*, *E. petersi*, and *Lysidice ninetta* have not been documented as invasive or of a pest-like nature. Although not listed as alien to South Africa, the amphinomid *Eurythoe complanata* will be discussed further as it occurred in high numbers on four of the six replicates of live rock. The biogeographical ranges of the 7 identified genera could not be confirmed due to genus-level identifications. However, after a review of the literature, the starfish *Asterina*, the fanworm *Bispira*, and the pilumnid crab *Heteropanope* genera are of particular interest.

Table 3.4: Species-level identifications assigned to live rock associated taxa sampled in this study using a combination of molecular and morphological data. *Highlighted species are known to be invasive. The biogeographic statuses were assigned with regards to South African waters and according to the framework proposed by Robinson et al. (2016). The number after the live rock source indicates the replicate number.*

Phylum	Class	Order	Family	Species	Sample	No. of specimens	Live rock source	Documented distribution	Biogeographic status
Annelida	Polychaeta	Amphinomida	Amphinomidae	*Eurythoe complanata*	72; 74 - 81	9	Kenya 1	Mediterranean Sea, Indo-Pacific, Atlantic Ocean. Tropical and subtropical (Arias et al., 2013; Gibbs, 1978)	Native
					82 - 85; 88; 90 - 92	7	Kenya 2		
					161	1	Indonesia 1		
					103 - 106; 122 - 128	11	Indonesia 2		
		Eunicida	Eunicidae	*Eunice harassii*	95	1	Kenya 2	Northeast Atlantic, Western Indian Ocean, Mediterranean Sea, Red Sea (Bellan, 2001; Wehe and Fiege, 2002)	Alien, in a contained facility
				Eunice petersi	73	1	Kenya 1	Indo-West Pacific: Red Sea, New Caledonia (Wehe and Fiege, 2002), Mozambique (Fauchald, 1992)	Alien, in a contained facility
				Lysidice ninetta	86 & 87	2	Kenya 2	Indo-Pacific, Atlantic Ocean (Day, 1967) Mediterranean Sea (Bellan, 2001)	Alien, in a contained facility
		Phyllodocida	Chrysopetalidae	*Bhawania cryptocephala*	89	1	Kenya 2	Red Sea, Indian Ocean, Pacifc Ocean (Hartman, 1954), Gulf of Aden, New Caledonia, Philippine Islands (Fauvel, 1953)	Native
Cnidaria	Anthozoa	Actiniaria	Aiptasiidae	***Aiptasia pulchella***	51 - 67	17	Indonesia 3	Pacific Ocean (Howe et al., 2012), Bay of Biscay (Gruet et al., 1976), Mediterranean Sea (Miralles et al., 2018a)	Alien, in a contained facility
		Alcyonacea	Xeniidae	*Xenia umbellata*	146 - 159	14	Kenya 1	Tropical Indo-Pacific (Fabricius and Alderslade, 2001; Williams, 1992), Red Sea (Vine, 1986)	Native
Mollusca	Bivalvia	Mytilida	Mytilidae	*Septifer bilocularis*	144 & 145	2	Indonesia 2	Indo-Pacific: East and South Africa (Branch et al., 1994), Red Sea (Vine, 1986), Hong Kong (Morton, 1991), Japan, Australia, eastern Polynesia and New Caledonia (Poutiers, 1998)	Native

3.3 Discussion

The aim of this study was to determine if live rock is a vector for marine alien invasive species into South Africa. In total, 174 marine invertebrate specimens were harvested from the combined replicates of Kenyan and Indonesian live rock. A combination of morphological and molecular identifications allocated 67 specimens to 8 species, and 53 specimens to 7 genera, accounting for 69% of the total identifications assigned. Accurate distribution ranges, biogeographic satuses with respect to South African waters (i.e. native or alien), and invasion statuses could only be reported for 8 species. Four of the eight species are alien (in a contained facility) to South Africa, with one of them listed as invasive (*Aiptasia pulchella*).

3.3.1 First official record of an invasive live rock associated species in South Africa

Seventeen specimens of the alien *Aiptasia pulchella* were harvested from the third Indonesian live rock replicate. This was the second-most commonly detected species. *A. pulchella* is alien to South African waters, and although it is not listed on the GRIS or GISD databases, it is listed as an invasive species on DAISIE. It is also referred to as being invasive and observed to have a pest- and weedy-like nature in numerous studies (Steen, 1986; Grizel and Héral, 1991; Chen et al., 2008; Colin, 2009; Howe et al., 2012). *A. pulchella* is found throughout the tropical and subtropical oceans of the Indo-Pacific (Howe et al., 2014) with a documented distribution that spans French Polynesia, Japan, Hawaii, the central American coast of the eastern Pacific (Colin and Arneson, 1995; Howe et al., 2012), the Bay of Biscay (Gruet et al., 1976), and the Mediterranean Sea (Miralles et al., 2018a). There is no literature that describes the native environmental conditions (i.e. salinity and thermal ranges, substrate type) in which *A. pulchella* thrives and it is therefore not possible to predict with certainty if it could survive in South African waters. However, taking the documented distribution ranges into consideration, *A. pulchella* is more likely to survive and thrive if it was released into the subtropical waters along the east coast of South Africa. Only one *Aiptasia* species is native to South African waters, namely *A. parva*, and its distribution occurs from East London to Sodwana Bay (Laird and Griffiths, 2016).

The third Indonesian live rock replicate was the only replicate that was purchased from a pet store that had placed it into their holding aquaria. There is a possibility that the pet store was dishonest when claiming that the live rock had only been in their aquaria for one day for the sake of making a sale. It is therefore not certain if the *A. pulchella* specimens sampled in this study were imported with the live rock or if the specimens originated from the pet store's holding aquaria and attached themselves to the live rock. The latter is a possibility as glass anemones (*Aiptasia* sp.) were observed in marine aquaria in 5 of the 9 pet stores inventoried (Chapter 5.3). Irrespective of the mechanism, the *A. pulchella* organisms could have been introduced to a hobbyist's aquarium if they had purchased the live rock.

There is much uncertainty in the literature regarding the identity of *A. pulchella*, with most commentators referring to its sister species *A. pallida* and *Exaiptasia pallida* as the invasive glass anemone (Calado and Narciso, 2005; Grajales and Rodríguez, 2016). Often, the entire genus is regarded as pest-like by marine hobbyists and is loosely referred to as *Aiptasia* (Brough, 2015; Barrington, 2018). Grajales and Rodríguez (2014, 2016) have attempted to revise the systematics of the genus *Aiptasia* and the greater family Aiptasiidae by morphological and phylogenetic methods. Molecular data has revealed that the genus is not monophyletic (Grajales and Rodríguez, 2016). Without a species-level identification, it is rather challenging to arrive at an accurate invasion status.

The phylogenetic reconstruction of the sampled taxa grouped them with *Aiptasia pulchella* with high support (100/99) (Figure 3.11). Due to the poor coverage of Aiptasiidae COI sequence data in GenBank and BOLD, only 6 unpublished reference sequences reported as *Aiptasia pulchella* could be used in the phylogenetic analysis. Taking Grajales and Rodríguez's (2016) findings into account, it could be possible that the reference sequence *Aiptasia pulchella* (CNIDC587-17) was incorrectly identified due to its detached positioning within the Aiptasiidae clade. Consequently, one of the 17 sampled sea anemones (66) in this study could be of a different species.

Aiptasia sp. are not new to hitch-hiking, with studies reporting their introduction on oysters for aquaculture purposes (Grizel and Héral, 1991), by hull fouling (Farrapeiran et al., 2007; Canning-Clode et al., 2013) and on macroalgae, live rock, and corals purchased for marine hobbyists' aquaria (Calfo, 2004; Rhyne et al., 2004; Odom, 2012; Ram, 2013; Howe et al., 2014). Their rapid and weedy proliferation is a consequence of their ability to self-fertilize, which is a characteristic observed in founder organisms that have been introduced and establish themselves in new areas (Schlesinger et al., 2010). This justifies the observed competition between *Aiptasia* sp. and the notorious invader *Caulerpa racemosa* (Keith, 2016). Undoubtedly, this is a species of concern and there is an urgent need to evaluate the risk of its introduction and establishment in South Africa's coastal waters.

3.3.2 Live rock associated species of concern

Both Kenyan and Indonesian live rock displayed macroalgae assemblages and corraline algae and this serves as a preliminary confirmation that live rock is a vector for the aforementioned taxa into South Africa. Further investigations into their identities are necessary to quantify the risk posed by live rock as an introduction vector thereof. Although species-level identifications were not confirmed for all the live rock associated mussels (family Mytilidae), it is worthwhile to note that live rock could be a potential additional vector for the introduction of mytilid invaders. The following live rock associated taxa sampled in this study are of particular interest and will be elaborated on below:

(i) The common fireworm, *Eurythoe complanata*

Although native to South African waters, *E. complanata* is of particular interest as it was the most numerous species harvested from four of the six live rock replicates (Table 3.4). *E. complanata* has been considered a cosmopolitan species having a wide circumtropical distribution (Arias et al., 2013). With the aid of molecular phylogenetic analyses, Barroso, Klautau and Paiva (2010) recently demonstrated that *E. complanata* consists of at least three cryptic species. As a result, the biogeographic status of this species is questionable (Zenetos et al., 2010, 2012) and needs to be re-evaluated as this coincides with the observation that highly invasive species are likely to reach nearly cosmopolitan distribution ranges (Hutchings and Kupriyanova, 2018).

Of the 24 *Eurythoe* sp. COI sequences deposited in GenBank, only 5 positive hits were produced when a BLAST search was performed against the queried sequence. When performing a search in BOLD, 83 *Eurythoe complanata* matches produced similarities over a range of 80.06 – 100%. Although most likely attributed to morphological misidentifications that were used when depositing the sequences, part of this vast range of identity scores could also be explained by Barroso, Klautau and Paiva's (2010) observations of the *E. complanata* cryptic species complex.

With regard to its reproductive habits, *E. complanata* shows high biological plasticity and often goes through cycles of asexual and sexual reproduction. During asexual cycles, these polychaete worms can fragment into two or more parts and subsequently regenerate their anterior and posterior ends (Kudenov, 1974). Furthermore, *E. complanata*'s rapid growth and regeneration rates, together with its long-enduring rostraria larvae, permit its large dispersal capability

(Kudenov, 1974). These are characteristics that have given members of the family Amphinomidae their high invasive potential (Arias et al., 2013; Schulze et al., 2017).

E. complanata has been reported to breed well in marine aquaria and can become quite prolific (Mascow, 2011). Hobbyists believe that *E. complanata* forms part of the beneficial 'clean-up crew' in a marine aquarium by feeding on excess organic matter and thereby contributes to the overall health of the 'mini-ecosystem' (anonymous, pers. comm., 2017). This benefit of *E. complanata* has resulted in an American company deliberately breeding and selling this species to marine hobbyists who then introduce them into their aquaria (Sustainable Aquatics, 2019). However, one drawback for hobbyists is the fact that *E. complanata* is covered with bristles containing a potent venom that can cause a painful sting when touched or handled (Nakamura et al., 2008).

(ii) The starfish genus, *Asterina*

The *Asterina* sp. starfish is a pest that is commonly observed by marine aquarium hobbyists and it is assumed that it is introduced by the addition of live rock (Helgason, 2016; Haselden, 2017; Fenner, 2019). The Global Register of Introduced and Invasive Species (GRIIS) database lists *Asterina burtoni* as invasive. Streftaris and Zenetos (2006) define *A. burtoni* as one of the 100 'worst invasives' in the Mediterranean Sea after the species was introduced and outcompeted the native *A. gibbosa* (Achituv, 1973; Galil and Zenetos, 2002; Galil, 2014). Although a species-level identification was not made for the *Asterina* sp. starfish in this study, it is likely that it could share a similar prolific fissiparous asexual reproduction method that facilitated the rapidly attained population density of its invasive relation *A. burtoni* (Karako et al., 2002). Since *Asterina* sp. often cause an unsightly plague across aquaria glass and have on the rare occasion been reported to prey on coral (Whitby, 2015), marine aquarium hobbyists often turn to a means of biological control by adding the harlequin shrimp (*Hymenocera picta*) (Dennison, 2019).

(iii) The fanworm genus, *Bispira*

When harvested, it was noted that the 15 *Bispira* sp. specimens were part of a dense and complex aggregation of soft and flexible tubes housing approximately 30 individuals. Not all individuals were sampled as they were difficult to harvest from within their tubes. This observation is characteristic of *Bispira* species (Dávila-Jiménez et al., 2017). Although not transported through the aquarium trade, AquaNIS reports the successful introduction of *B.*

polyomma and *B. fabricii* into European waters. The *Bispira* genus belongs to the family Sabellidae, to which a number of invasive fanworms belong, such as the infamous *Sabella spallanzanii* (Read et al., 2011; Ahyong et al., 2017) and *Branchiomma bairdi* (Khedhri et al., 2017). The presence of fanworms on live rock is generally regarded as an asset to marine aquaria due to their filter-feeding ability which aids in biologcal filtration and the subsequent removal of excess organic waste from the water column (Giangrande et al., 2005).

(v) The pilumnid crab genus, *Heteropanope*

GRIIS and DAISIE database searches confirm that the species *Heteropanope laevis* (synonyms: *Glabropilumnus laevis*, *Pilumnus laevis*) is an alien invasive in Mediterranean waters and it is also listed by Streftaris and Zenetos (2006) as one of the 100 'worst invasives'. *H. laevis* is, however, native to South African waters and the greater Indian Ocean (Grosholz, 2011). The pilumnid in this study may be *H. glabra* according to a Brachuryan taxonomic expert (Dr R. Naderloo, pers. comm., 2017) after morphological examination (Figure 3.7). This species is not native to South African waters and is distributed within the Indo-West Pacific and eastern Atlantic regions (Kaullysing et al., 2015).

Although their work is not peer-reviewed, marine aquarium hobbyists Charles and Linda Raabe enlisted the help of Dr Peter Castro (California State Polytechnic University) and curator Sandy Trautwein (Aquarium of the Pacific in Long Beach California) in identifying crabs associated with live rock obtained from a number of fellow hobbyists (Raabe and Raabe, 2016). Together they identified crabs belonging to 12 families: Xanthidae, Majidae, Porcellanidae, Galatheidae, Dromiidae, Portunidae, Parthenopidae, Calappidae, Leucosiidae, Cryptochiridae, Pinnotheridae, and Inachidae (Raabe and Raabe, 2016). Their observations provide insight into the extensive crab diversity that are associated with live rock within the marine aquarium trade.

3.3.3 Non-targeted pathogenic taxa

The risk posed by live rock through the introduction of alien invasive species is gradually being recognized, with most of the attention focused on the transmission of invasive macroalgae, i.e. *Caulerpa taxifolia* and *Caulerpa racemosa* (Stam et al., 2006; Walters et al., 2006, 2011; Vranken et al., 2018). A risk rarely addressed is the transmission and introduction of disease-causing organisms (Williams et al., 2015). Although not part of the aim of this study, non-target DNA was amplified from live rock associated taxa and identified as species of the coral disease causative agent genus, *Vibrio* (Olivotto et al., 2009; Arboleda and Reichardt, 2010) (Table 6.13 - Appendix B). As established in this study, polychaetes are commonly associated with live rock, yet they themselves are assosciated with a number of parasites and known to transmit diseases. Sussman et al. (2003) identified the coral-predator polychaete, *Hermodice carunculata*, as the vector for the coral-bleaching pathogen *Vibrio shiloi*, and Vijayan et al. (2005) report polychaetes as a vector for the white spot syndrome virus (WSSV) which has caused frequent disease outbreaks in the shrimp farming industry in Asia and India. It is evident that live rock poses a realistic risk of introducing diseases into South Africa.

It is likely that non-target DNA amplified as a result of poor DNA extraction from live rock specimens that started to decompose. It is suggested that future studies should use narcotising agents to remove all organisms from the surface of and within the live rock as soon as the live rock is purchased from the pet store. This is to ensure that the sampling of live and whole specimens will occur and will more accurately represent the diversity supported by the live rock.

3.3.4 Efficacy of DNA barcoding as a tool for live rock associated taxa identification

This study aimed to identify marine invertebrates spanning 6 phyla. COI barcodes generated species assignments with a 90% success rate which is significantly higher in comparison to the 13.5% achieved by that of the ITS region. The identification success rate refered to above is based on the number of informative species-level identifications produced through BLAST and BOLD searches of the queiried sequence combined with further phylogenetic inference. The diversity of marine invertebrate reference sequences within the GenBank and BOLD databases with relation to the organisms sequenced in this study was not nearly comprehensive enough to produce species-level identification hits. This is especially the case for the brittle stars, starfish, mussels, gastropods, and arthropods that were sampled in this study. This demonstrates that marine aquaria host a significant amount of unknown biological diversity.

In addition to the low ITS success rate, 12 ITS sequences produced no BLAST or BOLD hits. Although the BOLD Identification System (IDS) for ITS is the default identification tool for fungal barcodes, it does accept searches for sequences from any given ITS region and returns a species-level identification where possible (Ratnasingham and Hebert, 2007). The ITS sequences produced in this study were searched against the BOLD database, however no informative hits were generated and most displayed identities well below 80%. As a result, only BLAST search hits were listed for the ITS region (Table 6.12 - Appendix B).

The availability of COI reference sequences on both GenBank and BOLD databases for each major taxon analysed outnumbered that of the ITS searches, except for the soft coral family, Xeniidae. As a result, phylogenetic trees based on COI data were generated for 8 of the 9 major taxons. This is directly in line with Bucklin, Steinke and Blanco-Bercial (2011) who list corals and sea anemones as “problem children” for which COI is not a suitable barcoding region (McFadden et al., 2011).

Although this study was able to use phylogenetic reconstruction methods of COI sequence data to validate the identification of *A. pulchella*, questions arise regarding the accuracy thereof as the coverage of COI sequence data for Aiptasiidae members within the GenBank and BOLD databases was minimal. Grajales and Rodríguez (2016) demonstrate the complexity of the evolutionary relationships of Aiptasiidae with a number of barcoding regions needed to infer species identifications. This is a realistic issue that raises doubts regarding the practicality and rapidity of barcoding for biosecurity management practices. One standardized barcoding region (i.e. COI) has long been shown to not produce the resolving power needed for successful identifications across all existing phyla (Bucklin et al., 2011; Nagarajan et al., 2018). A more efficient and cost-effective molecular identification workflow could be achieved by assigning specific barcoding regions to different taxa groups and subsequently building barcoding libraries therewith. Ultimately, the comprehensiveness of the reference sequences in the databases searched determines the accuracy of molecular identifications.

Furthermore, molecular identifications by DNA barcoding is only as accurate as the morphological identification used to assign the reference specimens contained within the databases being searched. This is a critical first step in assembling an informative reference database for non-expert users (Collins, 2012). It was challenging to assign species identifications to such a wide variety of organisms even with the assistance of a taxonomic expert per taxa group where available. This study demonstrates the importance of accurate morphological identifications where species assignments could not be resolved using molecular methods as biogeographic statuses and distribution ranges cannot be deduced without a species-level identification.

CHAPTER 4: MARINE AQUARIUM HOBBYIST SURVEY

4.1 Methods and materials

An online survey was conducted to gain insight into the current awareness and knowledge of marine aquarium pest species, and the aquarium-keeping habits of aquarium hobbyists and pet store staff in South Africa.

4.1.1 Survey design

Hobbyists, of all ages and experience levels, were targeted for the purpose of this study. The Google Forms tool (https://docs.google.com/forms) was used to generate the questionnaire. Eleven questions were created with suitable pre-determined answers (Appendix C). The taxa associated with live rock listed in question 10 were frequently discussed aquarium pests on the Marine Aquariums of South Africa (MASA) forum and Facebook page, and were therefore chosen as pre-determined answers for this question. Questions marked with an asterisk (*) required an answer in order for the respondent to be able to submit the questionnaire. A combination of multiple choice answers (where only one answer can be selected) and checkboxes (where more than one answer can be selected) were used. For questions 6 to 10, questionnaire respondents were allowed to choose more than one answer if applicable. Therefore, percentages were calculated for each individual answer (i.e. method or organism) by taking the number of counts for each individual answer in proportion to the number of questionnaire respondents who selected that answer.

4.1.2 Survey distribution

An online mode of delivery was chosen for the survey due to the geographical range (i.e. South Africa) targeted, to deter bias from the respondents' answer, to not distract or make the respondent feel uncomfortable when answering the questions, and to allow for anonymity. The survey was advertised and distributed on the MASA forum and Facebook page, shared on appropriate Facebook pages and groups, emailed to known aquarium hobbyists, and shared over WhatsApp. The MASA forum was specifically targeted as it has 10 441 members. All pet shops assessed in Chapter 5 were approached and their co-operation in completing and distributing the survey was requested. The survey was conducted over a period of six months.

4.1.3 Cross-tabulation analysis

The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 26.0 (IBM Software Group, Chicago, USA). A frequency distribution was obtained per survey question and are presented in Apprendix D. Multiple response variable sets were defined for every survey question where more than one answer could be selected (i.e. live rock type (Question 4); quarantine method (Question 5); live rock associated taxa observed on live rock (Question 6); and live rock associated taxa identified as aquarium pests (Question 7). Cross-tabulation analyses were performed to investigate relationships between the survey question data. To test for independence between variable sets, a Pearson's chi-squared (χ^2) analysis was performed with a level of significance set at 5% ($p < 0.05$).

4.2 Results

4.2.1 Marine aquarium hobbyist survey

Sixty-three marine aquarium hobbyists responded to the questionnaire over the course of six months. The greatest proportion of questionnaire respondents are found in the trading-hub provinces of South Africa (Figure 4.1). The marine aquarium hobbyists surveyed are from a variety of expertise levels, and most are aquarium hobbyists with 2-5 years of experience (Figure 4.2). Approximately two-thirds of the surveyed hobbyists admitted to trading fish, coral frags, invertebrates, and/or unwanted organisms with fellow hobbyists (Figure 4.3). A wide variety of live rock types were kept by the surveyed hobbyists, with live rock from a previously established marine aquarium being the most common. Approximately a fifth of all questionnaire respondents did not know where their live rock came from. Locally cultured rock was the least preferred type of live rock to be kept. Fiji dominated the live rock import trade, followed by Kenya and Indonesia, respectively (Figure 4.4).

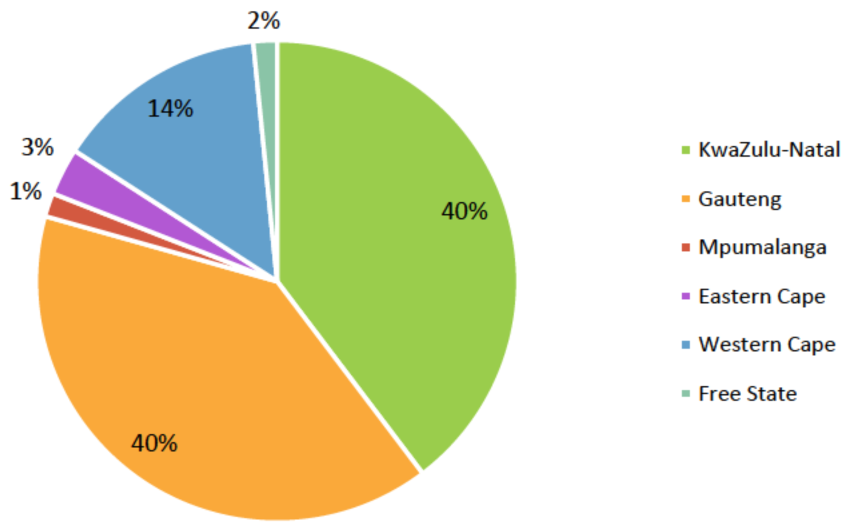


Figure 4.1: Provinces of residence of the surveyed marine aquarium hobbyists in South Africa.

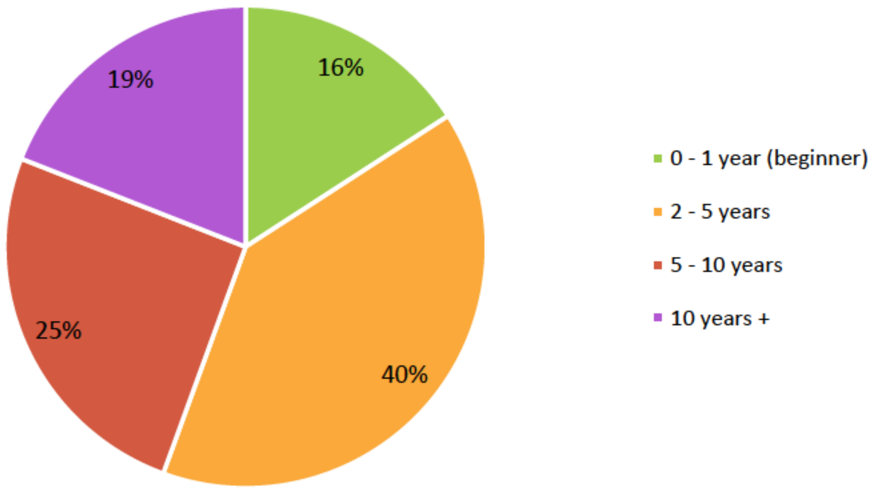


Figure 4.2: Expertise level of the surveyed marine aquarium hobbyists in South Africa.

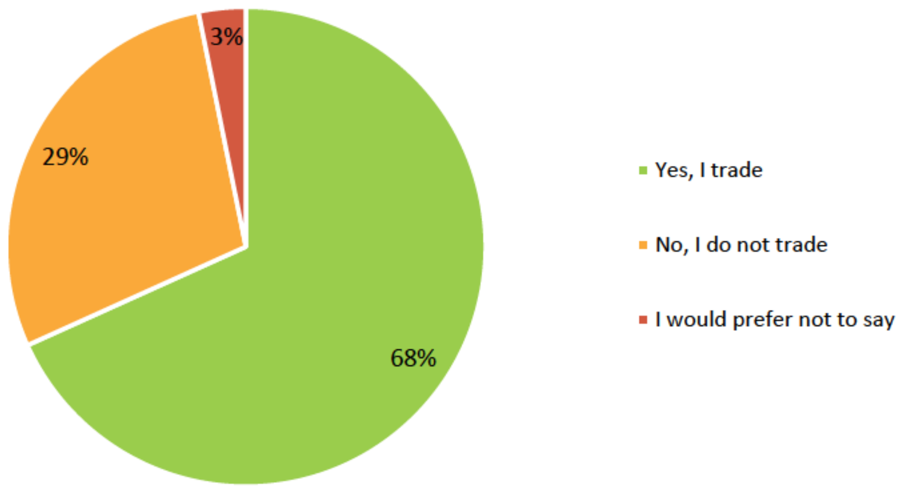


Figure 4.3: Proportion of the surveyed marine aquarium hobbyists who do/do not take part in the trading of fish, corals, invertebrates, and/or unwanted organisms with fellow aquarium hobbyists.

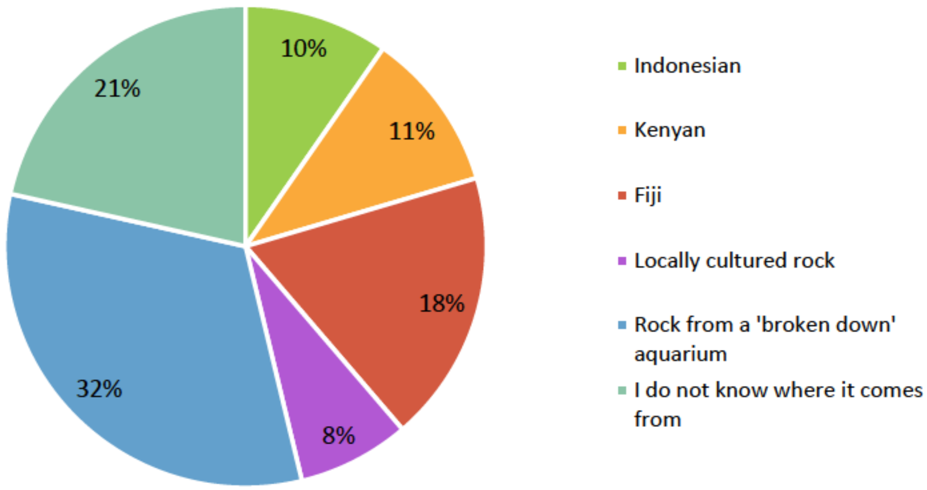


Figure 4.4: Types of live rock kept by the surveyed marine aquarium hobbyists in South Africa.

More than half of all questionnaire respondents like the organisms associated with their live rock. Most questionnaire respondents said that they trust that the pet shop quarantined the live rock before they purchased it (Figure 4.5). Polychaetes and starfish/brittle stars were by far the most commonly observed groups of live rock associated species in the surveyed marine aquarium hobbyists' aquaria. These were followed by gastropods, sponges, and algae/seaweed. It is evident that all major taxa groups are associated with live rock (Figure 4.6). Algae/seaweed, sea anemones, flatworms, and polychaetes, respectively, were the most commonly reported pest live rock associated taxa. Just less than a third of all questionnaire respondents reported that none of the live rock associated taxa groups had become a pest (Figure 4.7).

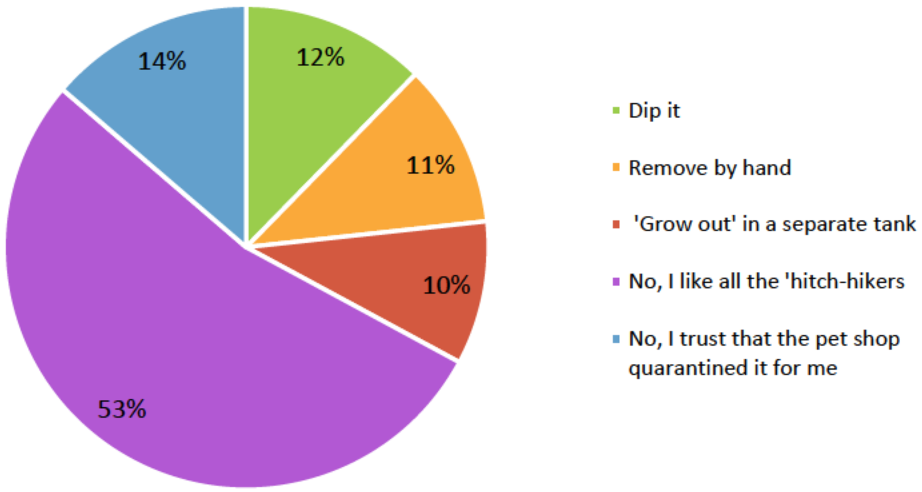


Figure 4.5: Methods used by the surveyed marine aquarium hobbyists to get rid of pest organisms associated with live rock.

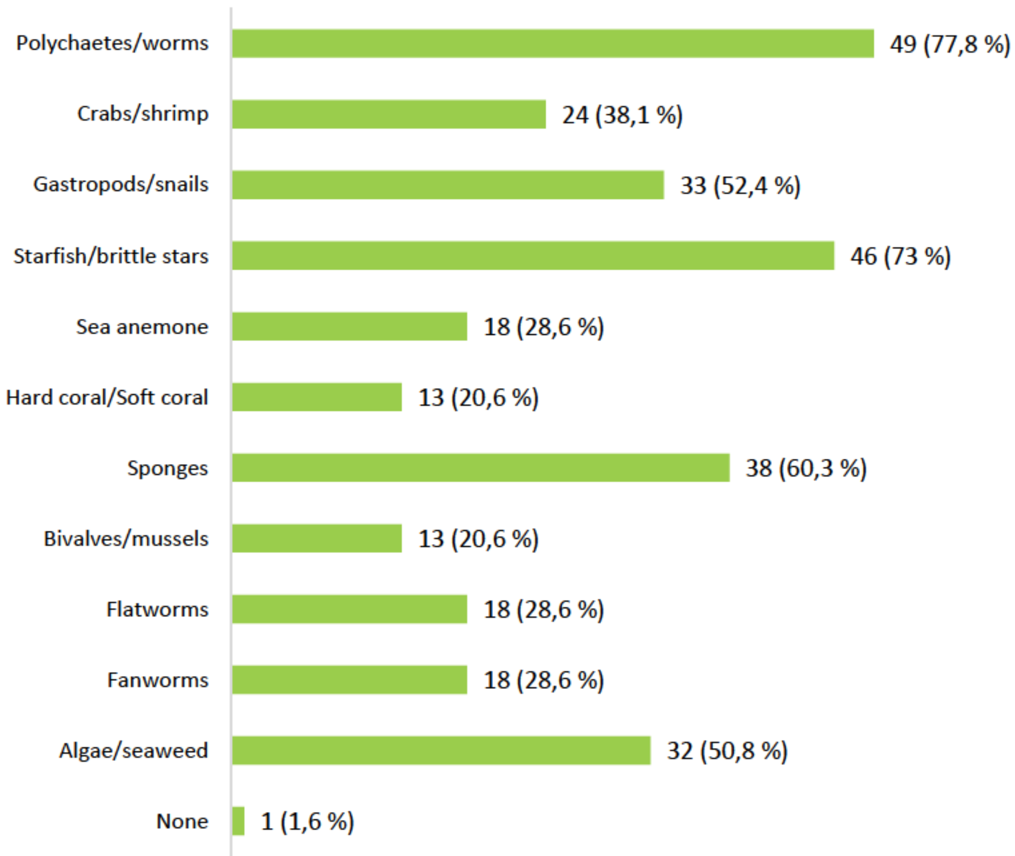


Figure 4.6: Live rock associated taxa observed by the surveyed marine aquarium hobbyists.

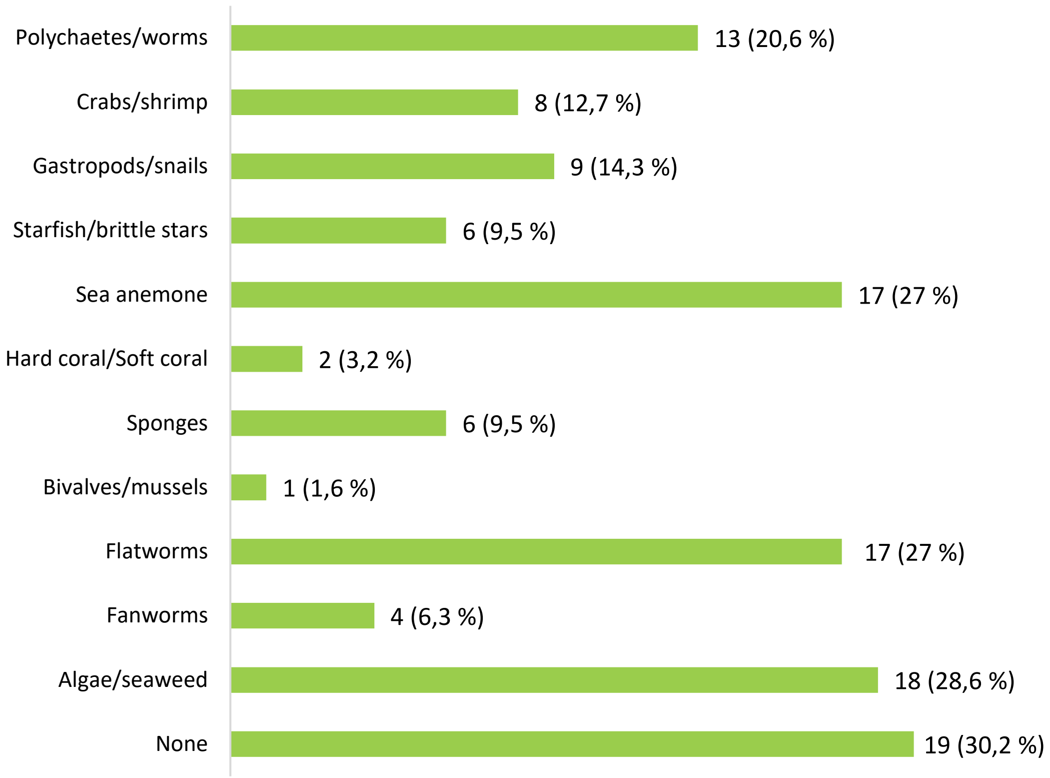


Figure 4.7: Live rock associated taxa that have become a pest in the surveyed hobbyist's marine aquaria.

Removing organisms by hand was by far the most commonly sought-after method to get rid of pest taxa associated with live rock. This is the easiest and cheapest method that can be used, and can be done before putting the live rock into the aquarium. Using marine fish or invertebrates to feed off the pest organisms, and injecting the organisms with a chemical or liquid solution, are the second-most commonly used methods to eradicate pest taxa associated with live rock by the surveyed marine aquarium hobbyists (Figure 4.8). Throwing pest organisms into the rubbish bin was the most commonly used method to dispose of taxa associated with live rock. This method was followed by throwing the pest organisms into the garden or down the toilet/drain. Of the 63 surveyed marine aquarium hobbyists, 8 admitted to releasing pest organisms into the ocean (Figure 4.9).

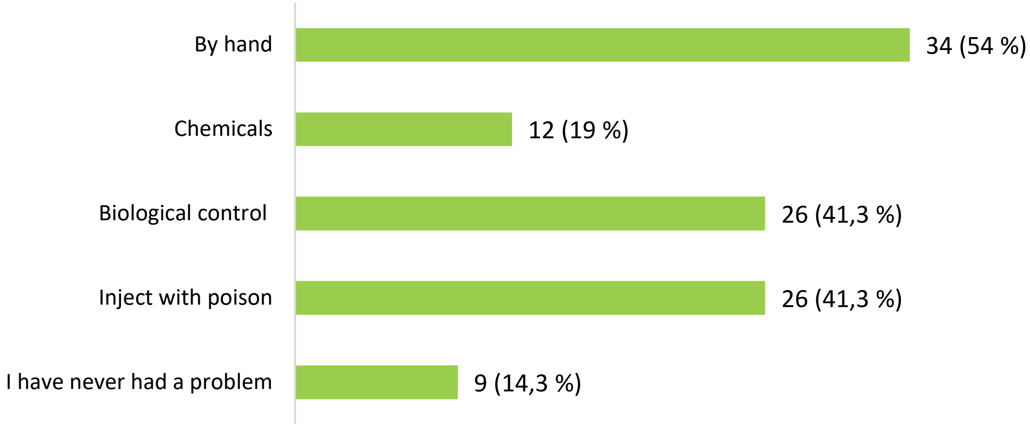


Figure 4.8: Methods used to get rid of pest taxa associated with live rock by the surveyed marine aquarium hobbyists.

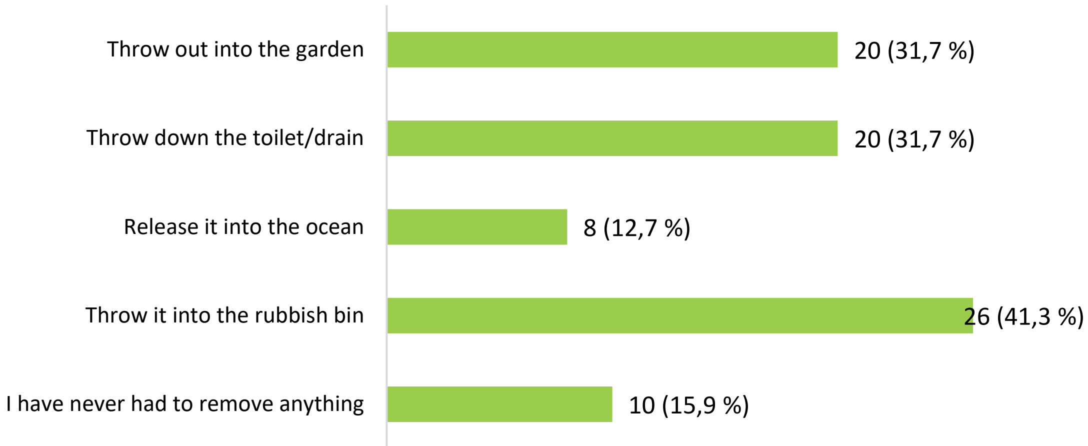


Figure 4.9: Methods of disposal of pest taxa associated with live rock used by the surveyed marine aquarium hobbyists.

Aiptasia sp. (glass anemone) was identified by nearly all (96.7%) of the questionnaire respondents. The results from Figure 4.10 confirm that these five taxa associated with live rock are perceived as aquarium pests by the surveyed marine aquarium hobbyists across South Africa. These results coincide with the results from Figure 4.7, which show that sea anemones, algae, flatworms, and starfish/brittle stars are all commonly observed pest taxa associated with live rock.

Figure 4.11 shows that approximately 40% of the questionnaire respondents acknowledge that the marine aquarium trade has the potential to distribute pest organisms into and around South Africa. Almost a third of the surveyed aquarium hobbyists do not know enough about this issue to comment. Sixteen percent of the questionnaire respondents can confidently say that the marine aquarium trade does not play a role in moving pest organisms around South Africa.

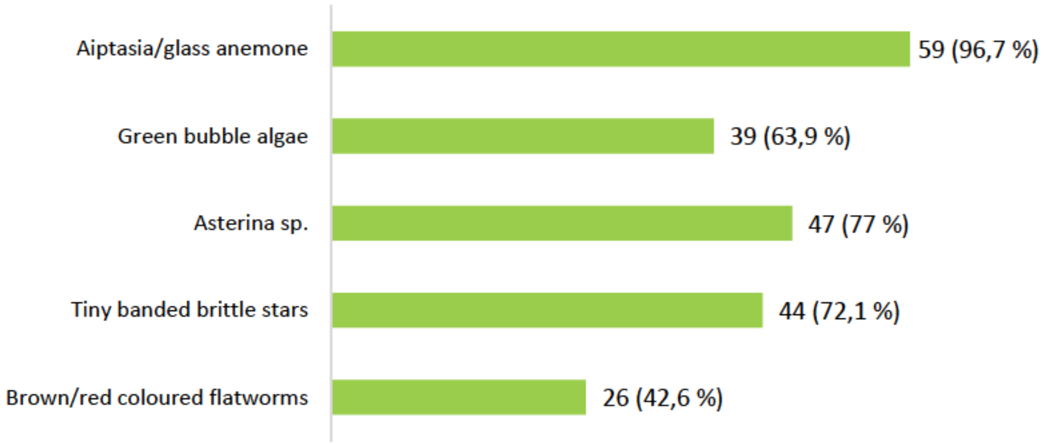


Figure 4.10: Commonly-observed pest taxa associated with live rock identified by the surveyed marine aquarium hobbyists.

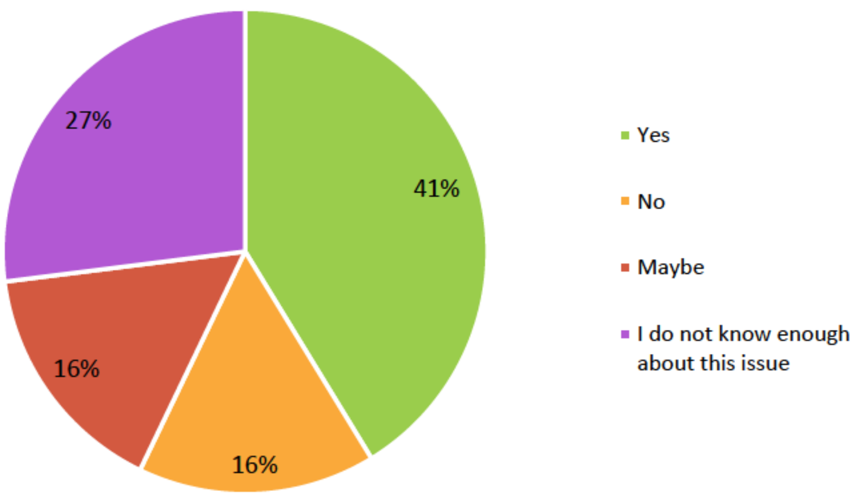


Figure 4.11: Proportion of the surveyed marine aquarium hobbyists' answers to the question: Do you think that the aquarium pet trade has the potential to move pest organisms into and around South Africa?

4.2.2 Cross-tabulation analysis

A cross-tabulation between province of residence (Question 1) and the type of live rock kept by marine aquarium hobbyists in South Africa (Question 4) is presented in Table 4.1. Of the 63 marine aquarium hobbyists that keep live rock, the majority live in KwaZulu-Natal (40.0%) and Gauteng (40.0%). The majority of KwaZulu-Natal (48.0%) and Gauteng (48.0%) hobbyists acquire their live rock from old 'broken down' aquaria. Indonesian, Kenyan, and Fiji live rock is most common in the major marine pet trading hubs of South Africa: KwaZulu-Natal, Gauteng, and the Western Cape. Locally cultured live rock is the least common type of live rock kept by marine aquarium hobbyists across all provinces. A requirement for the chi-square test of independence is that the expected value of the number of sample observations in each level of the variable is at least 5. More than 20% of the cells in Table 4.1 have expected cell counts less than 5, therefore the chi-square results ($\chi^2 = 43.064$, df = 25, p-value = 0.014) may be invalid.

Table 4.2 presents a cross-tabulation between expertise level (Question 2) and live rock quarantine methods used by marine aquarium hobbyists in South Africa (Question 5). The majority of marine aquarium hobbyists across all expertise levels (53.4%) reported liking the organisms associated with their live rock and therefore do not make use of any quarantine methods. There is no noticeable relationship between expertise level and live rock quarantine methods on closer observation of Table 4.2. This observation is supported by the Pearson chi-square analysis which concludes that the variables are independent ($\chi^2 = 17.023$, df = 15, p-value = 0.318). However, more than 20% of the cells in Table 4.2 have expected cell counts less than 5, therefore the chi-square results may be invalid.

A cross-tabulation between live rock type (Question 4) and invertebrates observed on live rock by marine aquarium hobbyists in South Africa (Question 6) is presented in Table 4.3. Of all taxa groups associated with Indonesian live rock, polychaetes (88.9%) were the most commonly observed, followed by starfish/brittle stars (77.8%), crabs/shrimp (66.7%), gastropods (66.7%), and sponges (66.7%). All marine aquarium hobbyists that keep Kenyan live rock also observed polychaetes on their live rock. Gastropods, starfish/brittle stars, sea anemone, flatworms, and algae/seaweed were observed by 60.0% of marine aquarium hobbyists that keep Kenyan live rock. Marine aquarium hobbyists that keep Fiji live rock most commonly observed starfish/brittle stars (87.5%) and polychaetes (81.3%). Polychaetes were the most commonly observed live rock associated taxa reported by those that kept locally cultured rock (85.7%) and rock from old 'broken down' aquaria (76.7%). The Pearson chi-square analysis ($\chi^2 = 68.779$, df =

55, p-value = 0.100) concluded that there is no correleation between live rock type and live rock associated taxa. However, chi-square results may be invalid as more than 20% of the cells in Table 4.3 have expected cell counts less than 5.

Table 4.4 presents a cross-tabulation between live rock type (Question 4) and invertebrates identified as aquarium pests by marine aquarium hobbyists in South Africa (Question 7). Seventy percent of marine aquarium hobbyists that keep Kenyan live rock also observed pest sea anemones on their live rock. Of the hobbyists that keep locally cultured live rock, 57% also report polychaetes and gastropods as live rock associated pests. The Pearson chi-square statistic is significant at the 0.05 level (χ^2 = 109.503, df = 55, p-value = 0.001) and it therefore can be said that there is a correlation between live rock type and live rock associated marine aquarium pests. However, more than 20% of the cells in Table 4.4 have expected cell counts less than 5, therefore the chi-square results may be invalid.

A cross-tabulation between expertise level (Question 2) and methods of pest control used by marine aquarium hobbyists in South Africa (Question 8) is presented in Table 4.5. Beginner (0-1 year) marine aquarists most often resort to removing pests by hand and disposing of them (70%) and injecting coral/sea anemones with chemicals or liquid solutions (60%). Sixty-four percent of aquarists with 2-5 years of experience remove pests by hand and dispose of them. Aquarists with 5-10 years of experience most commonly resort to biological control (43.8%), removing pests by hand and disposing of them (43.8%), and injecting coral/sea anemones with chemicals or liquid solutions (43.8%). Biological control is the most common method of controlling pests used by aquarists with ten or more years of experience. The Pearson chi-square analysis (χ^2 = 15.870, df = 15, p-value = 0.391) concluded that there is no correlation between expertise level and methods of pest control used by marine aquarium hobbyists. More than 20% of the cells in Table 4.5 have expected cell counts less than 5, therefore the chi-square results (χ^2 = 43.064, df = 25, p-value = 0.014) may be invalid.

Table 4.6 presents a cross-tabulation between province of residence (Question 1) and methods of pest disposal used by marine aquarium hobbyists in South Africa (Question 9). KwaZulu-Natal residents mostly resort to throwing pest organisms into the rubbish bin (44.0%) and out into the garden (40.0%). Forty-four percent of Gauteng residents dispose of pest organisms down the toilet/drain, followed by disposing of them out into the garden (36.0%) and into the rubbish bin (36.0%) as the most common methods of pest disposal. Western Cape residents mostly resort to throwing pest organisms into the rubbish bin (44.0%). Disposing of pest organisms down the toilet/drain is a method used by residents in all coastal provinces (i.e. KwaZulu-Natal, Western Cape, and the Eastern Cape). Releasing pest organisms into the ocean is a method of disposal used by 24% of KwaZulu-Natal and 22.2% of Western Cape residents. The Pearson chi-square analysis (χ^2 = 36.990, df = 25, p-value = 0.058) produced an insignificant chi-square statistic at the 0.05 level. Therefore, it can be concluded that there is no correlation between province of residence and the method of disposal of pest organisms used by marine aquarium hobbyists in South Africa.

A cross-tabulation between expertise level (Question 2) and marine aquarium pests recognized by marine aquarium hobbyists in South Africa (Question 10) is presented in Table 4.7. *Aiptasia* sp. was recognized by nearly all hobbyists across all expertise levels, with 91.3% of the 2-5 year hobbyists as the exception. Overall, the hobbyists with 5-10 years of experience recognized most of the marine aquarium pests. The Pearson chi-square statistic is significant at the 0.05 level (χ^2 = 41.358, df = 15, p-value = 0.001), therefore, it can be ascertained that there is a correlation between expertise level and marine aquarium pests recognized by marine aquarium hobbyists in South Africa.

Table 4.8 presents a cross-tabulation between expertise level (Question 2) and responses to Question 11: "Do you think that the aquarium pet trade has the potential to move pest organisms into and around South Africa?". Forty-one percent of marine aquarium hobbyists believe that the aquarium pet trade has the potential to move pest organisms into and around South Africa. This is the most commonly held view of hobbyists across all expertise levels, that is: 30% of beginners, 44% with 2 - 5 years experience, 43.8% with 5 - 10 years experience, and 41.7% with 10 or more years of experience. As expertise level increases, fewer marine aquarium hobbyists report to not know enough about the issue at hand. The Pearson chi-square statistic is insignificant at the 0.05 level (χ^2 = 5.587, df = 9, p-value = 0.780). As a result, there is no correlation between expertise level and views regarding the aquarium pet trade as a pathway for pest organisms. However, more than 20% of the cells in Table 4.8 have expected cell counts less than 5, therefore the chi-square results may be invalid.

Table 4.1: Cross-tabulation between province of residence and the type of live rock kept by marine aquarium hobbyists in South Africa. *Percentages are based on row total counts. Total counts are based on total number of respondents. The grand total is the sum of column total counts.*

			Question 4: What type of live rock do you keep in your aquarium?						
			Indonesian	Kenyan	Fiji	Locally cultured rock	Rock from an old 'broken down' aquarium	I do not know where it comes from	Total count
Question 1: Which province do you live in?	KwaZulu-Natal	Count	1	1	4	3	12	11	25
		% within province	4.0%	4.0%	16.0%	12.0%	48.0%	44.0%	
	Gauteng	Count	4	3	6	2	12	7	25
		% within province	16.0%	12.0%	24.0%	8.0%	48.0%	28.0%	
	Mpumalanga	Count	0	0	1	0	1	0	1
		% within province	0.0%	0.0%	100.0%	0.0%	100.0%	0.0%	
	Eastern Cape	Count	0	1	0	0	2	0	2
		% within province	0.0%	50.0%	0.0%	0.0%	100.0%	0.0%	
	Western Cape	Count	3	4	4	1	2	2	9
		% within province	33.3%	44.4%	44.4%	11.1%	22.2%	22.2%	
	Free State	Count	1	1	1	1	1	0	1
		% within province	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%	
		Total count	9	10	16	7	30	20	63

Table 4.2: Cross-tabulation between expertise level and live rock quarantine methods used by marine aquarium hobbyists in South Africa. *Percentages are based on row total counts. Total counts are based on total number of respondents. The grand total is the sum of column total counts.*

			Question 5: Do you quarantine your live rock before you put it into your tank?					
			I dip it	I remove unwanted 'hitch-hikers' by hand	I let it 'grow out' in a separate tank	No, I like all the organisms that come with the rock	No, I trust that the pet shop did it for me	Total count
Question 2: How long have you kept a marine aquarium for?	0 - 1 year (beginner)	Count	2	2	1	3	3	10
		% within expertise level	20.0%	20.0%	10.0%	30.0%	30.0%	
	2 - 5 years	Count	4	5	4	15	4	25
		% within expertise level	16.0%	20.0%	16.0%	60.0%	16.0%	
	5 - 10 years	Count	1	1	2	13	1	16
		% within expertise level	6.3%	6.3%	12.5%	81.3%	6.3%	
	10 years +	Count	2	0	0	8	2	12
		% within expertise level	16.7%	0.0%	0.0%	66.7%	16.7%	
		Total count	9	8	7	39	10	63

Table 4.3: Cross-tabulation between live rock type and invertebrates observed on live rock by marine aquarium hobbyists in South Africa. *Percentages are based on row total counts. Total counts are based on total number of respondents. The grand total is the sum of column total counts.*

			Question 6: What types of invertebrates have you noticed come off of your live rock?												
			Polychaetes/worms	Crabs/shrimp	Gastropods/snails	Starfish/brittle stars	Sea anemone	Hard coral/Soft coral	Sponges	Bivalves/mussels	Flatworms	Fanworms	Algae/seaweed	None	Total count
Question 4: What type of live rock do you keep in your aquarium?	Indonesian	Count	8	6	6	7	5	5	6	2	4	3	4	0	9
		% within live rock type	88.9%	66.7%	66.7%	77.8%	55.6%	55.6%	66.7%	22.2%	44.4%	33.3%	44.4%	0.0%	
	Kenyan	Count	10	4	6	6	6	3	5	2	6	3	6	0	10
		% within live rock type	100.0%	40.0%	60.0%	60.0%	60.0%	30.0%	50.0%	20.0%	60.0%	30.0%	60.0%	0.0%	
	Fiji	Count	13	8	8	14	5	5	9	3	5	4	7	0	16
		% within live rock type	81.3%	50.0%	50.0%	87.5%	31.3%	31.3%	56.3%	18.8%	31.3%	25.0%	43.8%	0.0%	
	Locally cultured rock	Count	6	2	5	4	3	2	1	3	4	1	4	0	7
		% within live rock type	85.7%	28.6%	71.4%	57.1%	42.9%	28.6%	14.3%	42.9%	57.1%	14.3%	57.1%	0.0%	
	Rock from an old 'broken down' aquarium	Count	23	10	16	23	8	4	19	9	10	13	19	1	30
		% within live rock type	76.7%	33.3%	53.3%	76.7%	26.7%	13.3%	63.3%	30.0%	33.3%	43.3%	63.3%	3.3%	
	I don't know where it comes from	Count	14	7	10	14	7	3	11	2	4	4	7	0	20
		% within live rock type	70.0%	35.0%	50.0%	70.0%	35.0%	15.0%	55.0%	10.0%	20.0%	20.0%	35.0%	0.0%	
		Total count	49	24	34	46	18	13	37	13	18	17	31	1	63

Table 4.4: Cross-tabulation between live rock type and invertebrates identified as aquarium pests by marine aquarium hobbyists in South Africa. *Percentages are based on row total counts. Total counts are based on total number of respondents. The grand total is the sum of column total counts.*

			Question 7: Which invertebrates have become a pest or a nuisance in your aquarium?												
			Polychaetes/worms	Crabs/shrimp	Gastropods/ snails	Starfish/ brittle stars	Sea anemone	Hard coral/ Soft coral	Sponges	Bivalves/ mussels	Flatworms	Fanworms	Algae/seaweed	None	Total count
Question 4: What type of live rock do you keep in your aquarium?	Indonesian	Count	3	4	2	2	4	1	3	1	4	1	3	3	9
		% within live rock type	33.3%	44.4%	22.2%	22.2%	44.4%	11.1%	33.3%	11.1%	44.4%	11.1%	33.3%	33.3%	
	Kenyan	Count	1	3	1	2	7	1	1	1	3	1	3	2	10
		% within live rock type	10.0%	30.0%	10.0%	20.0%	70.0%	10.0%	10.0%	10.0%	30.0%	10.0%	30.0%	20.0%	
	Fiji	Count	4	3	2	4	5	1	4	1	6	2	4	4	16
		% within live rock type	25.0%	18.8%	12.5%	25.0%	31.3%	6.3%	25.0%	6.3%	37.5%	12.5%	25.0%	25.0%	
	Locally cultured rock	Count	4	1	4	2	3	1	1	1	3	2	2	1	7
		% within live rock type	57.0%	14.3%	57.0%	28.6%	42.9%	14.3%	14.3%	14.3%	42.9%	28.6%	28.6%	14.3%	
	Rock from an old 'broken down' aquarium	Count	6	3	6	3	7	2	2	1	9	2	12	8	30
		% within live rock type	20.0%	10.0%	20.0%	10.0%	23.3%	6.7%	6.7%	3.3%	30.0%	6.7%	40.0%	26.7%	
	I don't know where it comes from	Count	4	2	2	2	6	0	1	0	3	0	5	8	20
		% within live rock type	20.0%	10.0%	10.0%	10.0%	30.0%	0.0%	5.0%	0.0%	15.0%	0.0%	25.0%	40.0%	
		Total count	13	8	9	6	17	2	6	1	17	4	18	19	63

Table 4.5: Cross-tabulation between expertise level and methods of pest control used by marine aquarium hobbyists in South Africa. *Percentages are based on row total counts. Total counts are based on total number of respondents. The grand total is the sum of column total counts.*

			Question 8: If you have had any pesty invertebrates in your aquarium, how do you manage to control them?					
			I remove them by hand and dispose of them	Chemicals	Biological control	Inject coral/sea anemones with chemical or liquid solution	No, I have never had a problem with pesty organisms	Total count
Question 2: How long have you kept a marine aquarium for?	0 - 1 year (beginner)	Count	7	2	4	6	1	10
		% within expertise level	70.0%	20.0%	40.0%	60.0%	10.0%	
	2 - 5 years	Count	16	5	8	6	5	25
		% within expertise level	64.0%	20.0%	32.0%	24.0%	20.0%	
	5 - 10 years	Count	7	4	7	7	2	16
		% within expertise level	43.8%	25.0%	43.8%	43.8%	12.5%	
	10 years +	Count	4	1	7	5	0	12
		% within expertise level	33.3%	8.3%	58.3%	41.7%	0.0%	
		Total count	34	12	26	24	8	63

Table 4.6: Cross-tabulation between province of residence and the methods of pest disposal used by marine aquarium hobbyists in South Africa. *Percentages are based on row total counts. Total counts are based on total number of respondents. The grand total is the sum of column total counts.*

			Question 9: If you have had to remove any invertebrate/fish/algae from your tank, where do you dispose of it?					
			Out into the garden	Down the toilet/drain	Release it into the ocean	Thrown it into the rubbish bin	I have never had to remove anything from my aquarium	Total count
Question 1: Which province do you live in?	KwaZulu-Natal	Count	10	4	6	11	3	25
		% within province	40.0%	16.0%	24.0%	44.0%	12.0%	
	Gauteng	Count	9	11	0	9	4	25
		% within province	36.0%	44.0%	0.0%	36.0%	16.0%	
	Mpumalanga	Count	0	0	0	1	0	1
		% within province	0.0%	0.0%	0.0%	100.0%	0.0%	
	Eastern Cape	Count	1	2	0	0	0	2
		% within province	50.0%	100.0%	0.0%	0.0%	0.0%	
	Western Cape	Count	0	1	2	4	3	9
		% within province	0.0%	11.1%	22.2%	44.4%	33.3%	
	Free State	Count	1	1	0	1	0	1
		% within province	100.0%	100.0%	0.0%	100.0%	0.0%	
		Total count	21	19	8	26	10	63

Table 4.7: Cross-tabulation between expertise level and marine aquarium pests recognized by marine aquarium hobbyists in South Africa. *Percentages are based on row total counts. Total counts are based on total number of respondents. The grand total is the sum of column total counts.*

			Question 10: Do you recognize any of the following marine aquarium pests?					
			Aiptasia sp./ glass anemone	Green bubble algae	*Asterina* sp./ tiny white starfish	Tiny banded brittle stars	Brown/red flatworms	Total count
Question 2: How long have you kept a marine aquarium for?	0 - 1 year (beginner)	Count	10	1	5	4	1	10
		% within expertise level	100.0%	10.0%	50.0%	40.0%	10.0%	
	2 - 5 years	Count	21	16	20	16	9	23
		% within expertise level	91.3%	69.6%	87.0%	69.6%	39.1%	
	5 - 10 years	Count	16	13	14	14	10	16
		% within expertise level	100.0%	81.3%	87.5%	87.5%	62.5%	
	10 years +	Count	12	9	8	10	6	12
		% within expertise level	100.0%	75.0%	66.7%	83.3%	50.0%	
		Total count	59	39	47	44	26	61

Table 4.8: Cross-tabulation between expertise level and response to "Do you think that the aquarium pet trade has the potential to move pest organisms into and around South Africa?" by marine aquarium hobbyists. *Percentages are based on row total counts. Total counts are based on total number of respondents. The grand total is the sum of column total counts.*

			Question 11: Do you think that the aquarium pet trade has the potential to move pest organisms into and around South Africa?				
			Yes	No	Maybe	I do not know enough about this issue	Total count
Question 2: How long have you kept a marine aquarium for?	0 - 1 year (beginner)	Count	3	2	2	3	10
		% within expertise level	30.0%	20.0%	20.0%	30.0%	
	2 - 5 years	Count	11	2	7	5	25
		% within expertise level	44.0%	8.0%	28.0%	20.0%	
	5 - 10 years	Count	7	4	4	1	16
		% within expertise level	43.8%	25.0%	25.0%	6.3%	
	10 years +	Count	5	2	4	1	12
		% within expertise level	41.7%	16.7%	33.3%	8.3%	
		Total count	26	10	17	10	63

4.2.3 Pet store trading observations and habits of concern

Pet shop owners and the staff of all 9 pet stores visited across South Africa were not willing to participate in a formal interview or complete the marine aquarium hobbyist survey. Only one of the nine pet shops sent out the hobbyist survey to their customers on their WhatsApp messaging group. To supplement the lack of formal data, information was gathered through informal conversations regarding the import, packaging, disposal of water and organisms, and general marine aquarium-keeping habits, and is discussed hereafter.

International stock of marine fish, corals, live rock, and invertebrates arrive in concealed styrofoam boxes, sometimes after being held for long periods of time at customs awaiting clearance. Marine fish and invertebrates arrive packaged in individual plastic bags filled with water, whereas live rock and corals usually arrive 'dry' and wrapped individually with wet newspaper. The styrofoam containers holding 'dry' stock often collects a shallow layer of water containing sand and organisms that have escaped or fallen off live rock or the substrate to which the corals are attached (pers. obs.). Depending on the hardiness of the soft or hard coral, it might also arrive in its own individual plastic bag filled with water. These packets have also been seen to collect a layer of sand and organisms (pers. obs.).

The discarding of the water that collects at the bottom of the styrofoam containers, and that which holds individual fish, invertebrates, and sometimes corals, is of concern. Pet shop staff have been observed to directly throw this water down drains. A few staff members have said that they know that they should use bleach to cure the water before discarding it, but it becomes a hassle and curing agents are of expense (anonymous, pers. comm., 2017). Informal discussions with pet store employees revealed that the pet stores do not enforce quarantine of live rock due to a number of reasons: they trust that the exporter did this for them; marine aquarium hobbyists request that they do not cure the live rock as it strips it of its aesthetic value; curing agents are of extra expense; and some marine aquarium hobbyists prefer uncured live rock as they value the biological filtering and decomposition services provided by the live rock associated organisms.

The invasive glass sea anemone (*Aiptasia* sp.) was observed in 5 of the 9 pet stores inventoried in this study (pers. obs.). The exact species cannot be confirmed as this was by sheer observation, however, it is of concern that invertebrates, coral, and live rock residing in the same holding aquaria as *Aiptasia* sp. are being traded.

4.3 Discussion

The aim of this study was to determine the role that pet stores and hobbyists play in the introduction, movement, and trade of marine fish, invertebrates, and live rock around South Africa and to evaluate their current awareness and knowledge of marine aquarium pest species. Overall, this survey was not well received by pet stores and aquarium hobbyists. Every effort was made to distribute the survey and resulted in only 63 responses, even after it was posted onto the MASA forum which hosts more than 10 000 marine aquarium hobbyists.

The potential reasons for this lack in participation of the marine aquarium hobbyist survey is based on a number of anonymous personal communications with hobbyists and pet store staff during 2015 and 2016. Hobbyists conveyed that the results of the survey would be "damaging to the trade" and therefore did not want to participate in it. Many pet stores convey that they struggle to supply the demand for live rock by hobbyists as the paperwork has become too tedious and troublesome. A sense of fear was evident among pet store staff that the outcome of this study would result in a ban on the import of live rock. The demand for, and value of, live rock by marine aquarium hobbyists is so great that it is highly unlikely that they would intentionally release it into the ocean. Informal conversations with hobbyists revealed that if they wanted to get rid of their live rock, they would most likely sell it to fellow hobbyists. As stated in Chapter 3.1.1, prices ranged from R100 to R390 per kilogram of live rock purchased.

The survey revealed that the purchase and/or exchange of live rock and organisms often happens informally, through internet forums, or in hobbyist clubs. This contribution to the diversity of organisms within the marine aquarium trade in South Africa remains unexplored, and would without a doubt be very difficult to monitor. Furthemore, it is evident that this informal exchange of organisms presents an additional pathway for the transmission of alien invasive species.

Live rock from a previously established aquarium (referred to in the questionnaire as a 'broken down' aquarium) was the most common source for the survey respondents. This study newly recognizes this as a mechanism of secondary spread of alien species amongst hobbyists within the marine aquarium trade in South Africa. The survey results show that Fijian live rock dominated the import trade. This is unusual as when sourcing live rock from pet stores across the country for the live rock associated taxa analysis (Chapter 3), only Kenyan and Indonesian live rock were attainable. The results might refer to live rock purchased before this study commenced.

More than half of all survey respondents believe live rock associated taxa to be an asset to their aquaria. Polychaetes and starfish/brittle stars were the most commonly observed live rock associated taxa by hobbyists. This is in accordance with the taxa harvested from live rock in Chapter 3. Although more than half reported them as beneficial, just over two-thirds of the questionnaire respondents reported live rock associated species as pests in their aquaria. Reefkeeping (available at www.reefkeeping.com), a popular online magazine amongst hobbyists, suggests that "until they are at plague proportions, they are more of a nuisance than a threat to our inhabitants" (Whitby, 2015). It further suggests that performing routine maintenance and keeping nutrients limited will help prevent the proliferation of live rock associated pests. Also, new live rock or coral should be dipped using specialized curing chemicals, those listed by Ram (2013) as an example, before adding them to the main aquarium. There is also a wealth of online information and published research regarding biological control methods for pests, in particular the glass sea anemone, *Aiptasia* sp. (Rhyne et al., 2004; Calado and Narciso, 2005).

Throwing pest organisms into the rubbish bin was the most commonly used method to dispose of pest organisms. This method was followed by throwing the pest organisms into the garden or down the toilet/drain. This study highlights the urgent need for responsible aquarium ownership awareness and education in South Africa. Of even greater concern is that 13% of the surveyed marine aquarium hobbyists admitted to intentionally releasing pest organisms associated with live rock into the ocean. Cross-tabulation analysis further ascertained that this method of pest disposal is used by marine aquarium hobbyists residing in coastal provinces (Table 4.6). It may be that these irresponsible methods of disposal are due to a lack of knowledge of the consequences of their actions, as only 40% of the questionnaire respondents acknowledged that the marine aquarium trade has the potential to distribute pest organisms into and around South Africa. Cross-tabulation analysis revealed that as expertise level increases, fewer marine aquarium hobbyists report to not know enough about the issue at hand (Table 4.8). This suggests the need for increased awareness and education of beginner marine aquarium hobbyists in the foundational aspects of invasion biology.

Nearly all (96.7%) of the questionnaire respondents identified *Aiptasia* sp. as a marine aquarium pest. It can therefore be inferred that *Aiptasia* sp. is distributed across South African marine aquaria since the questionnaire respondents reside in most of the provinces. This further supports the invasion concern for *Aiptasia* sp. as emphasized in Chapter 3. Further research is needed to determine the identities of the pest organisms that are being released by hobbyists and what, if any, ecological impacts they might have. The species that pose the greatest threat to South African waters could then be targeted by hobbyist education initiatives (Odom, 2012).

The cross-tabulation analyses provided further valuable insight into the relationship between the variables investigated in the marine aquarium hobbyist survey. However, the design of the survey (i.e. the inclusion of multiple response questions) was not appropriate for analysis by cross-tabulation. Firstly, when cross-tabulating two multiple response variable sets (as seen with Table 4.3 and 4.4), it is impossible to determine which taxa was observed on what type of live rock by the questionnaire respondent as they were allowed to select all the answers that applied. Unfortunately, it was therefore impossible to accurately determine if there is a relationship between live rock type and the type of live rock associated organisms. Furthermore, a requirement for the chi-square test of independence is that the expected value of the number of sample observations in each level of the variable set is at least 5. When cross-tabulating a single response with a multiple response variable set, or a multiple response with another multiple response variable set, more than 20% of the cells in the resulting cross-tabulation have expected cell counts less than 5 due to a wider variety of answers to choose from. Therefore, as seen with most of the cross-tabulations (except for Table 4.7), the Pearson chi-square analysis results may be invalid. These survey design errors have been highlighted for consideration when designing future research questionnaires that aim to gain a greater understanding into the relationships between marine aquarium vectors and their associated taxa. Overall, this survey has provided valuable insight into the previously unknown hobby habits of South African marine aquarists and their current awareness and knowledge of marine aquarium pest species.

CHAPTER 5: MARINE PET STORE STOCK INVENTORIES

5.1 Methods and materials

5.1.1 Data collection

To assess the identity and quantity of marine fish, corals, and invertebrates species traded in South Africa, three pet stores that specialize in marine aquarium keeping were selected in Cape Town, Durban, and Pretoria/Johannesburg. These regions represent the major marine pet trading hubs in South Africa (pers. obs.). During 2015 and 2016, each pet store within each major trading hub was visited three times each (totalling 27 visits nationally). Details regarding the pet stores remained anonymous, therefore a lettering system was used to represent each pet store, with each visit allocated a roman numeral.

In order to determine the most accurate quantities and species of marine fish, corals, and invertebrates traded, pet stores were contacted in advance of the visit to enquire when a new shipment would arrive. The pet stores were then visited on the day of stock arrival, or as close to that day as possible. During each visit, a Samsung S5 cell phone was used to video and photograph all marine fish, corals, and invertebrates.

5.1.2 Morphological identification and quantification

All marine fish were identified using the FishBase database (Froese and Pauly 2002) and Dr. Burgess's Atlas of Marine Aquarium Fishes (Burgess et al., 1997). A frequency of occurrence (%) was calculated per species by dividing the number of pet store visits that stocked the species by the total number of pet store visits nationally (i.e. 27 pet store visits). Distribution ranges and biogeographic statuses (i.e. native, alien) with respect to South African waters were derived for each species from the FishBase database (Froese and Pauly 2002).

5.2 Results

Johannesburg/Pretoria pet stores stocked the most marine fish (129 ± 40,9) per pet store, followed with about half the amount by Durban (62 ± 41,8), and Cape Town (55 ± 38.4), respectively (Table 6.26 - Appendix E). Raw data counts are presented in Table 6.25 of Appendix E per species, with common names and distribution ranges listed for each species in Table 6.28 of Appendix E due to lack of space. Marine fish species were imported from a number of countries from around the world, namely: Sri Lanka, the USA (Hawaii), Indonesia, Philippines, Vietnam, Kenya, Israel, and Fiji. Durban and Cape Town pet store staff explained that the majority of their stock is ordered from a larger supplier based in Johannesburg/Pretoria who places orders with international wholesalers.

The inventories reveal that a total of 228 marine fish species from 34 families and 7 orders are being kept for sale across South Africa. The Acanthuridae family of surgeonfish, tangs, and unicornfish dominate the import and trade of marine fish (15 %), followed by the Pomacanthidae angelfish (13 %), the Pomacentridae clownfish and damselfish (11 %), the Labridae wrasse (11 %), the Chaetodontidae butterflyfish (8 %), and the Gobiidae gobies (8 %), respectively (Figure 5.1). The order Perciformes (ray-finned fish) account for 25 of these families and 209 of the species. Although revealing the same trend as in Figure 5.1, a breakdown of the composition of families within the Perciformes order can be found in Table 6.27 of Appendix E.

The top 20 most frequently imported marine aquarium fish in South Africa are listed in Table 5.1, which is derived from data displayed in Tables 6.25 and 6.28 (see Appendix E). Among all the 27 pet store inventories compiled nationally, the bluestreak cleaner wrasse (*Labroides dimidiatus*) was the most frequently imported marine fish (63 %), followed by the bluegreen chromis (*Chromis viridis*) (48.1 %), and the yellow tang (*Zebrasoma flavescens*) (48.1 %) (Table 5.1).

Of the 228 species identified, 136 (60 %) are alien to South African waters (Table 6.28 - Appendix E). The AquaNIS database lists *Zebrasoma xanthurum*, *Acanthurus chirurgus*, and *Heniochus acuminatus* as having been introduced to foreign bodies of water, with *Z. xanthurum* most likely released through the marine aquarium trade into the Mediterranean Sea (Guidetti et al., 2016). *A. coeruleus* and *Z. flavescens* are also defined as introduced alien species (Langeneck et al., 2015), with the latter reported as having been released from a marine aquarium (Weitzmann et al., 2016). After performing extensive database searches and literature reviews it was ascertained that none of the species identified in this study have been reported as invasive globally.

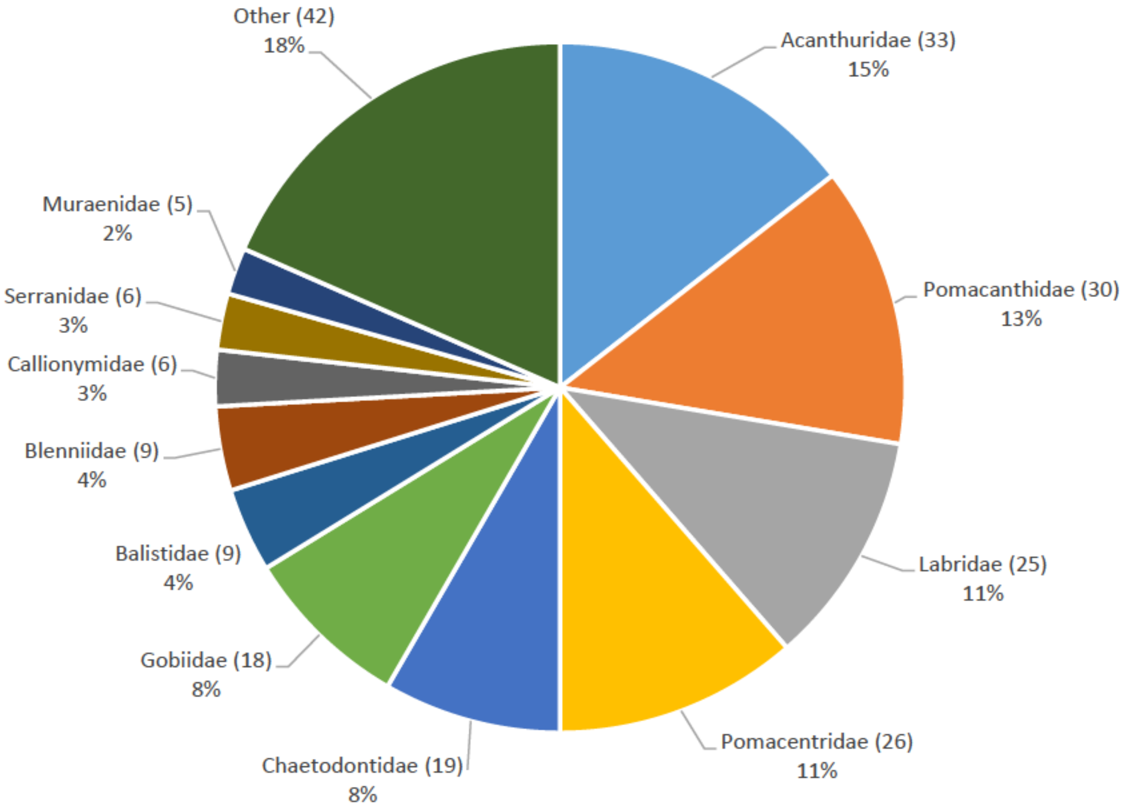


Figure 5.1: Composition by family of marine aquarium fish imported into South Africa. *Data for the top 11 families are provided, with the remainder grouped as 'other'. The number proceeding the family name in parentheses signifies the number of species recorded within each family.*

Table 5.1: Top 20 most frequently imported marine aquarium fish in South Africa showing their distribution ranges, frequency of occurence (%) in a pet store nationally, and the maximum number of stock per species counted. *Distribution ranges were derived from FishBase (Froese and Pauly 2002). Highlighted species are alien to South African waters. The frequency of occurrence (%) is based on the number of pet store visits that stocked the species divided by the total number of pet store visits nationally. The maximum number of stock per species counted is based on any pet store visit from any region.*

Species	Common name	Distribution	Frequency of occurence (%)	Maximum stock count
1. *Labroides dimidiatus*	Bluestreak cleaner wrasse	IndoPacific	63,0	26
2. *Chromis viridis*	Bluegreen chromis	IndoPacific	48,1	38
3. *Zebrasoma flavescens*	**Yellow tang**	Pacific Ocean	48,1	12
4. *Amphiprion ocellaris*	**Ocellaris clownfish, Common clownfish**	IndoWest Pacific	40,7	34
5. *Chelmon rostratus*	**Copperband butterflyfish**	Western Pacific	40,7	7
6. *Paracanthurus hepatus*	Regal tang, Palette surgeonfish, Hippo tang	IndoPacific	37,0	15
7. *Valenciennea puellaris*	**Diamond watchman goby, Orangespotted sleepergoby**	IndoPacific	37,0	9
8. *Salarias fasciatus*	**Lawnmower blenny, Sailfin blenny, Algae blenny**	IndoPacific	37,0	7
9. *Ecsenius bicolor*	**Bicolor blenny, Flame tail blenny, Red tail blenny**	IndoPacific	37,0	4
10. *Forcipiger flavissimus*	Yellow longnose butterflyfish, Forceps butterflyfish	IndoPacific, Micronesia, Eastern Pacific	37,0	4
11. *Pomacanthus imperator*	Emperor angelfish	IndoPacific	37,0	4
12. *Pterapogon kauderni*	**Banggai cardinalfish**	Banggai Islands, Indonesia	33,3	23
13. *Centropyge bispinosa*	Coral beauty, Twospined angelfish	IndoPacific	33,3	6
14. *Synchiropus splendidus*	**Mandarinfish, Mandarin dragonet**	Western Pacific	33,3	5
15. *Ecsenius midas*	Midas blenny, Lyretail blenny, Golden blenny	IndoPacific	29,6	6

Species	**Common name**	**Distribution**	**Frequency of occurence (%)**	**Maximum stock count**
16. *Zebrasoma veliferum*	**Sailfin tang**	Western Indian Ocean, Pacific Ocean	29,6	6
17. *Sphaeramia nematoptera*	**Pajama cardinalfish**	IndoPacific	25,9	19
18. *Zanclus cornutus*	Moorish idol	IndoPacific, Eastern Pacific	25,9	7
19. *Chaetodon vagabundus*	Vagabond butterflyfish	IndoPacific	25,9	4
20. *Naso lituratus*	**Orange spine surgeonfish, Lipstick tang, Clown tang**	Eastern Pacific	25,9	3

5.3 Discussion

Extensive sampling of fish for sale in marine aquarium pet stores across South Africa revealed that the number of fish species in the trade is vast (n = 228), 60 % are alien, and that this pathway for the introduction of marine fish and invertebrate species deserves greater attention through regulations and research. As far as the author is aware, this is the first study that has attempted to identify and quantify the marine fish species traded on a national scale in South Africa. As discussed in Chapter 2.6.4, Everett and van der Elst (2008) conducted preliminary quantifications of species traded in the marine aquarium trade in Durban.

Overall, the Acanthuridae surgeonfish, tangs, and unicornfish are the most popular family of marine fish imported (15%), followed by the Pomacanthidae angelfish (13%), the Pomacentridae clownfish and damselfish (11%), the Labridae wrasse (11%), the Chaetodontidae butterflyfish (8%), and the Gobiidae gobies (8%), respectively. These results are in line with a number of studies that list these marine fish families as the most common within the marine aquarium trade (Wabnitz et al., 2003; Everett and van der Elst, 2008; Rhyne et al., 2017). An alternative to traditional pet store purchasing that became apparent in this study is that of the online market. The extent of this trade was not examined and therefore part of the marine aquarium trade diversity in South Africa remains unexplored.

Although none of the species inventoried in this study were listed as invasive globally, five species were recorded on AquaNIS and in the literature as being introduced into foreign bodies of water. *Zebrasoma xanthurum* (Guidetti et al., 2016) and *Z. flavescens* (Weitzmann et al., 2016) were sighted in the Mediterranean Sea and hypothesized as being introduced through the marine aquarium trade.

Previous studies have demonstrated that the probability of introduction of aquarium organisms is significantly higher in regions near to large coastal-lying cities (Guidetti et al., 2016; Tricarico et al., 2016). In Chapter 4 it was confirmed that marine aquarium hobbyists release unwanted organisms into the ocean. The flow of trade in these organisms is greater inland (Pretoria/Johannesburg) than it is in coastal cities, with pet stores often receiving stock twice a month (pers. obs.). Pet stores in Pretoria/Johannesburg stocked almost double the quantity than that of pet stores in Durban and Cape Town. As there is no access to the ocean where trade flow is at its greatest, it is hypothesized that the total risk of introducing unwanted pests from marine aquaria is reduced. However, pest organisms from inland areas are still an introduction risk to coastal waters through the variety of informal trading pathways revealed in Chapter 4. Although the strength of this vector has not been formally defined, there is no doubt that this is a potential pathway for the introduction of alien invasive marine aquarium fish and invertebrate organisms into South African waters.

While the international trade targets invertebrates which constitute only one tenth of the number of marine fish species (Rhyne et al., 2012), the most notorious and successful invaders are those of marine invertebrates and algae (Lowe et al., 2000). As mentioned in the limitations of this study (Chapter 1.3), the identification and quantification of macroalgae, corals, and other marine invertebrates was not part of the aim of this study. This would provide valuable insight into the overall diversity traded, and would be necessary to highlight species of invasion risk to South Africa. It is difficult to infer accurate species-level identifications for corals and invertebrates based on photographs alone, and sampling of the specimens would not be feasible. The combined effort of regulatory officials, pet stores, and taxonomic experts would be necessary to successfully quantify and accurately identify the aforementioned taxa.

Importation for the ornamental aquarium trade is listed among the top five pathways of non-native introductions of species, and accounts for more than one-third of the aquatic species on the IUCN 100 Worst Invaders list (Padilla and Williams, 2004; Semmens et al., 2004; Strecker et al., 2011). This highlights the importance of accurate species-level identifications. While conducting stock inventories for marine aquarium fish in this study, it was noted that a variety of common names are used to refer to a single species. Also, where present, lists of species for sale displayed in pet stores do not reflect scientific names for the common names given. In one instance, it was challenging to assign an identification to a racoon butterflyfish (*Chaetodon lunula*) as this species displays different colouring and shaping depending on their sourced region. Another noted example is that of the commonly traded Emperor Angelfish (*Pomacanthus imperator*), where a specimen was almost misidentified as juveniles look very similar to adults of a different species, such as that of the Chrysurus Angelfish (*Pomacanthus chrysurus*). Sometimes, the imported species that arrive are very different to what was ordered (anonymous, pers. comm., 2017). The pet store staff are then required to make an identification to their best knowledge, which may result in misidentifications. It is evident that it is extremely easy to misidentify species that are brought into the country through the marine aquarium trade, which consequently affects biosecurity management practices.

Although industry reporting and monitoring is fundamentally important to the management of the marine aquarium trade, there is currently no comprehensive system for tracking import and export data of marine species traded in South Africa. Invoice accuracy and reliability has been questioned by other researchers (Wabnitz et al., 2003; Rhyne et al., 2012; Militz et al., 2016). Further research should investigate discrepancies between invoice data and the identities of box contents. It is recommended that a national online database be established and that the identities and quantities of all imported marine fish and invertebrates are entered, similar to that of The Marine Aquarium Biodiversity and Trade Flow online database (https://www.aquariumtradedata.org/) developed by Rhyne et al. (2015). Information from this database can be used to determine trade flow and species that pose a risk of invasion, but can also provide a wealth of information for monitoring vulnerable populations that could be threatened by the South African marine aquarium trade market. Furthermore, recommendations regarding control and management measures can be suggested using reliable data.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

The marine aquarium trade has been recognized as a pathway for the transmission of numerous alien species globally (Odom, 2012; Williams et al., 2015; Allen et al., 2017; Vranken et al., 2018). As far as the author is aware, this is the first study, globally, that has quantified and identified the diversity of marine invertebrates associated with live rock. The results confirm that live rock utilized in marine aquaria is a vector for alien invasive species in South Africa. A combination of DNA barcoding and morphological identifications discovered 17 specimens of the alien invasive live rock associated *Aiptasia pulchella* on one of the 6 replicates of live rock examined in the study. While this assessment of live rock associated taxa only presents a small sample of the potentially invasive species introduced into pet stores in South Africa, it provides an insight into the diversity of marine invertebrates associated with live rock which is then transferred to hobbyists' aquaria. Further studies should aim to identify and quantify the macroalgae assemblages associated with live rock. Although not part of the aim of this study, non-target DNA amplification exposed the coral pathogenic bacteria *Vibrio* sp., which further verifies that the marine aquarium trade is also a vector for diseases. This pathway is a potential biosecurity risk to the marine aquarium trade and to South African aquatic ecosystems and requires urgent attention.

Live rock was observed to transmit and introduce organisms via a number of mechanisms of secondary spread within South Africa: the trade between fellow hobbyists; the sale of live rock from a hobbyist's previously established aquarium to a pet stores; the re-purchase of the aforementioned live rock by another hobbyist; the transmission of pest organisms, already established within pet store aquaria, to imported live rock; and ultimately, and potentially the most devastating, the release of pest live rock associated organisms by hobbyists into the ocean (Chapter 4). In Chapter 4.2.2, it was observed that corals and the substrate to which they are attached might have the potential to transfer non-target taxa. Consequently, this could also be a vector for alien invasive species in South Africa through the same mechanisms of secondary spread listed here for live rock.

The marine aquarium hobbyist survey revealed that the disposal methods of pest organisms are of concern, in particular the release of unwanted or pest live rock associated species into the ocean. Furthermore, informal trading and exchanges between aquarium hobbyists as well as purchases from online pet stores are further avenues for the introduction of alien invasive organisms and constitute a noteworthy portion of the marine aquarium organism diversity that also requires investigaton.

A broad range of climatic conditions is characteristic of South African coastal waters, with sea surface temperatures varying between 20°C and 28°C along the warm tropical east coast, and between 10°C and 18°C along the cool temperate west coast (Griffiths et al., 2010). Given that the distribution of a marine species is largely governed by temperature (Tittensor et al. 2010), it is expected that the invasion potential of a species is greatest in thermally similar areas to that of its native range (Swart and Robinson, 2019). Most marine aquarium fishes traded are native to tropical regions of the world, and cannot tolerate temperatures below 18°C (Eme and Bennett, 2009; Velasco-Blanco et al., 2019). Therefore, it is more likely that a marine aquarium introduction will result in a successful establishment in the warmer coastal waters off the east coast of South Africa compared to the cooler southern and western waters. This inference is key to the prioritization of conservation and management initiatives of regions that are more likely to be invasible in South Africa.

It is suggested that South African universities, conservation organizations, and the Department of Agriculture, Forestry and Fisheries (DAFF) combine their efforts to provide proactive education to marine aquarium hobbyists and pet store staff in order to promote responsible aquarium ownership skills, including, but not limited to: how to safely remove and dispose of aquarium pests; the importance of quarantining organisms and live rock before introducing them to an aquarium; information on key life history characteristics (aggressiveness, reproduction methods/growth rate, maximum size, etc.) to prevent hobbyists from purchasing pets that eventually become unwanted; and how to use chemicals to treat aquarium water before disposing of it (Livengood and Chapman, 2007; Chang et al., 2009; Walters et al., 2011; Vranken et al., 2018). Foundational aspects of invasion biology (e.g. What are alien species? Why are they problematic?) should also be explored through public awareness and education campaigns.

With these vectors and numerous mechanisms of secondary spread in mind, van Kleunen et al. (2015) demonstrated that rates of establishment are higher for organisms spreading within a region (intra-regional) than for those introduced from international sources (inter-regional) as a result of higher propagule pressure and shorter geographical distances. Although based on plants, their observations are highly similar to the logistics demonstrated in this study for the marine aquarium trade in South Africa. Durban and Cape Town pet stores revealed that most of their stock 'piggy-backs' on the orders of Johannesburg/Pretoria pet stores who import from international sources (Chapter 5.2). The transfer of trade stock from Johannesburg/Pretoria pet stores to the Durban and Cape Town pet stores is another potential mechanism of secondary spread of pest organisms within the marine aquarium trade in South Africa. Therefore, the marine aquarium trade, and the multitude of vectors associated therewith, are of concern and should be further addressed by formal observations.

Despite the high diversity and volume of marine fish assessed in this study, there are few regulations and laws enforced for the monitoring and control thereof (van der Walt et al., 2017). It is recommended that legislation be revisited to enforce pet store owners to keep records of species imported. Further studies should conduct stock inventories that compare box contents to the species names on invoices. Photographs do not provide the detail necessary to infer species-level identifications for most invertebrates, and since it is not feasible to sample aquarium stock on a national scale, experts in the taxonomy of a variety of taxa would need to be recruited. To achieve this, the compliance of pet stores will be required which may necessitate the assistance of regulatory officials from DAFF. This should resolve discrepancies in trade data and provide a further level of screening for alien invasive species introduced by accident. Identified organisms that show pest-like characteristics or are predicted to be of invasion risk should also be investigated through the examination of their environmental conditions and temperature tolerances in order to predict their ability to tolerate and thrive in South African waters. These methods have been successfully used by Chang et al. (2009) and Vranken et al. (2018) to highlight aquarium species of concern.

If biosecurity measures are to be improved, a rapid and reliable means for species identification is required (Bucklin et al., 2011). This study has demonstrated that the success of DNA barcoding for the sole purpose of species identification is highly dependent on the availability and species coverage of reference sequences in the GenBank and BOLD online databases. It is recommended that a combination of morphological and molecular data be used to assign accurate species-level identifications where biosecurity matters are concerned. Depending on the diversity of the taxa being sampled, a number of barcoding regions may need to be amplified to resolve or produce species-level identifications. Creating a comprehensive database for biological diversity requires a global effort, and it is of utmost importance that researchers submit their DNA sequenced data to public online databases (Radulovici et al., 2010).

The ecosystems and geographic regions most susceptible to alien introductions by the South African marine aquarium trade should be identified to allow for the implementation of inspection points with quarantine measures. The early detection of imported species is vital as the prevention of introductions of alien species is more cost-effective than the eradication and control of the resulting post-introduced invaders (Simberloff et al. 2013; Ojaveer et al., 2015; Faulkner et al., 2016). The number of alien species increasingly being introduced into South Africa and the rest of Africa (Robinson et al., 2005; Faulkner et al., 2017; Marr et al., 2017) suggests that current biosecurity measures and efforts are insufficient and deserve greater recognition in policy- and regulation-making, and in any future monitoring and control programmes that aim to mitigate the many vectors of alien invasive species introductions (Faulkner et al., 2016).

Since there is a marked lack of knowledge on marine alien species introduced through the marine aquarium trade in South Africa, this study has provided significant preliminary insight into this introduction pathway, along with its associated vectors, and serves as a foundation for further research. The research presented here has been conducted with the marine aquarium hobbyist in mind: the intention is not to spoil the popular trade with restrictions, but rather to increase knowledge surrounding the sustainability and associated conservation impacts thereof, and to this end education initiatives are paramount.

REFERENCES

Acharya, K.P. (2014). Invasive species: genetics, characteristics and trait variation along a latitudinal gradient. PhD thesis. Norwegian University of Science and Technology, Trondheim, Norway, 74 pp.

Achituv, Y. (1973). On the distribution and variability of the Indo-Pacific sea star *Asterina wega* (Echinodermata: Asteroidea) in the Mediterranean Sea. Marine Biology, 18: 333–336.

Acosta, H., Forrest, B.M. (2009). The spread of marine non-indigenous species via recreational boating: a conceptual model for risk assessment based on fault tree analysis. Ecological Modelling, 220(13-14): 1586–1598.

Ahyong, S.T., Kupriyanova, E., Burghardt, I., Sun, Y., Hutchings, P.A., Capa, M., Cox, S.L. (2017). Phylogeography of the invasive Mediterranean fan worm, *Sabella spallanzanii* (Gmelin, 1791), in Australia and New Zealand. Journal of the Marine Biological Association of the United Kingdom, 97(5): 985–991.

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle, in: Petrov, B. & Csaki, F. (Eds.) Proceedings of the Second International Symposium on Information Theory. Akadémia Kiado. Budapest, Hungary, pp. 267–281.

Alexander, M.E., Simon, C.A., Griffiths, C.L., Peters, K., Sibanda, S., Miza, S., Groenewald, B., Majiedt, P., Sink, K.J., Robinson, T.B. (2016). Back to the future: reflections and directions of South African marine bioinvasion research. African Journal of Marine Science, 38 (1): 141–144.

Allen, P., Barquero, M., Bermúdez, E., Calderón, J., Hilje, B., Pineda, W., Saborío-Rodríguez, G., Arguedas, V., Chacón-Madrigal, E. (2017). Calling for more accurate information in aquarium trade: analysis of live-fish import permits in Costa Rica. Management of Biological Invasions, 8: 533–542.

Allendorf, F., Lundquist, L. (2003). Population biology, evolution, and control of invasive species. Conservation Biology, 17: 24–30.

Altschul, S., Gish, W., Miller, W., Myers, E., Lipman, D. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215: 403–410.

Anderson, L. (2005). California's reaction to *Caulerpa taxifolia*: a model for invasive species rapid response. Biological Invasions, 7: 1003–1016.

Anton, A., Geraldi, N.R., Lovelock, C.E., Apostolaki, E.T., Bennett, S., Cebrian, J., Krause-Jensen, D., Marbà, N., Martinetto, P., Pandolfi, J.M. (2019). Global ecological impacts of marine exotic species. Nature Ecology and Evolution, 3: 787–800.

Antozzi, W.O. (1997). The developing live rock aquaculture industry. Report SERO-ECON-98-10. National Marine Fisheries Service, Southeast Regional Office. St. Petersburg, Florida, pp. 11.

Appleton, A., Miranda, N. (2015). Two Asian freshwater snails newly introduced into South Africa and an analysis of alien species reported to date. African Invertebrates, 56: 1–17.

Arboleda, M., Reichardt, W. (2010). *Vibrio* sp. causing Porites ulcerative white spot disease. Diseases of Aquatic Organisms, 90: 93–104.

Arellano-Méndez, L., Mora-Olivo, A., Zamora-Tovar, C., de la Rosa-Manzano, E. (2017). First report of the invasive red lionfish *Pterois volitans* (Linnaeus, 1758) (Scorpaenidae) on the coast of Tamaulipas, Mexico. BioInvasions Records, 6: 255–258.

Arias, A., Barroso, R., Anadon, N., Paiva, P. (2013). On the occurrence of the fireworm *Eurythoe complanata* complex (Annelida, Amphinomidae) in the Mediterranean Sea with an updated revision of the alien Mediterranean amphinomids. Zookeys, 337: 19–33.

Ashton, G., Boos, K., Shucksmith, R., Cook, E. (2006). Risk assessment of hull fouling as a vector for marine non-natives in Scotland. Aquatic Invasions, 1: 214–218.

Bacela-Spychalska, K., Grabowski, M., Rewicz, T., Konopacka, A., Wattier, R. (2013). The "killer shrimp" *Dikerogammarus villosus* (Crustacea, Amphipoda) invading Alpine lakes: overland transport by recreational boats and scuba-diving gear as potential entry vectors? Aquatic Conservation-Marine and Freshwater Ecosystems, 23: 606–618.

Barreiros, J.P. (2017). Invasions changing marine biodiversity. Journal of Aquaculture & Marine Biology, 5(2): 00111.

Barrington, K. (2018). Getting rid of *Aiptasia* in your reef aquarium [WWW Document]. Rate My Fish Tank. URL https://www.ratemyfishtank.com/blog/getting-rid-of-aiptasia-in-your-reef-aquarium (accessed 29 February 2019).

Barroso, R., Klautau, M., Paiva, A. (2010). *Eurythoe complanata* (Polychaeta: Amphinomidae), the 'cosmopolitan' fireworm, consists of at least three cryptic species. Marine Biology, 157: 69–80.

Bates, A., McKelvie, C., Sorte, C., Morley, S., Jones, N., Mondon, J., Bird, T., Quinn, G. (2013). Geographical range, heat tolerance and invasion success in aquatic species. Proceedings of the Royal Society B, 280: 1–8.

Bellan, G. (2001). Polychaeta, in: Costello, M., Emblow, C., White, R. (Eds.) European register of marine species: a check-list of the marine species in Europe and a bibliography of guides to their identification. Collection Patrimoines Naturels, 50: 214-231.

Benayahu, Y., Loya, Y. (1985). Settlement and recruitment of a soft coral: why is *Xenia macrospiculata* a successful colonizer? Bulletin of Marine Science, 36: 177–188.

Benayahu, Y., Van Ofwegen, L.P., Mcfadden, C.S. (2018). Evaluating the genus *Cespitularia* MilneEdwards & Haime, 1850 with descriptions of new genera of the family Xeniidae (Octocorallia, Alcyonacea). Zookeys, 754: 63–101.

Bernatchez, L. (2016). On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes. Journal of Fish Biology, 89: 2519–2556.

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V., Wilson, J.R., Richardson, D.M. (2011). A proposed unified framework for biological invasions. Trends in Ecology & Evolution, 26: 333–339.

Blundell, A.G., Mascia, M.B. (2005). Discrepancies in reported levels of international wildlife trade. Conservation Biology, 19: 2020–2025.

Bock, D.G., Caseys, C., Cousens, R.D., Hahn, M.A., Heredia, S.M., Hübner, S., Turner, K.G., Whitney, K.D., Rieseberg, L.H. (2015). What we still don't know about invasion genetics. Molecular Ecology, 24: 2277–2297.

Bolton, T.F., Graham, W.M. (2006). Jellyfish on the rocks: bioinvasion threat of the international trade in aquarium live rock. Biological Invasions, 8: 651–653.

Bourne, S.D., Hudson, J., Holman, L.E., Rius, M. (2018). Marine invasion genomics: revealing ecological and evolutionary consequences of biological invasions, in: Oleksiak, M.F. & Rajora, O.P. (Eds.) Population genetics: marine organisms, population genomics. Springer International Publishing, pp. 1–36.

Branch, G.M., Griffiths, C.L., Branch, M.L., Beckley, L.E. (1994). Two oceans. A guide to the marine life of southern Africa. David Philip. Cape Town, South Africa, 360 pp.

Brough, C. (2015). *Aiptasia* pests - getting rid of glass anemones [WWW Document]. Animal-World: pet and animal information. URL http://animal-world.com/Aquarium-Coral-Reefs/Aiptasia-Pests (accessed 29 February 2019).

Bruckner, A.W. (2005). The importance of ornamental reef fish trade in the wider Caribbean. International Journal of Tropical Biology and Conservation, 53: 127–138.

Brunel, S., Fernández-Galiano, E., Genovesi, P., Heywood, V.H., Kueffer, C., Richardson, D.M. (2013). Invasive alien species: a growing but neglected threat? in: Late lessons from early warnings: science, precaution, innovation. European Environment Agency. Copenhagen, Denmark, pp. 486 - 508.

Bucklin, A., Steinke, D., Blanco-Bercial, L. (2011). DNA barcoding of marine metazoa. Annual Review of Marine Science, 3: 471–508.

Burgess, W., Axelrod, H., Hunziker III, R. (1997). Dr. Burgess's atlas of marine aquarium fishes. T.F.H. Publications Inc. United States of America, 1023 pp.

Calado, R., Narciso, L. (2005). Ability of Monaco shrimp *Lysmata seticaudata* (Decapoda: Hippolytidae) to control the pest glass anemone *Aiptasia pallida* (Actiniaria: Aiptasidae). Helgoland Marine Research, 59: 163–165.

Calfo, A. (2004). Aquarium culture of the aeolid nudibranch *Berghia*, predator on the nuisance anemone *Aiptasia*. Reefkeeping, 2(12): 2–13.

Canning-Clode, J., Fofonoff, P., McCann, L., Carlton, J., Ruiz, G. (2013). Marine invasions on a subtropical island: fouling studies and new records in a recent marina on Madeira Island (Eastern Atlantic Ocean). Aquatic Invasions, 8: 261-270.

Carlton, J. (2001). Introduced species into US coastal waters: environmental impacts and management priorities. Pew Oceans Commission. Arlington, Virginia, 28 pp.

Carlton, J.T., Fowler, A.E. (2018). Ocean rafting and marine debris: a broader vector menu requires a greater appetite for invasion biology research support. Aquatic Invasions, 13: 11–15.

Carroll, D., Kempf, S. (1990). Laboratory culture of the aeolid nudibranch *Berghia verrucicornis* (Mollusca, Opisthobranchia): some aspects of its development and life history. Biological Bulletin, 179: 243–253.

CBD (2014). Pathways of introduction of invasive species, their prioritization and management. [WWW Document]. URL https://www.cbd.int/doc/meetings/sbstta/sbstta-18/official/sbstta-18-09-add1-en.pdf (accessed 1 March 2020).

Chan, F., Briski, E. (2017). An overview of recent research in marine biological invasions. Marine Biology, 164: 1–10.

Chang, A., Grossman, J., Sabol Spezio, T., Weiskel, H., Blum, J., Burt, J., Muir, A., Piovia-Scott, J., Veblen, K., Grosholz, E. (2009). Tackling aquatic invasions: risks and opportunities for the aquarium fish industry. Biological Invasions, 11: 773–785.

Charif, D., Lobry, J. (2007). seqinr 1.0-2: a contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis, in: Bastolla, U., Porto, M., Roman, H., Vendruscolo, M. (Eds.) Structural approaches to sequence evolution: molecules, networks, populations. Springer Verlag. New York, pp. 207–232.

Chase, M.W., Cowan, R.S., Hollingsworth, P.M., van den Berg, C. (2007). A proposal for a standardised protocol to barcode all land plants. Taxon, 56: 295–299.

Chen, C., Soong, K., Chen, C.A. (2008). The smallest oocytes among broadcast-spawning Actiniarians and a unique lunar reproductive cycle in a unisexual population of the sea anemone, *Aiptasia pulchella* (Anthozoa: Actiniaria). Zoological Studies, 47: 37–45.

Chen, J., Li, Q., Kong, L., Yu, H. (2011). How DNA barcodes complement taxonomy and explore species diversity: the case study of a poorly understood marine fauna. PLoS ONE, 6(6): e21326.

Clarke Murray, C., Pakhomov, E.A., Therriault, T.W. (2011). Recreational boating: a large unregulated vector transporting marine invasive species. Diversity and Distributions, 17: 1161–1172.

Clegg, S.M., Degnan, S.M., Kikkawa, J., Moritz, C., Estoup, A., Owens, I.P.F. (2002). Genetic consequences of sequential founder events by an island-colonizing bird. Proceedings of the National Academy of Sciences, 99: 8127–8132.

Clusella-Trullas, S., Garcia, R. (2017). Impacts of invasive plants on animal diversity in South Africa: a synthesis. Bothalia - African Biodiversity & Conservation, 47: 1–12.

Coissac, E., Hollingsworth, P.M., Lavergne, S., Taberlet, P. (2016). From barcodes to genomes: extending the concept of DNA barcoding. Molecular Ecology, 25: 1423–1428.

Colautti, R.I., MacIsaac, H.I. (2004). A neutral terminology to define “invasive” species. Diversity and Distributions, 10: 135–141.

Colin, P. (2009). Marine environments of Palau. Indo-Pacific Press. San Diego, California, 215 pp.

Colin, P., Arneson, C. (1995). Tropical Pacific invertebrates: a field guide to the marine invertebrates occurring on tropical Pacific coral reefs, seagrass beds and mangroves. The Coral Reef Research Foundation. Coral Reef Press, 304 pp.

Collins, R.A. (2012). Identification of ornamental fishes for biosecurity. PhD thesis. Lincoln University, New Zealand, 194 pp.

Concepcion, G., Kahng, S., Crepeau, M., Franklin, E., Coles, S., Toonen, R. (2010). Resolving natural ranges and marine invasions in a globally distributed octocoral (genus *Carijoa*). Marine Ecology Progress Series, 401: 113–127.

DAFF (2015). Guide to the authorisation requirements for aquaculture in South Africa. Department of Agriculture, Forestry and Fisheries. Cape Town, South Africa, 42 pp.

David, M., Magaletti, E., Kraus, R., Marini, M. (2019). Vulnerability to bioinvasions: current status, risk assessment and management of ballast water through a regional approach – the Adriatic Sea. Marine Pollution Bulletin, 147: 1-7.

Davidson, A.M., Jennions, M., Nicotra, A.B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology Letters, 14: 419–431.

Dávila-Jiménez, Y., Tovar-Hernández, M., Simoes, N. (2017). The social feather duster worm *Bispira brunnea* (Polychaeta: Sabellidae): aggregations, morphology and reproduction. Marine Biology Research, 13(7): 782-796.

Day, J. (1967). A monograph on the Polychaeta of Southern Africa, part I (Errantia) and part II (Sedentaria). Trustees of the British Museum, Natural History. London, 878 pp.

de Lange, W.J., van Wilgen, B.W. (2010). An economic assessment of the contribution of biological control to the management of invasive alien plants and to the protection of ecosystem services in South Africa. Biological Invasions, 12: 4113–4124.

de Silva, S. (2012). Aquaculture: a newly emergent food production sector - and perspectives of its impacts on biodiversity and conservation. Biodiversity and Conservation, 21: 3187–3220.

Dennison, R. (2019). Harlequin shrimp care guide, in: Dennison, R. (Ed.) The Fishkeeper. Dennison Publishing. Durban, South Africa, 10(2): pp. 8-10.

DeWitt, T., Scheiner, S. (2004). Phenotypic variation from single genotypes: a primer, in: DeWitt, T. & Scheiner, S. (Eds.) Phenotypic plasticity: functional and conceptual approaches. Oxford University Press. Oxford, England, 11 pp.

Dey, V. (2016). The global trade in ornamental fish. INFOFISH International, 4: 52–55.

Dias, P.J., McKenzie, C.H., Wells, F.E., Pederson, J.A., Carlton, M.L.C. (2017). Keeping up with marine bioinvasions: building bridges, crossing borders and moving forward at the international conference on marine bioinvasions. Management of Biological Invasions, 8: 137–140.

Early, R., Bradley, B., Dukes, J., Lawler, J., Olden, J., Blumenthal, D., Gonzalez, P., Grosholz, E., Ibañez, I., Miller, L., Sorte, C., Tatem, A. (2016). Global threats from invasive alien species in the twenty-first century and national response capacities. Nature Communications, 7: 1–9.

Egoh, B.E., Ntshotsho, P., Maoela, M.A., Blanchard, R., Ayompe, L.M., Rahlao, S. (2020). Setting the scene for achievable post-2020 convention on biological diversity targets: a review of the impacts of invasive alien species on ecosystem services in Africa. Journal of Environmental Management, 261: 110-171.

Ellender, B., Weyl, O. (2014). A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. Aquatic Invasions, 9: 117–132.

Ellis, S., Sharron, L. (1999). The culture of soft corals (order: Alcyonacea) for the marine aquarium trade. Publication Number 137. Waimanalo, Hawaii, USA, 77 pp.

Ellul, T., Evans, J., Schembri, P.J. (2019). Invasion alert: rapid range expansion of *Caulerpa taxifolia var. distichophylla* in Maltese waters (central Mediterranean). BioInvasions Records, 8: 1–10.

Elton, C. (1958). The ecology of invasions by animals and plants. Methuen, London, 181 pp.

Eme, J., Bennett, W.A. (2009). Critical thermal tolerance polygons of tropical marine fishes from Sulawesi, Indonesia. Journal of Thermal Biology, 34: 220–225.

Estoup, A., Beaumont, M., Sennedot, F., Moritz, C., Cornuet, J. (2004). Genetic analysis of complex demographic scenarios: spatially expanding populations of the cane toad, *Bufo marinus*. Evolution (N. Y), 58: 2021–2036.

Everett, B., van der Elst, R. (2008). An assessment of the marine ornamental fisheries of KwaZulu-Natal, South Africa from 1990 to 2003 (ORI Unpublished Report 269). Durban, South Africa, 15 pp.

Fabricius, K., Alderslade, P. (2001). Soft corals and sea fans: a comprehensive guide to the tropical shallow water genera of the central-west Pacific, the Indian Ocean and the Red Sea. Australian Institute of Marine Science. Townsville, Australia, 264 pp.

Famà, P., Wysor, B., Kooistra, W., Zuccarello, G. (2002). Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast tuf*A* gene. Journal of Phycology, 38: 1040–1050.

Farrapeira, C., Melo, A., Barbosa, D., Silva, K. (2007). Ship hull fouling in the Port of Recife, Pernambuco. Brazilian Journal of Oceanography, 55(3): 207–221.

Fauchald, K. (1977). The polychaete worms. Definitions and keys to the orders, families and genera. Natural History Museum of Los Angeles County. Science Series, 28: 1-188.

Fauchald, K. (1992). A review of the genus *Eunice* (Eunicidae: Polychaeta) based upon type material. Smithsonian Contributions to Zoology, 523: 1-422.

Faulkner, K., Hurley, B., Robertson, M., Rouget, M., Wilson, J. (2017). The balance of trade in alien species between South Africa and the rest of Africa. Bothalia - African Biodiversity & Conservation, 47: 1–16.

Faulkner, K., Robertson, M., Rouget, M., Wilson, J. (2016). Understanding and managing the introduction pathways of alien taxa: South Africa as a case study. Biological Invasions, 18: 73–87.

Faulkner, K.T., Burness, A., Byrne, M.J., Kumschick, S., Peters, K., Robertson, M.P., Saccaggi, D.L., Weyl, O.L.F., Williams, V.L. (2020). South Africa's pathways of introduction and dispersal and how they have changed over time, in: van Wilgen, B.W., Measey, J., Richardson, D.M., Wilson, J.R., Zengeya, T.A. (Eds.) Biological invasions in South Africa. Invading Nature - Springer Series in Invasion Ecology 14. Cham, Switzerland, pp 313-354.

Fauvel, P. (1953). The fauna of India including Pakistan, Ceylon, Burma and Malaya: Annelida, Polychaeta. The Indian Press, Ltd. Allahabad, India, 507 pp.

Fenner, B. (2019). FAQs about *Asterina* (tiny, white...) Sea Stars, Compatibility/Control [WWW Document]. Wet Web Media. URL http://www.wetwebmedia.com/asterinacompf.htm (accessed 20 February 2019).

Ferreira, C., Luiz, O., Floeter, S., Lucena, M., Barbosa, M., Rocha, C., Rocha, L. (2015). First record of invasive lionfish (*Pterois volitans*) for the Brazilian coast. PLoS ONE, 10: 1–5.

Fletcher, L.M., Zaiko, A., Atalah, J., Richter, I., Dufour, C.M., Pochon, X., Wood, S.A., Hopkins, G.A. (2017). Bilge water as a vector for the spread of marine pests: a morphological, metabarcoding and experimental assessment. Biological Invasions, 19: 2851–2867.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3: 294–299.

Frézal, L., Leblois, R. (2008). Four years of DNA barcoding: current advances and prospects. Infection, Genetics and Evolution, 8: 727–736.

Froese, R., Pauly, D. (Eds.) (2002). FishBase [WWW Document]. URL http://www.fishbase.org/ (accessed 16 August 2018).

Fuller, T., Hughey, J. (2013). Molecular investigation of the invasive sponge *Hymeniacidon sinapium* (de Laubenfels, 1930) in Elkhorn Slough, California. Aquatic Invasions, 8: 59–66.

Fusco, G., Minelli, A. (2010). Phenotypic plasticity in development and evolution: facts and concepts. Philosophical Transactions of the Royal Society B: Biological Sciences, 365: 547–556.

Galil, B. (2014). Seeing red: alien species along the Mediterranean coast of Israel. Aquatic Invasions, 2: 281–312.

Galil, B., Zenetos, A. (2002). A sea change - exotics in the Eastern Mediterranean Sea, in: Leppakoski, E., Olenin, S., Gollasch, S. (Eds.) Invasive aquatic species of Europe: distribution, impact and management. Kluwer Academic Publishers. Dordrecht, Boston, London, pp. 325–336.

Geburzi, J.C., McCarthy, M.L. (2018). How do they do it? Understanding the success of marine invasive species, in: Jungblut, S., Liebich, V., Bode, M. (Eds.) YOUMARES 8 - Oceans across boundaries: learning from each other. Springer. Cham, Switzerland, pp. 109–144.

Gertzen, E., Familiar, O., Leung, B. (2008). Quantifying invasion pathways: fish introductions from the aquarium trade. Canadian Journal of Fisheries and Aquatic Sciences, 65: 1265–1273.

Ghalambor, C., McKay, J., Carroll, S., Reznick, D. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology, 21: 394–407.

Giangrande, A., Cavallo, A., Licciano, M., Mola, E., Pierri, C., Trianni, L. (2005). Utilization of the filter feeder polychaete *Sabella spallanzanii* Gmelin (Sabellidae) as bioremediator in aquaculture. Aquaculture International, 13: 129–136.

Gibbs, P.E. (1978). Macrofauna of the intertidal sand flats on low wooded islands, Northern Great Barrier Reef. Philosophical Transactions of the Royal Society of London Series B, 284(999): 81-97.

Gollasch, S., Hewitt, C.L., Bailey, S., David, M. (2019). Introductions and transfers of species by ballast water in the Adriatic Sea. Marine Pollution Bulletin, 147: 8–15.

González, J., Grijalba-Bendeck, M., Arturo, A., Ricardo, B. (2009). The invasive red lionfish, *Pterois volitans* (Linnaeus 1758), in the southwestern Caribbean Sea. Aquatic Invasions, 4: 507–510.

Grabowski, M., Bacela, K., Konopacka, A. (2007). How to be an invasive gammarid (Amphipoda: Gammaroidea) - comparison of life history traits. Hydrobiologia, 590: 75–84.

Grajales, A., Rodríguez, E. (2014). Morphological revision of the genus *Aiptasia* and the family Aiptasiidae (Cnidaria, Actiniaria, Metridioidea). Zootaxa, 3826: 55–100.

Grajales, A., Rodríguez, E. (2016). Elucidating the evolutionary relationships of the Aiptasiidae, a widespread cnidarian-dinoflagellate model system (Cnidaria: Anthozoa: Actiniaria: Metridioidea). Molecular Phylogenetics and Evolution, 94: 252–263.

Gravuer, K. (2004). Determinants of the introduction, naturalisation, and spread of *Trifolium* species in New Zealand. MSc thesis. Lincoln University, New Zealand, 165 pp.

Green, E. (2003). International trade in marine aquarium species: using the global marine aquarium database, in: James, C. & Cato, C. (Eds.) Marine ornamental species. Iowa State Press. Iowa, USA, pp. 29–48.

Green, E., Hendry, H. (1999). Is CITES an effective tool for monitoring trade in corals? Coral Reefs, 18: 403–407.

Griffiths, C., Robinson, T., Lange, L., Mead, A. (2010). Marine biodiversity in South Africa: an evaluation of current states of knowledge. PLoS ONE, 5: 1–13.

Grizel, H., Héral, M. (1991). Introduction into France of the Japananes oyster (*Crassostrea gigas*). ICES Journal of Marine Science, 47: 399–403.

Grosholz, E. (2011). Crabs, in: Simberloff, D. & Rejmánek, M. (Eds.) Encyclopedia of biological invasions - Encyclopedias of the Natural World, No. 3. University of California Press Ltd. London, England, pp. 125-128.

Grosholz, E., Crafton, R., Fontana, R., Pasari, J., Williams, S., Zabin, C. (2015). Aquaculture as a vector for marine invasions in California. Biological Invasions, 17: 1471–1484.

Gruet, Y., Heral, M., Robert, J.M. (1976). Premières observations sur l'introduction de la faune associée au naissain d'huîtres Japonaises Crassostrea gigas (Thunberg), importé sur la côte Atlantique Française. CBM-Cahiers de Biologie Marine, 17(2): 173-184.

Guidetti, P., Magnani, L., Navone, A. (2016). First record of the acanthurid fish *Zebrasoma xanthurum* (Blyth, 1852) in the Mediterranean Sea, with some considerations on the risk associated with the aquarium trade. Mediterranean Marine Science, 17: 147–151.

Hall, T. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95–98.

Harrower, C.A., Scalera, R., Pagad, S., Schönrogge, K., Roy, H.E. (2018). Guidance for interpretation of CBD categories on introduction pathways. Technical note prepared by IUCN for the European Commission, 100 pp.

Hartman, O. (1954). Bikini and nearby atolls, Marshall Islands; marine annelids from the northern Marshall Islands. Geological Survey Professional Paper 260-Q. United States Government Printing Office. Washington, pp. 619–644.

Haselden, R. (2017). *Asterina*? Beneficial or pest? [WWW Document]. Reef2Reef Forum. URL https://www.reef2reef.com/threads/asterina-beneficial-or-pest.315717/ (accessed 20 March 2019).

Haverkort-Yeh, R. (2013). A taxonomic survey of Saudi Arabian Red Sea octocorals (Cnidaria: Alcyonacea). Marine Biodiversity, 43: 279–291.

Hebert, P., Cywinska, A., Ball, S., DeWaard, J. (2003a). Biological identifications through DNA barcodes. Proceedings of the Royal Society, 270: 313–321.

Hebert, P., Ratnasingham, S., DeWaard, J. (2003b). Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society, 270: 596–599.

Helgason, N. (2016). *Asterina* starfish, an introduction to this common reef aquarium critter [WWW Document]. Reef Build. Inc. URL https://reefbuilders.com/2016/03/30/asterina-starfish-an-introduction-to-this-common-reef-aquarium-critter/ (accessed 20 March 2019).

Hellberg, M. (2006). No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evolutionary Biology, 6: 1–8.

Herath, H., Wijewardene, L. (2014). Ornamental fish trade in Sri Lanka: an economic perspective. International Research Journal of Environmental Sciences, 3: 40–45.

Herndon, T. (1998). Open-ocean culture of live rock: an environment-friendly approach to aquaculture with many benefits. Today's Aquarist, 6(9): 4–7.

Hoeksema, B. (2007). Delineation of the Indo-Malayan centre of maximum marine biodiversity: the Coral Triangle, in: Renema, W. (Ed.) Biogeography, time and place: distributions, barriers and islands. Springer Publishing. Leiden, Netherlands, pp. 117–178.

Holland, B. (2000). Genetics of marine bioinvasions. Hydrobiologia, 420: 63–71.

Holmberg, R., Tlusty, M., Futoma, E., Kaufman, L., Morris, J., Rhyne, A. (2015). The 800-pound grouper in the room: asymptotic body size and invasiveness of marine aquarium fishes. Marine Policy, 53: 7–12.

Hoveka, L., van der Bank, M., Boatwright, J., Bezeng, B., Yessoufou, K. (2016). The noncoding trnH-psbA spacer, as an effective DNA barcode for aquatic freshwater plants, reveals prohibited invasive species in aquarium trade in South Africa. South African Journal of Botany, 102: 208–216.

Howe, P., Reichelt-Brushett, A., Clark, M. (2012). *Aiptasia pulchella*: a tropical cnidarian representative for laboratory ecotoxicological research. Environmental Toxicology and Chemistry, 31: 2653–2662.

Howe, P., Reichelt-Brushett, A., Clark, W. (2014). Effects of Cd, Co, Cu, Ni and Zn on asexual reproduction and early development of the tropical sea anemone *Aiptasia pulchella*. Ecotoxicology, 23: 1593–1606.

Hu, Z., Guiry, M., Duan, D. (2009). Using the ribosomal internal transcribed spacer (ITS) as a complement marker for species identification of red macroalgae. Hydrobiologia, 635: 279–287.

Huber, M. (2010). Compendium of bivalves. A full-color guide to 3300 of the world's marine bivalves. A Status on Bivalvia after 250 Years of Research. ConchBooks. Hackenheim, Germany, 901 pp.

Hulme, P. (2008). Phenotypic plasticity and plant invasions: is it all Jack? Functional Ecology, 22: 3–7.

Hulme, P. (2009). Trade, transport and trouble: managing invasive species pathways in an era of globalization. Journal of Applied Ecology, 46: 10–18.

Hutchings, P., Kupriyanova, E. (2018). Cosmopolitan polychaetes - fact or fiction? Personal and historical perspectives. Invertebrate Systematics, 32(1): 1–9.

IUCN (2000). IUCN Guidelines for the prevention of biodiversity loss caused by alien invasive species. Species Survival Commission. Gland, Switzerland, 25 pp.

Janes, M., Mary, A. (2012). Synopsis of the family Xeniidae (Cnidaria: Octocorallia): status and trends. Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia, 9–13 July 2012. The new age of integrated coral taxonomy, 5 pp.

Janes, M., Mcfadden, C., Chanmethakul, T. (2014). A new species of *Ovabunda* (Octocorallia, Xeniidae) from the Andaman Sea, Thailand with notes on the biogeography of this genus. Zookeys, 431: 1–17.

Johnston, E.L., Dafforn, K.A., Clark, G.F., Rius, M., Floerl, O. (2017). How anthropogenic activities affect the establishment and spread of non-indigenous species post-arrival. Oceanography and Marine Biology: An Annual Review, 55: 389–420.

Jousson, O., Pawlowski, J., Zaninetti, L., Zechman, F., Dini, F., Di Guiseppe, G., Woodfield, R., Millar, A., Meinesz, A. (2000). Invasive alga reaches California. Nature, 408: 157–158.

Karako, S., Achituv, Y., Katcoff, D. (2002). *Asterina burtoni* (Asteroidea; Echinodermata) in the Mediterranean and the Red Sea: does asexual reproduction facilitate colonization? Marine Ecology Progress Series, 234: 139–145.

Katsanevakis, S., Crocetta, F. (2014). Pathways of introduction of marine alien species in European waters and the Mediterranean – a possible undermined role of marine litter. Marine litter in the Meditteranean and the Black Seas. CIESM Workshop. Tirana, Albania, pp. 61–68.

Katsanevakis, S., Wallentinus, I., Zenetos, A., Leppäkoski, E., Çinar, M., Oztürk, B., Grabowski, M., Golani, D., Cardoso, A. (2014). Impacts of invasive alien marine species on ecosystem services and biodiversity: a pan-European Review. Aquatic Invasions, 9(4): 391-423.

Kaullysing, D., Padate, V.P., Rivonker, C.V. (2015). New records of *Epixanthus frontalis* and *Heteropanope glabra* (Decapoda: Brachyura: Oziidae, Pilumnidae) for Goa, India, with identification keys of their respective genera. Indian Journal of Geo-Marine Sciences, 44(8): 1191–1199.

Keith, I. (2016). Marine invasive species in the Galapagos marine reserve. PhD thesis. University of Dundee, United Kingdom, 282 pp.

Keller, L., Waller, D. (2002). Inbreeding effects in wild populations. Trends in Ecology & Evolution, 17: 230–241.

Keller, R., Lodge, D. (2007). Species invasions from commerce in live aquatic organisms: problems and possible solutions. Bioscience, 57: 428–436.

Keller, R., Geist, J., Jeschke, J., Kühn, I. (2011). Invasive species in Europe: ecology, status, and policy. Environmental Sciences Europe, 23: 1–17.

Kelley, A.L. (2014). The role thermal physiology plays in species invasion. Conservation Physiology, 2: 1-7.

Khedhri, I., Tovar-hernández, M., Bonifácio, P., Ahmed, A., Aleya, L. (2017). First report of the invasive species *Branchiomma bairdi* McIntosh, 1885 (Annelida: Sabellidae) along the Tunisian coast (Mediterranean Sea). BioInvasions Records, 6: 139–145.

Kimberg, P., Woodford, D., Weyl, O., Hui, C., Richardson, D., Msezane, T., van der Walt, K., Swartz , E., Chimimba, C., Zengeya, T., Ellender, B. (2014). Understanding the unintended spread and impact of alien and invasive fish species - development of management guidelines for South African inland waters. WRC Report No. 2039/1/14, Water Research Commission. Pretoria, South Africa, 130 pp.

King, T.A. (2019). Wild caught ornamental fish: a perspective from the UK ornamental aquatic industry on the sustainability of aquatic organisms and livelihoods. Journal of Fish Biology, 94: 925–936.

Kinziger, A., Nakamoto, R., Anderson, E., Harvey, B. (2011). Small founding number and low genetic diversity in an introduced species exhibiting limited invasion success (speckled dace, *Rhinichthys osculus*). Ecology and Evolution, 1: 73–84.

Klein, J., Verlaque, M. (2008). The *Caulerpa racemosa* invasion: a critical review. Marine Pollution Bulletin, 56: 205–225.

Klinck, J. (2009). The alien invasive moss *Campylopus introflexus* in the Danish coastal dune system. MSc thesis. Copenhagen University, Denmark, 105 pp.

Kolar, C., Lodge, D. (2002). Ecological predictions and risk assessment for alien fishes in North America. Science, 298: 1233–1236.

Kolar, C., Lodge, D. (2001). Progress in invasion biology: predicting invaders. Trends in Ecology & Evolution, 16: 199–204.

Kress, W., Prince, L., Williams, K. (2002). The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. American Journal of Botany, 89: 1682–1696.

Kudenov, J. (1974). The reproductive biology of *Eurythoe complanata* (Pallas, 1766), (Polychaeta: Amphinomidae). PhD thesis. University of Arizona, Tucson, USA, 128 pp.

Laird, M., Griffiths, C. (2016). Additions to the South African sea anemone (Cnidaria, Actiniaria) fauna, with expanded distributional ranges for known species. African Invertebrates, 57: 15–37.

Lambrinos, J. (2004). How interactions between ecology and evolution influence contemporary invasion dynamics. Ecology, 85: 2061–2070.

Langeneck, J., Boyer, M., De Cecco, P., Luciani, C., Marcelli, M., Vacchi, M. (2015). First record of *Acanthurus chirurgus* (Perciformes: Acanthuridae) in the Mediterranean Sea, with some distributional notes on Mediterranean Acanthuridae. Mediterranean Marine Science, 16: 427–431.

Lawson Handley, L., Estoup, A., Evans, D., Thomas, C., Lombaert, E., Facon, B., Aebi, A., Roy, H. (2011). Ecological genetics of invasive alien species. BioControl, 56: 409–428.

Leal, M., Nunes, C., Kempf, S., Reis, A., da Silva, T.L., Serôdio, J., Cleary, D., Calado, R. (2013). Effect of light, temperature and diet on the fatty acid profile of the tropical sea anemone *Aiptasia pallida*. Aquaculture Nutrition, 19: 818–826.

Lecaillon, G. (2004). The “C.A.R.E.” (collect by artificial reef eco-friendly) system as a method of producing farmed marine animals for the aquarium market: an alternative solution to collection in the wild. SPC Live Reef Fish Information Bulletin, 12: 17–20.

Leuven, R., van der Velde, G., Baijens, I., Snijders, J., van der Zwart, C., Lenders, H., bij de Vaate, A. (2009). The river Rhine: a global highway for dispersal of aquatic invasive species. Biological Invasions, 11: 1989–2008.

Li, Y., Zheng, X., Yang, X., Ou, D., Lin, R., Liu, X. (2017). Effects of live rock on removal of dissolved inorganic nitrogen in coral aquaria. Acta Oceanologica Sinica, 36: 87–94.

Lin, Y., Gao, Z., Zhan, A. (2015). Introduction and use of non-native species for aquaculture in China: status, risks and management solutions. Reviews in Aquaculture, 7: 28–58.

Lins, D., de Marco, P., Andrade, A., Rocha, R. (2018). Predicting global ascidian invasions. Diversity and Distributions, 24: 692–704.

Livengood, E., Chapman, F. (2007). The ornamental fish trade: an Introduction with perspectives for responsible aquarium fish. University of Florida IFAS Extension FA124, pp. 1-8.

Lowe, S., Browne, M., Boudjelas, S., De Poorter, M. (2000). 100 of the world's worst invasive alien species: a selection from the global invasive species database. The IUCN Invasive Species Specialist Group. Auckland, New Zealand, 12 pp.

Ma, K., Simard, N., Stewart-Clark, S., Bernier, R., Nadeau, M., Willis, J. (2016). Early detection of the non-indigenous colonial ascidian *Diplosoma listerianum* in eastern Canada and its implications for monitoring. Management of Biological Invasions, 7: 365–374.

Mantelatto, M., da Silva, A., dos Santos Louzada, T., McFadden, C., Creed, J. (2018). Invasion of aquarium origin soft corals on a tropical rocky reef in the southwest Atlantic, Brazil. Marine Pollution Bulletin, 130: 84–94.

Marr, S., Ellender, B., Woodford, D., Alexander, M., Wasserman, R., Ivey, P., Zengeya, T., Weyl, O. (2017). Evaluating invasion risk for freshwater fishes in South Africa. Bothalia - African Biodiversity & Conservation, 47: 1–10.

Martin, G., Coetzee, J. (2011). Fresh water aquatic plant invasion risks posed by the aquarium trade, aquarists and the internet trade in South Africa. Water SA, 37: 371–380.

Mascow, M. (2011). *Eurythoe complanata*, Animal Diversity Web [WWW Document]. University of Michigan, Museum of Zoology. URL https://animaldiversity.org/accounts/Eurythoe_complanata/ (accessed 18 February 2019).

McFadden, C., Benayahu, Y., Pante, E., Thoma, J., Andrew, P. (2011). Limitations of mitochondrial gene barcoding in Octocorallia. Molecular Ecology Resources, 11: 19–31.

Mcfadden, C., Reynolds, A., Janes, M. (2014). DNA barcoding of xeniid soft corals (Octocorallia: Alcyonacea: Xeniidae) from Indonesia: species richness and phylogenetic relationships. Systematics and Biodiversity, 12: 247–257.

McMahon, R. (2002). Evolutionary and physiological adaptations of aquatic invasive animals: *r* selection versus resistance. Canadian Journal of Fisheries and Aquatic Sciences, 59: 1235–1244.

Mead, A., Carlton, J., Griffiths, C., Rius, M. (2011). Revealing the scale of marine bioinvasions in developing regions: a South African re-assessment. Biological Invasions, 13: 1991–2008.

Mendoza, R., Contreras, S., Koleff, P., Álvarez, P. (2010). Aquarium trade as a pathway for the introduction of invasive species into Mexico, in: De Carlo, F. & Basano, A. (Eds.) Freshwater Ecosystems and Aquaculture Research. Nova Science Publishers Inc. Hauppauge, New York, pp. 209–224.

Meyer, C.P., Paulay, G. (2005). DNA barcoding: error rates based on comprehensive sampling. PLoS Biology, 3: e422.

Militz, T., Kinch, J., Foale, S., Southgate, P. (2016). Fish rejections in the marine aquarium trade: an initial case study raises concern for village-based fisheries. PLoS ONE, 11: 1–14.

Miller, S. (2007). DNA barcoding and the renaissance of taxonomy. Proceedings of the National Academy of Sciences, 104: 4775–4776.

Minchin, D., Gollasch, S. (2002). Vectors - how exotics get around, in: Leppäkoski, E., Gollasch, S., Olenin, S. (Eds.) Invasive aquatic species of Europe: distribution, impact and management. Kluwer Academic Publishers. London, pp. 183–192.

Minchin, D., Gollasch, S., Wallentinus, I. (2005). Vector pathways and the spread of exotic species in the sea. ICES Cooperative Research Report. Copenhagen, Denmark, 29 pp.

Mineur, F., Cook, E., Minchin, D., Bohn, K., MacLeod, A., Maggs, C. (2012). Changing coasts: marine aliens and artificial structures. Oceanography and Marine Biology: An Annual Review, 50: 189–234.

Mioduchowska, M., Jan, M., Gołdyn, B., Kur, J., Sell, J. (2018). Instances of erroneous DNA barcoding of metazoan invertebrates: are universal cox1 gene primers too "universal"? PLoS ONE, 13: e0199609.

Miralles, L., Ardura, A., Clusa, L., Garcia-Vazquez, E. (2018a). DNA barcodes of Antipode marine invertebrates in Bay of Biscay and Gulf of Lion ports suggest new biofouling challenges. Scientific Reports, 8: 16214.

Miralles, L., Gomez-Agenjo, M., Rayon-Viña, F., Gyrait, G., Garcia-Vazquez, E. (2018b). Alert calling in port areas: marine litter as possible secondary dispersal vector for hitchhiking invasive species. Journal for Nature Conservation, 42: 12–18.

Molnar, J., Gamboa, R., Revenga, C., Spalding, M. (2008). Assessing the global threat of invasive species to marine biodiversity. Frontiers in Ecology and the Environment, 6: 485–492.

Monticini, P. (2010). The ornamental fish trade - production and commerce of ornamental fish: technical-managerial and legislative aspects. Globefish Research Programme, Vol. 102, FAO. Rome, Italy, 132 pp.

Morris, J., Akins, J. (2009). Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago. Environmental Biology of Fishes, 86: 389–398.

Morton, B. (1991). A molluscan fouling community dominated by *Septijer virgatus* (Bivalvia: Mytilidae) in Hong Kong. Malacologia Review, 24(1-2): 115-118.

Mouton, A., Basson, L., Impson, D. (2001). Health status of ornamental freshwater fishes imported to South Africa: a pilot study. Aquarium Science and Conservation, 3: 313–319.

Muhammad Hamizan, Y., Shahbudin, S., Noor Faizul Hadry, N., Mahfuzah, Y., Rafindde, R., Mohd Fikri Akmal, K., Mohd, Husaini, R. (2015). The potential of artificial live rock as subtrate for coral spat and epibenthic organisms. Jurnal Teknologi (Sciences & Engineering), 77: 25–29.

Murray, J., Watson, G., Giangrande, A., Licciano, M., Bentley, M. (2012). Managing the marine aquarium trade: revealing the data gaps using ornamental polychaetes. PLoS ONE 7(1): e29543.

Nagarajan, M., Parambath, A., Prabhu, V. (2018). DNA barcoding: a potential tool for invasive species identification. DNA Barcoding and Molecular Phylogeny, 1: 73–85.

Nakamura, K., Tachikawa, Y., Kitamura, M., Ohno, O., Suganuma, M., Uemura, D. (2008). Complanine, an inflammation-inducing substance isolated from the marine fireworm *Eurythoe complanata*. Organic & Biomolecular Chemistry, 6: 2058–2060.

Naylor, R., Williams, S., Strong, D. (2001). Aquaculture - a gateway for exotic species. Science, 294(5547): 1655–1656.

Nei, M., Maruyama, T., Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. Evolution, 29: 1-10.

Neves, C., da Rocha, R. (2008). Introduced and cryptogenic species and their management in Paranagua Bay, Brazil. Brazilian Archives of Biology and Technology, 51: 623–633.

Nilsen, A., Fossa, S. (2003). Reef secrets: starting right, selecting fishes and invertebrates, advanced biotope techniques. Microcosm. Neptune City, New Jersey, 448 pp.

Novak, S. (2007). The role of evolution in the invasion process. Proceedings of the National Academy of Sciences, 104: 3671–3672.

Nunes, A., Zengeya, T., Measey, G., Weyl, O. (2017). Freshwater crayfish invasions in South Africa: past, present and potential future. African Journal of Aquatic Science, 42: 309–323.

Occhipinti-Ambrogi, A. (2007). Global change and marine communities: alien species and climate change. Marine Pollution Bulletin, 55: 342–352.

Odom, R. (2012). The next “killer” algae? Assessing and mitigating invasion risk for aquarium strains of the marine macroalgal genus *Chaetomorpha*. MSc thesis. University of Central Florida, Orlando, Florida, 63 pp.

Ogawa, T., Brown, C. (2001). Ornamental reef fish aquaculture and collection in Hawaii. Aquarium Science and Conservation, 3: 151–169.

Ojaveer, H., Galil, B.S., Campbell, M.L., Carlton, J.T., Canning-Clode, J., Cook, E.J., Davidson, A.D., Hewitt, C.L., Jelmert, A., Marchini, A., McKenzie, C.H., Minchin, D., Occhipinti-Ambrogi, A., Olenin, S., Ruiz, G. (2015). Classification of non-indigenous species based on their impacts: considerations for application in marine management. PLoS Biology, 13(4): e1002130.

Ojaveer, H., Galil, B.S., Carlton, J.T., Alleway, H., Goulletquer, P., Lehtiniemi, M., Marchini, A., Miller, W., Occhipinti-Ambrogi, A., Peharda, M., Ruiz, G., Williams, S., Zaiko, A. (2018). Historical baselines in marine bioinvasions: implications for policy and management. PLoS ONE, 13(8): e0202383.

Olivotto, I., Holt, G.J., Carnevali, O. (2009). Advances in marine ornamental aquaculture: breeding and rearing studies, in: Davin, T. & Brannet, A. (Eds.) Coral reefs: biology, threats and restoration. Nova Science Publisher Inc., pp. 1–40.

Padilla, D., Williams, S. (2004). Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. Frontiers in Ecology and the Environment, 2: 131–138.

Pagenkopp Lohan, K.M., Hill-Spanik, K.M., Torchin, M.E., Strong, E.E., Fleischer, R.C., Ruiz, G.M. (2015). Molecular phylogenetics reveals first record and invasion of *Saccostrea* species in the Caribbean. Marine Biology, 162: 957–968.

Paradis, E., Claude, J., Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. Bioinformatics, 20: 289–290.

Parks, J., Pomeroy, R., Balboa, C. (2003). The economics of live rock and live coral aquaculture, in: Cato, J. & Brown, C. (Eds.) Part III: the invertebrates. A. Live rock cultivation. Iowa State Press, pp. 185–206.

Patoka, J., Magalhães, A.L., Kouba, A., Faulkes, Z., Jerikho, R., Vitule, J.R. (2018). Invasive aquatic pets: failed policies increase risks of harmful invasions. Biodiversity and Conservation, 27: 3037–3046.

Poutiers, J.M. (1998). Bivalves. Acephala, Lamellibranchia, Pelecypoda, in: Carpenter, K.E. & Niem, V.H. (Eds.) FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 1. Seaweeds, corals, bivalves, and gastropods. Rome, Italy, pp. 123-362.

Prakash, S., Ajith Kumar, T., Raghavan, R., Rhyne, A., Tlusty, M., Subramoniam, T. (2017). Marine aquarium trade in India: challenges and opportunities for conservation and policy. Marine Policy, 77: 120–129.

R Core Team (2013). R: a language and environment for statistical computing. Vienna, Austria. Available online at https://www.R-project.org/.

Raabe, C., Raabe, L. (2016). Crabs [WWW Document]. A hitchhiker's guide to reefs. URL http://chucksaddiction.thefishestate.net/hitchcrabs.html (accessed 11 February 2019).

Radulovici, A., Archambault, P., Dufresne, F. (2010). DNA barcodes for marine biodiversity: moving fast forward? Diversity, 2: 450–472.

Ram, S. (2013). Compositions for control of malicious marine anemones. US 8,404,262 B2.

Rambaut, A. (2012). FigTree v 1.4.0. United Kingdom. Available online at http://tree.bio.ed.ac.uk/software/figtree.

Randall, J. (1987). Introductions of marine fishes to the Hawaiian Islands. Bulletin of Marine Science, 41: 490–502.

Ratnasingham, S., Hebert, P. (2007). BOLD: the Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes, 7: 355–364.

Read, G., Inglis, G., Stratford, P., Ahyong, S. (2011). Arrival of the alien fanworm *Sabella spallanzanii* (Gmelin, 1791) (Polychaeta: Sabellidae) in two New Zealand harbours. Aquatic Invasions, 6: 273–279.

Rech, S., Salmina, S., Borrell Pichs, Y., García-Vazquez, E. (2018). Dispersal of alien invasive species on anthropogenic litter from European mariculture areas. Marine Pollution Bulletin, 131: 10–16.

Revell, L. (2011). phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution, 3: 217–223.

Rhyne, A., Lin, J., Deal, K. (2004). Biological control of aquarium pest anemone *Aiptasia pallida* verrill by peppermint shrimp *Lysmata risso*. Journal of Shellfish Research, 23: 227–229.

Rhyne, A., Rotjan, R., Bruckner, A., Tlusty, M. (2009). Crawling to collapse: ecologically unsound ornamental invertebrate fisheries. PLoS ONE, 4(12): e8413.

Rhyne, A., Tlusty, M. (2011). Trends in the marine aquarium trade: the influence of global economics and technology. AACL International Bioflux, 5: 99–103.

Rhyne, A., Tlusty, M., Schofield, P., Kaufman, L., Morris, J. (2012). Revealing the appetite of the marine aquarium fish trade: the volume and biodiversity of fish imported into the United States. PLoS ONE 7(5): e35808.

Rhyne, A., Tlusty, M., Holmberg, R., Szczebak, J. (2015). Marine aquarium biodiversity and trade flow [WWW Document]. Roger Williams University and New England Aquarium. URL www.aquariumtradedata.org (accessed 15 September 2015).

Rhyne, A., Tlusty, M., Szczebak, J., Holmberg, R. (2017). Expanding our understanding of the trade in marine aquarium animals. PeerJ, 5: e2949.

Ricciardi, A. (2016). Tracking marine alien species by ship movements. Proceedings of the National Academy of Sciences of the United States of America, 113(20): 5470–5471.

Richardson, D.M., Pyšek, P., Carlton, J.T. (2011). A compendium of essential concepts and terminology in biological invasions, in: Richardson, D.M. (Ed.) Fifty years of invasion ecology: the legacy of Charles Elton. Blackwell, Chichester, pp 409–420.

Robertson, D., Dominguez-Dominguez, O., Victor, B., Simoes, N. (2018). An Indo-Pacific damselfish (*Neopomacentrus cyanomos*) in the Gulf of Mexico: origin and mode of introduction. PeerJ, 6: e4328.

Robinson, T., Griffiths, C., McQuaid, C., Rius, M. (2005). Marine alien species of South Africa - status and impacts. African Journal of Marine Science, 27: 297–306.

Robinson, T.B., Alexander, M.E., Simon, C.A., Griffiths, C.L., Peters, K., Sibanda, S., Miza, S., Groenewald, B., Majiedt, P., Sink, K. (2016). Lost in translation? Standardising the terminology used in marine invasion biology and updating South African alien species lists. African Journal of Marine Science, 38: 129–140.

Robinson, T.B., Peters, K., Brooker, B. (2020). Coastal invasions: the South African context, in: van Wilgen, B.W., Measey, J., Richardson, D.M., Wilson, J.R., Zengeya, T.A. (Eds.) Biological Invasions in South Africa. Invading Nature - Springer Series in Invasion Ecology 14. Cham, Switzerland, pp. 229-247.

Ruiz Allais, J., Amaro, M., McFadden, C., Halász, A., Benayahu, Y. (2014). The first incidence of an alien soft coral of the family Xeniidae in the Caribbean, an invasion in eastern Venezuelan coral communities. Coral Reefs, 33: 287.

Sakai, A., Allendorf, F., Holt, J., Lodge, M., Molofsky, J., With, K., Cabin, R., Cohen, J., Norman, C., Mccauley, D., Neil, P., Parker, M., Thompson, J., Weller, S. (2001). The population biology of invasive species. Annual Review of Ecology and Systematics, 32: 305–332.

Sandel, V., Martínez-Fernández, D., Wangpraseurt, D., Sierra, L. (2015). Ecology and management of the invasive lionfish *Pterois volitans*/*miles* complex (Perciformes: Scorpaenidae) in Southern Costa Rica. Revista de Biología Tropical, 63(1): 213–221.

Schlesinger, A., Kramarsky-Winter, E., Rosenfeld, H., Armoza-Zvoloni, R., Loya, Y. (2010). Sexual plasticity and self-fertilization in the sea anemone *Aiptasia diaphana*. PLoS ONE, 5(7): e11874.

Schliep, K. (2011). Phangorn: phylogenetic analysis in R. Bioinformatics, 27: 592–593.

Schulze, A., Grimes, C., Rudek, T. (2017). Tough, armed and omnivorous: *Hermodice carunculata* (Annelida: Amphinomidae) is prepared for ecological challenges. Journal of the Marine Biological Association of the United Kingdom, 97: 1075–1080.

Semmens, B., Buhle, E., Salomon, A., Pattengill-Semmens, C. (2004). A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. Marine Ecology Progress Series, 266: 239–244.

Shearer, T., van Oppen, M., Romano, S., Wörheide, G. (2002). Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Molecular Ecology, 11: 2475–2487.

Sherman, C., Lotterhos, K., Richardson, M., Tepolt, C., Rollins, L., Palumbi, S., Miller, A. (2016). What are we missing about marine invasions? Filling in the gaps with evolutionary genomics. Marine Biology, 163: 1–24.

Simberloff, D., Martin, J-L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., Vilà, M. (2013). Impacts of biological invasions - what's what and the way forward. Trends in Ecology & Evolution, 28(1): 58–66.

Smith, K., Behrens, M., Max, L., Daszak, P. (2008). U.S. drowning in unidentified fishes: scope, implications, and regulation of live fish import. Conservation Letters, 1: 103–109.

Smith, E.R.C., Bennion, H., Sayer, C.D., Aldridge, D.C., Owen, M. (2020). Recreational angling as a pathway for invasive non-native species spread: awareness of biosecurity and the risk of long distance movement into Great Britain. Biological Invasions, 22: 1135–1159.

Stam, W., Olsen, J., Zaleski, S., Murray, S., Brown, K., Walters, L. (2006). A forensic and phylogenetic survey of *Caulerpa* species (Caulerpales, Chlorophyta) from the Florida coast, local aquarium shops, and e-commerce: establishing a proactive baseline for early detection. Journal of Phycology, 42: 1113–1124.

Statzner, B., Bonada, N., Dolédec, S. (2007). Biological attributes discriminating invasive from native European stream macroinvertebrates. Biological Invasions, 10: 517–530.

Steen, R.G. (1986). Evidence for heterotrophy by zooxanthellae in symbiosis with *Aiptasia pulchella*. Biological Bulletin, 170: 267–278.

Stemmer, K., Burghardt, I., Mayer, C., Reinicke, G.B., Wägele, H., Tollrian, R. (2012). Morphological and genetic analyses of xeniid soft coral diversity (Octocorallia; Alcyonacea). Organisms, Diversity & Evolution, 13: 135–150.

Stoeckle, M., Janzen, D., Hallwachs, W., Hanken, J., Baker, J. (2003). Taxonomy, DNA, and the Barcode of Life. BioScience, 53: 796-797.

Strayer, D., Eviner, V., Jeschke, J., Pace, M. (2006). Understanding the long-term effects of species invasions. Trends in Ecology & Evolution, 21: 645–651.

Strecker, A., Campbell, P., Olden, J. (2011). The aquarium trade as an invasion pathway in the Pacific Northwest. Fisheries, 36: 74–85.

Streftaris, N., Zenetos, A. (2006). Alien marine species in the Mediterranean - the 100 'worst invasives' and their impact. Mediterranean Marine Science, 7: 87–118.

Sussman, M., Loya, Y., Fine, M., Rosenberg, E. (2003). The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen *Vibrio shiloi*. Environmental Microbiology, 5: 250–255.

Sustainable Aquatics (2019). SA marine polychaetes – live marine bristleworms [WWW Document]. Sustainable Aquatics. URL http://sustainableaquatics.com/feeds/sa-marine-polychaetes-live-marine-bristleworms/ (accessed 26 January 2019).

Swart, C., Robinson, T.B.R. (2019). Horizon scanning for alien predatory crabs: insights from South Africa. African Journal of Marine Science, 41(2): 125-135.

Sylvester, F., Kalaci, O., Leung, B., Lacoursière-Roussel, A., Murray, C., Choi, F., Bravo, M., Therriault, T., Macisaac, H. (2011). Hull fouling as an invasion vector: can simple models explain a complex problem? Journal of Applied Ecology, 48: 415–423.

Tepolt, C.K. (2015). Adaptation in marine invasion: a genetic perspective. Biological Invasions, 17: 887–903.

Thaler, A., Freitag, A., Bergman, E., Fretz, D., Saleu, W. (2015). Robots as vectors for marine invasions: best practices for minimizing transmission of invasive species via observation-class ROVs. Tropical Conservation Science, 8: 711–717.

Theoharides, K.A., Theoharides, K.A., Dukes, J.S. (2007). Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. New Phytologist, 176: 256–273.

Thomaz, S.M., Kovalenko, K.E., Havel, J.E., Kats, L.B. (2014). Aquatic invasive species: general trends in the literature and introduction to the special issue. Hydrobiologia, 746: 1–12.

Tittensor, D.P., Mora, C., Jetz, W., Lotze, H.K., Ricard, D., Berghe, E.V., Worm, B. (2010). Global patterns and predictors of marine biodiversity across taxa. Nature, 466: 1098–1101.

Tissot, B., Best, B., Borneman, E., Bruckner, A., Cooper, C., D'Agnes, H., Fitzgerald, T., Leland, A., Lieberman, S., Mathews Amos, A., Sumaila, R., Telecky, T., McGilvray, F., Plankis, B., Rhyne, A., Roberts, G., Starkhouse, B., Stevenson, T. (2010). How U.S. ocean policy and market power can reform the coral reef wildlife trade. Marine Policy, 34: 1385–1388.

Trégarot, E., Fumaroli, M., Arqué, A., Hellio, C., Maréchal, J.P. (2015). First records of the red lionfish (*Pterois volitans*) in Martinique, French West Indies: monitoring invasion status through visual surveys. Marine Biodiversity Records, 8: 1–7.

Tricarico, E., Junqueira, A., Dudgeon, D. (2016). Alien species in aquatic environments: a selective comparison of coastal and inland waters in tropical and temperate latitudes. Aquatic Conservation: Marine and Freshwater Ecosystems, 26: 872–891.

UNEP (2011). The strategic plan for biodiversity 2011–2020 and the Aichi biodiversity targets, in: COP CBD Tenth Meetng. Nagoya, Japan, 272 pp.

van der Walt, K.A., Mäkinen, T., Swartz, E.R., Weyl, O.L. (2017). DNA barcoding of South Africa's ornamental freshwater fish - are the names reliable? African Journal of Aquatic Science, 42: 155–160.

van Kleunen, M., Dawson, W., Essl, F., Pergl, J., Winter, M., Weber, E., Kreft, H., Weigelt, P., Kartesz, J., Nishino, M., Antonova, L., Barcelona, J., Cabezas, F., Cárdenas, D., Cárdenas-Toro, J., Castaño, N., Chacón, C., Chatelain, C., Ebel, A., Figueiredo, D., Fuentes, N., Groom, Q., Henderson, L., Kupriyanov, A., Masciadri, S., Meerman, J., Morozova, O., Moser, D., Nickrent, D., Patzelt, A., Pelser, P., Baptiste, M., Poopath, M., Schulze, M., Seebens, H., Shu, W., Thomas, J., Velayos, M., Wieringa, J., Pyšek, P. (2015). Global exchange and accumulation of non-native plants. Nature, 525: 100–103.

van Wilgen, B.W., Wilson, J.R.U. (Eds.) (2018). The status of biological invasions and their management in South Africa in 2017. South African National Biodiversity Institute, Kirstenbosch and DST-NRF Centre of Excellence for Invasion Biology, Stellenbosch.

Vander Zanden, M.J. (2005). The success of animal invaders. Proceedings of the National Academy of Sciences, 102: 7055–7056.

Velasco-Blanco, G., Re, A.D., Díaz, F., Ibarra-Castro, L., Abdo-de la Parra, M.I., Rodríguez-Ibarra, L.E., Rosas, C. (2019). Thermal preference, tolerance, and thermal aerobic scope in clownfish *Amphiprion ocellaris* (Cuvier, 1830) predict its aquaculture potential across tropical regions. International Aquatic Research, 11: 187–197.

Vermeij, G. (1996). An agenda for invasion biology. Biological Conservation, 78: 3–9.

Verna, D., Harris, B., Holzer, K., Minton, M. (2016). Ballast-borne marine invasive species: exploring the risk to coastal Alaska, USA. Management of Biological Invasions, 7: 199–211.

Vijayan, K., Stalin Raj, V., Balasubramanian, C., Alavandi, S., Sekhar, V., Santiago, T. (2005). Polychaete worms - a vector for white spot syndrome virus (WSSV). Diseases of Aquatic Organisms, 63: 107–111.

Vine, P. (1986). Red Sea invertebrates. Immel Publishing. London, United Kingdom, 224 pp.

Voight, J., Lee, R., Reft, A., Bates, A. (2012). Scientific gear as a vector for non-native species at deep-sea hydrothermal vents. Conservation Biology, 26: 938–942.

Vranken, S., Bosch, S., Peña, V., Leliaert, F., Mineur, F., De Clerck, O. (2018). A risk assessment of aquarium trade introductions of seaweed in European waters. Biological Invasions, 20: 1171–1187.

Wabnitz, C., Nahacky, T. (2014). Rapid aquarium fish stock assessment and evaluation of industry best practices in Kosrae, Federated States of Micronesia. Prepared by the Secretariat of the Pacific Community (SPC). Kosrae, Federated States of Micronesia, 24 pp.

Wabnitz, C., Taylor, M., Green, E., Razak, T. (2003). From ocean to aquarium: the global trade in marine ornamental species, UNEP-WCMC. Cambridge, United Kingdom, 65 pp.

Walters, L., Brown, K., Stam, W., Olsen, J. (2006). E-commerce and *Caulerpa*: unregulated dispersal of invasive species. Frontiers in Ecology and the Environment, 4: 75–79.

Walters, L., Odom, R., Zaleski, S. (2011). The aquarium hobby industry and invasive species: has anything changed? Frontiers in Ecology and the Environment, 9: 206–207.

Walther, G.R., Roques, A., Hulme, P.E., Sykes, M.T., Pysˇek, P., et al. (2009). Alien species in a warmer world: risks and opportunities. Trends in Ecology & Evolution, 24: 686– 693.

Webber, B., Scott, J. (2011). Rapid global change: implications for defining natives and aliens. Global Ecology and Biogeography, 21: 305–311.

Wehe, T., Fiege, D. (2002). Annotated checklist of the polychaete species of the seas surrounding the Arabian Peninsula: Red Sea, Gulf of Aden, Arabian Sea, Gulf of Oman, Arabian Gulf. Fauna of Arabia, 19: 7-238.

Weigand, H., Beermann, A., Fedor, Č., Costa, F., Csabai, Z., Geiger, M., Rimet, F., Rulik, B., Strand, M., Szucsich, N., Weigand, A., Willassen, E., Wyler, A., Bouchez, A., Borja, A., Zuzana, Č., Ferreira, S., Dijkstra, K., Eisendle, U., Freyhof, J., Gadawski, P., Graf, W., Haegerbaeumer, A., Van Der Hoorn, B.B., Japoshvili, B., Keresztes, L., Keskin, E., Leese, F., Macher, J.N., Mamos, T., Paz, G., Pe, V., Maric, D., Andreas, M., Price, B.W., Rinkevich, B., Teixeira, M.A.L., Várbíró, G., Ekrem, T. (2019). DNA barcode reference libraries for the monitoring of aquatic biota in Europe: gap-analysis and recommendations for future work. Science of the Total Environment, 678: 499–524.

Weitzmann, B., Mercader, L., Azzurro, E. (2016). First sighting of *Zebrasoma flavescens* (Teleostei: Acanthurida) and *Balistoides conspicillum* (Teleostei: Balistidae) in the Mediterranean Sea: two likely aquarium releases. Mediterranean Marine Science, 16: 147–150.

Whitby, P. (2015). Pests, parasites and things that go munch in the night. Part 2: public enemy number two: *Asterina* sp. starfish [WWW Document]. Reefkeeping 101, 8(6). URL http://www.reefkeeping.com/joomla/index.php/current-issue/article/45-reefkeeping-101- (accessed 26 February 2019).

White, T.J., Bruns, T., Lee, S., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis, M., Gelfand, H., Sninsky, J., White, T. (Eds.) PCR protocols: a guide to methods and applications. Academic Press Inc. San Diego, pp. 315–322.

Whitney, K.D., Gering, E. (2014). Five decades of invasion genetics. New Phytologist, 205: 472–475.

Wijesekara, R., Yakupitiyage, A. (2001). Ornamental fish industry in Sri Lanka: present status and future trends. Aquarium Sciences and Conservation, 3: 241–252.

Williams, G.C. (1992). Biogeography of the octocorallian coelenterate fauna of southern Africa. Biological Journal ofthe Linnean Society, 46: 351-401.

Williams, G.C., Van Syoc, R.J. (2007). Methods of preservation and anesthetization of marine invertebrates, in: Carlton, J. (Ed.) The Light and Smith Manual: intertidal invertebrates from central California to Oregon. University of California Press. Berkeley and Los Angeles, California, pp. 37–42.

Williams, S.L., Crafton, R.E., Fontana, R.E., Grosholz, E.D., Ha, G., Pasari, J.R., Zabin, C.J. (2015). A vector analysis of marine ornamental species in California. Management of Biological Invasions, 6: 13–29.

Williams, S.L., Smith, J.E. (2007). A global review of the distribution, taxonomy, and impacts of introduced seaweeds. Annual Review of Ecology, Evolution, and Systematics, 38: 327–359.

Williamson, A., Bax, N., Gonzalez, E., Geeves, W. (2002). Development of a regional risk management framework for APEC economies for use in the control and prevention of introduced pests. Asia-Pacific Economic Cooperation Secretariat. Technical Reports of the Marine Resource Conservation Working Group; CSIRO Centre for Research on Introduced Marine Pests. Hobart, Australia; Inter-American Centre for Sustainable Ecosystems Development. Santiago, Chile, 195 pp.

Williamson, M., Fitter, A. (1996). The varying success of invaders. Ecology, 77: 1661–1666.

Wong, B., Candolin, U. (2015). Behavioral responses to changing environments. Behavioral Ecology, 26: 665–673.

Wood, E. (2001). Collection of coral reef fish for aquaria: global trade, conservation issues and management strategies. Marine Conservation Society. United Kingdom, 80 pp.

Xiong, W., Shen, C., Wu, Z., Lu, H., Yan, Y. (2017). A brief overview of known introductions of non-native marine and coastal species into China. Aquatic Invasions, 12: 109–115.

Yuen, Y., Yamazaki, S., Nakamura, T., Tokuda, G., Yamasaki, H. (2009). Effects of live rock on the reef-building coral *Acropora digitifera* cultured with high levels of nitrogenous compounds. Aquacultural Engineering, 41: 35–43.

Zenetos, A., Gofas, S., Verlaque, M., Cinar, M., Garcia Raso, J., Bianchi, C., Morri, C., Azzurro, E., Bilecenoglu, M., Froglia, C., Siokou, I., Violanti, D., Sfriso, A., San Martin, G., Giangrande, A., Katagan, T., Ballesteros, E., Ramos-Espla, A., Mastrototaro, F., Ocana, O., Zingone, A., Gambi, M., Streftaris, N. (2010). Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union Marine Strategy Framework Directive (MSFD). Part 1. Spatial distribution. Mediterranean Marine Science, 11: 381–493.

Zenetos, A., Gofas, S., Morri, C., Rosso, A., Bianchi, C., Morri, C, Azzurro, E., Bilecenoglu, M., Froglia, C., Siokou, I., Violanti, D., Sfriso, A., San Martin, G., Giangrande, A., Katagan, T., Ballesteros, E., Ramos-Espla, A., Mastrototaro, F., Ocana, O., Zingone, A., Gambi, M., Streftaris, N. (2012). Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union’s Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways. Mediterranean Marine Science, 13: 328–352.

Zenni, R.D., Lamy, J.B., Lamarque, L.J., Porté, A.J. (2014). Adaptive evolution and phenotypic plasticity during naturalization and spread of invasive species: implications for tree invasion biology. Biological Invasions, 16: 635–644.

Zou, S., Fei, C., Wang, C., Gao, Z., Bao, Y., He, M., Wang, C. (2016). How DNA barcoding can be more effective in microalgae identification: a case of cryptic diversity revelation in *Scenedesmus* (Chlorophyceae). Scientific Reports, 6: 36822.

APPENDIX A: SEQUENCES SOURCED FROM GENBANK AND BOLD

Table 6.1: List of Actiniaria species used for the cytochrome *c* oxidase I (COI) phylogenetic analyses and their respective accession numbers mined from GenBank and Barcode of Life Database (BOLD) sample IDs. *Species used as the outgroup in the phylogenetic analyses are marked with an asterisk (*). A hyphen (-) indicates no available data for that field.*

Order	Superfamily	Family	Species	Accession number	BOLD ID
Actiniaria	Actinioidea	Actiniidae	*Anthopleura elegantissima* *	-	BBGCO054-14
			Anthopleura sola *	-	BIPP107-17
			Urticinopsis antarctica *	-	AADBE120-10
	Metridioidea	Aiptasiidae	*Aiptasia pulchella*	HG423148.1	CNIDC395-16
				HG423147.1	CNIDC584-17
				NC_022265.1	CNIDC587-17
		Diadumenidae	*Diadumene leucolena*	-	SERCI191-14
			Diadumene lineata	-	SERCI193-14
				-	KBCSM697-14
				-	GBCI6610-17
		Gonactiniidae	*Gonactinia prolifera*	-	SWEMA895-15
				-	SWEMA896-15
			Protanthea simplex	-	SWEMA951-15
		Halcampidae	*Halcampa chrysanthellum*	-	SWEMA897-15
			Halcampa duodecimcirrata	-	SWEMA899-15
				-	SWEMA900-15
		Hormathiidae	*Actinauge cristata*	-	KHA484-14
				-	KHA485-14
			Actinauge sp.	-	KHA204-14
			Hormathia digitata	-	GBMTG5014-16
			Hormathia nodosa	-	ARCMI601-14
				-	KHA070-14
			Stephanauge nexilis	-	ARCMI609-14
				-	KHA458-14
				-	KHA459-14
		Metridiidae	*Metridium farcimen*	-	KBCSM032-14
			Metridium senile	-	CQCS032-10
		Sagartiidae	*Sagartia ornata*	-	GBMTG5857-16
				-	SWEMA929-15
			Sagartia troglodytes	-	SWEMA930-15

Table 6.2: List of Sabellida species used for the cytochrome *c* oxidase I (COI) phylogenetic analyses and their respective accession numbers mined from GenBank and Barcode of Life Database (BOLD) sample IDs. *Species used as the outgroup in the phylogenetic analyses are marked with an asterisk (*). A hyphen (-) indicates no available data for that field.*

Order	Family	Species	Accession number	BOLD ID
Sabellida	Sabellidae	*Bispira manicata*	HQ015124.1	-
			HQ015124	-
		Bispira monroi	-	YCHAE298-08
		Bispira sp.	LT717721	-
			LT717716	-
			LT717718	-
			LT717719	-
		Branchiomma bairdi	KP254646	YCHAE292-08
			-	YCHAE304-08
		Branchiomma sp.	EU835657	-
		Chone ecaudata	-	CMBIA168-11
		Chone infundibuliformis	HQ024001	-
		Chone magna	HM473336	-
		Chone mollis	MG421022	-
			MG423454	-
		Euchone rosea	KT307641	-
		Eudistylia vancouveri	HM473367	-
			HM473368	-
		Laonome sp.	KP793137	-
			KP793136	-
		Manayunkia athalassia	DQ209265	-
		Manayunkia baicalensis	KF289880	-
		Manayunkia godlewskii	KF289841	-
		Manayunkia zenkewitschii	AJ428405	-
		Megalomma splendida	MF121324	-
			HM473463	-
		Myxicola aesthetica	HQ932482	-
		Myxicola infundibulum	HM473471	-
			HM473472	-
		Parasabella aberrans	KP938269	-
			LT717715	-
			KP938268	-
		Parasabella aulaconota	KP938259	-
			KP938261	-
		Parasabella crassichaetae	KP938264	-

		Perkinsiana fonticula	-	YCHAE266-08
			-	YCHAE280-08
		Pseudobranchiomma grandis	LT717723	-
		Pseudopotamilla reniformis	HQ023777	-
			HQ023778	-
			HQ023779	-
		Sabella pavonina	-	POLNB1407-14
		Sabella spallanzanii	KY472753	-
			KY472760	-
			KY472785	-
		Sabellomma cupoculata	KP938254	-
			KP938256	-
		Schizobranchia insignis	HM473678	-
			HM473673	-
		Stylomma palmatum	HQ015147	-
			HQ015146	-
	Serpulidae	*Pomatoceros triqueter* *	JX308732	-
		Serpula columbiana *	HM375142	-
		Serpula vermicularis *	GU672097	-

Table 6.3: List of Eunicida species used for the cytochrome *c* oxidase I (COI) phylogenetic analyses and their respective accession numbers mined from GenBank and Barcode of Life Database (BOLD) sample IDs. *Species used as the outgroup in the phylogenetic analyses are marked with an asterisk (*). A hyphen (-) indicates no available data for that field.*

Order	Family	Species	Accession number	BOLD ID
Eunicida	Dorvilleidae	*Dorvillea albomaculata*	EF464550	-
		Dorvillea erucaeformis	AY838868	-
		Dorvillea similis	DQ317857	-
		Iphitime paguri	EF464549	-
		Ophryotrocha geryonicola	GQ415476	-
		Ophryotrocha mediterranea	KR004757	-
		Ophryotrocha scutellus	GQ415488	-
	Eunicidae	*Eunice americana*	GQ497561	-
		Eunice amoureuxi	GQ497538	-
		Eunice antarctica	GQ497532	-
		Eunice antennata	DQ317858	-
		Eunice antillensis	GQ497533	-
		Eunice cariboea	DQ317859	-
		Eunice chicasi	-	YCHAE043-08
		Eunice gagzoi	-	YCHAE183-08
		Eunice insularis	GQ497537	-

		Eunice harassii	GQ497535	-
		Eunice lucei	GQ497529	-
		Eunice miurai	GQ497530	-
		Eunice mutilata	GQ497540	-
		Eunice rubra	-	YCHAE147-08
		Eunice thomasiana	GQ497563	-
		Eunice torquata	GQ497539	-
		Lysidice collaris	DQ470468	-
			DQ470466	-
			DQ470467	-
		Lysidice ninetta	DQ470392	-
			DQ470375	-
			GQ497564	-
			KR916860	-
		Marphysa bellii	KT307661	-
		Marphysa californica	GQ497552	-
		Marphysa disjuncta	-	CMBIA223-11
		Marphysa mixta	-	YCHAE073-08
		Marphysa mossambica	KX172164	-
		Palola sp.	DQ317809	GBAN0759-06
			DQ317812	-
		Palola viridis	JN558584	-
			JN558583	-
		Gallardoneris iberica	KT307645	-
		Lumbrineris cruzensis	-	HZPLY296-13
		Lumbrineris erecta	HM473450	-
		Lumbrineris heteropoda	-	HZPLY289-13
		Lumbrineris luti	-	CMBIA477-11
		Lumbrineris mixochaeta	-	CHONE199-11
	Lumbrineridae	*Lumbrineris tetraura*	-	HZPLY354-13
		Ninoe armoricana	KT307669	-
		Ninoe leptognatha	JF731020	-
		Ninoe nigripes	HQ024162	-
		Ninoe palmata	-	HZPLY360-13
		Ninoe tridentata	-	CMBIA246-11
		Arabella iricolor	GU362693	-
	Oenonidae	*Arabella semimaculata*	AY838866	-
		Oenone fulgida	AY838872	-
		Diopatra aciculata	AY838867	-
		Diopatra ceprea	-	GBMIN618-12
	Onuphidae	*Diopatra micrura*	GQ456162	-
		Diopatra neapolitana	KT992100	-
Sabellida	Sabellidae	*Bispira manicata* *	HQ015124	-

		Bispira monroi *	-	YCHAE298-08

Table 6.4: List of Amphinomida species used for the cytochrome *c* oxidase I (COI) phylogenetic analyses and their respective accession numbers mined from GenBank and Barcode of Life Database (BOLD) sample IDs. *Species used as the outgroup in the phylogenetic analyses are marked with an asterisk (*). A hyphen (-) indicates no available data for that field.*

Order	Family	Subfamily	Species	Accession number	BOLD ID
Amphinomida	Amphinomidae	Amphinominae	*Amphinome* sp.	-	YCHAE405-08
				-	YCHAE409-08
			Amphinome rostrata	JN086544	-
			Cryptonome conclava	JN086545	-
			Eurythoe complanata	KY972418.1	HZPLY1109-13
				KT878019	GBAN9554-17
			Eurythoe sp.	FJ429274	-
			Hermodice carunculata	KC017555	FPMAR158-08
				-	FPMAR011-08
			Hipponoe gaudichaudi	JN086551	-
			Linopherus ambigua	-	HZPLY160-12
				-	HZPLY161-12
			Paramphinome jeffreysii	KT592260	BXPOL053-07
			Pareurythoe borealis	JN086550	-
			Pherecardia sp.	JF905665	-
			Pherecardia striata	KC706793	-
		Archinominae	*Archinome jasoni*	JX028095	-
			Archinome levinae	JX028101	-
			Archinome rosacea	JX028105	-
			Archinome storchi	JX028068	-
			Archinome tethyana	JX028113	-
			Chloeia pinnata	-	CMBIA163-11
			Chloeia viridis	JN086546	FPMAR156-08
			Notopygos caribea	KM055019	-
			Notopygos kekooa	KM055009	-
			Notopygos mitsukurii	KM055008	-
			Notopygos ornata	JX028115	-
	Euphrosinidae	-	*Euphrosine foliosa* *	JN086547	-
				JN852945	-

Table 6.5: List of Phyllodocida species used for the cytochrome *c* oxidase I (COI) phylogenetic analyses and their respective accession numbers mined from GenBank and Barcode of Life Database (BOLD) sample IDs. *Species used as the outgroup in the phylogenetic analyses are marked with an asterisk (*). A hyphen (-) indicates no available data for that field.*

Order	Family	Species	Accession number	BOLD ID
Phyllodocida	Chrysopetalidae	*Bhawania cryptocephala*	KX525480	-
			KX525479	-
		Bhawania heteroseta	EU555053	-
			KP255102	-
			KP254256	-
		Bhawania reyssi	EU555054	-
		Paleanotus bellis		DISMA032-17
		Paleanotus sp.	EU555056	-
			JN588618	-
		Treptopale homalos	JN569295	-
			JN569294	-
		Treptopale paromolos	JN569307	GBAN4815-13
			JN588620	-
	Nereididae	*Alitta virens*	MG422752	-
			MG423323	-
		Ceratonereis sp.	-	LIPOL031-08
		Cheilonereis cyclurus	HM473330	-
		Hediste atoka	AB603855	-
			AB603850	-
		Hediste diadroma	AB996717	-
			AB996718	-
		Hediste diversicolor	EU300699	-
		Neanthes acuminata	KJ539100	-
			KJ539101	-
		Nectoneanthes oxypoda	-	HZPLY588-13
			-	HZPLY592-13
			-	HZPLY594-13
		Nereis aibuhitensis	JX503023	
		Nereis heterocirrata	HZPLY623-13	HZPLY623-13
		Nereis longior	HZPLY598-13	HZPLY598-13
		Nereis mediator	DISMA034-17	DISMA034-17
		Nereis multignatha	JX503019	-
		Nereis pelagica	MG423243	-
		Nereis riisei	JF293304	-
		Nereis sinensis	HZPLY603-13	HZPLY603-13
		Nereis vexillosa	KM611954	-
		Nereis zonata	HQ024405	-

		Perinereis brevicirris	JX503026	-
			JX503025	-
		Perinereis cultrifera	-	HZPLY609-13
			-	HZPLY620-13
		Perinereis helleri	JX420256	-
		Perinereis nuntia	JX420257	-
		Perinereis suluana	JX420255	-
			JX392072	-
		Platynereis bicanaliculata	HM473589	-
			HM473590	-
		Platynereis dumerilii	KF815726	-
			KF737174	-
		Platynereis massiliensis	KT124681	-
		Pseudonereis anomala	JX420262	-
			JX420263	-
		Pseudonereis gallapagensis	JF293309	-
			JF293310	-
		Pseudonereis variegata	JX503028	-
			JX503027	-
		Simplisetia erythraensis	EU835670	-
		Autolytus emertoni	-	CCPOL189-07
			-	CCPOL198-07
		Epigamia magna	MF121450	-
		Epigamia noroi	EF123747	-
		Eusyllis blomstrandi	-	GBAN1921-08
		Myrianida gidholmi	-	GBMIN861-12
		Myrianida longoprimicirrata	-	GBAN5476-14
		Myrianida pinnigera	-	GBMIN868-12
		Odontosyllis phosphorea	-	BCPOL381-08
		Pionosyllis sp.	-	BCPOL893-10
			-	BCPOL179-08
		Proceraea madeirensis	-	GBAN5480-14
	Syllidae	*Proceraea nigropunctata*	-	DISMA038-17
		Proceraea picta	-	GBAN1910-08
		Streptosyllis bidentata	-	GBAN1903-08
		Syllides convolutus	-	GBAN1902-08
		Syllides fulvus	-	GBAN1901-08
		Syllis compacta	-	GBAN1899-08
		Syllis elongata	-	BCPOL576-10
		Syllis ferrani	-	GBAN1896-08
		Syllis garciai	-	GBAN1895-08
		Syllis hyalina	-	GBAN1892-08
		Syllis nipponica	-	GBAN1889-08
		Trypanosyllis krohnii	-	GBAN9340-17
		Trypanosyllis luzonensis	-	GBAN9125-17

		Trypanosyllis zebra	-	GBAN1885-08
		Typosyllis sp.	-	KBPOL624-11
		Typosyllis pigmentata	-	BCPOL359-08
		Xenosyllis moloch	-	GBAN9319-17
Sabellida	Sabellidae	*Sabella pavonina* *	KF369181.1	-
		Sabella spallanzanii *	KY472787.1	-

Table 6.6: List of Valvatida species used for the cytochrome *c* oxidase I (COI) phylogenetic analyses and their respective accession numbers mined from GenBank. *Species used as the outgroup in the phylogenetic analyses are marked with an asterisk (*). A hyphen (-) indicates no available data for that field.*

Order	Family	Species	Accession number
Forcipulatida	Asteriidae	*Asterias amurensis* *	AF240032
		Asterias forbesi *	HM542086
		Asterias rubens *	MG421066
Valvatida	Asterinidae	*Anseropoda placenta*	KX458826
			KX458827
		Aquilonastra anomala	AY370754
		Aquilonastra coronata	AY370747
		Asterina gibbosa	MF279278.1
			KP768164.1
			KP768162.1
		Asterina martinbarriosi	MF279279.1
		Asterina pancerii	MF279275.1
			KP768174.1
			KP768173.1
		Asterina phylactica	KP768167.1
			KP768166.1
			KP768165.1
		Asterina sp.	KP768170.1
			KP768169.1
			KP768168.1
		Asterina stellifera	KX347562.1
			KP768175.1
			KC626149
		Asterinides sp.	KC626146
			KC626145
		Cryptasterina pacifica	U50057
		Cryptasterina pentagona	U50051
		Meridiastra atyphoida	AY370760
		Meridiastra calcar	U50046

		Meridiastra fissura	AY370758
		Meridiastra gunnii	EU869950
		Meridiastra medius	AY370749
		Meridiastra mortenseni	AY077748
		Meridiastra oriens	AY458467
		Paranepanthia aucklandensis	AY370751
		Paranepanthia grandis	AY370757
		Parvulastra exigua	EU870013
			AY370748
		Patiria chilensis	EU870017
			AY370745
		Patiria miniata	HQ231380
			KU495909.1
			HM542105.1
			JN259388
		Patiriella regularis	EU870022
			EU870021
		Stegnaster inflatus	AY077743
			AY370743

Table 6.7: List of Amphipoda species used for the cytochrome *c* oxidase I (COI) phylogenetic analyses and their respective accession numbers mined from GenBank. *Species used as the outgroup in the phylogenetic analyses are marked with an asterisk (*). A hyphen (-) indicates no available data for that field.*

Order	Family	Species	Accession number	BOLD ID
Amphipoda	Eusiridae	*Rhachotropis saskia* *	-	MH272110
		Rhachotropis thordisae *	-	MG521140
	Hyalidae	*Apohyale anceps*	MG320301	-
		Apohyale prevostii	KX223996	WWGSL246-08
			KX223997	WWGSL247-08
			-	WWGSL248-08
		Apohyale pugettensis	KM611619	-
			KM611793	-
			KM611623	-
			MG510761	-
		Parallorchestes americana	MG310450	-
			MG317229	-
			MG312892	-
		Parallorchestes cowani	MG316655	-
			MG320473	-
			MG318239	-

			MG315163	-
		Parhyale aquilina	KU565877	GBCMA11574-16
		Parhyale hawaiensis	AY639937	GBCMA12738-16
			EF989709	-
		Protohyale frequens	JX545473	-
			JX545472	-
		Protohyale jarrettae	MG315403	-
			MG314848	-
			MG315203	-
			MG315819	-
		Ptilohyale plumicornis	KU565876	GBCMA11572-16
			-	GBCMA11573-16
	Oedicerotidae	*Monoculodes pallidus* *	-	MG264758

Table 6.8: List of Isopoda species used for the cytochrome *c* oxidase I (COI) phylogenetic analyses and their respective accession numbers mined from GenBank.

Species used as the outgroup in the phylogenetic analyses are marked with an asterisk (*). A hyphen (-) indicates no available data for that field.

Order	Family	Species	Accession number	BOLD ID
Isopoda	Chaetiliidae	*Mesidotea entomon**	DQ889111	-
	Idoteidae	*Edotia sublittoralis*	-	CMBIA118-11
		Edotia triloba	FJ581626	-
			FJ581625	-
		Glyptidotea lichtensteini	AF255781	-
		Idotea sp.	MG936326	-
		Idotea baltica	AF241900	-
			AF241894	-
			AF241895	-
		Idotea chelipes	JQ425516	FCCOM321-11
				FCCOM322-11
		Idotea emarginata	KU530491	-
			KT209137	-
			AF241933	-
		Idotea granulosa	MG935344	-
			KT208546	-
			KT209363	-
		Idotea kirchanskii	GU443133	-
			GU443131	-
			GU443130	-
		Idotea linearis	KT209000	-
		Idotea metallica	AF241928	-

			AF241929	-
			KU530446	-
		Idotea neglecta	-	DSISO079-16
			LC067262	-
		Idotea ochotensis	LC067195	-
			LC067193	-
			KT209359	-
		Idotea pelagica	JQ425512	-
			KT209180	-
		Idotea resecata	JX545469	-
			JX545468	-
		Idotea urotoma	KU530527	-
		Pentidotea montereyensis	GU442885	GBCMI1556-13
			GU442884	-
		Pentidotea wosnesenskii	MG509710	-
			MG512904	-
		Stenosoma raquelae	JQ425510	-
		Stenosoma spinosum	JQ425508	-
		Stenosoma stephenseni	JF915297	-
			JF915298	-
		Synidotea bicuspida	MG318626	-
		Synidotea laevidorsalis	JX502963	-
		Synidotea wonkimi	KX171201	-
		Synisoma acuminatum	JN652045	-
			FJ905099	-
		Synisoma capito	FJ905097	GBCMI1581-13
		Synisoma lancifer	JN652100	-
			FJ905098	-
			FJ905091	-
		Synisoma nadejda	FJ905090	-
			FJ905053	-

Table 6.9: List of Alcyonacea species used for the internal transcribed spacer (ITS) phylogenetic analyses and their respective accession numbers mined from GenBank. *Species used as the outgroup in the phylogenetic analyses are marked with an asterisk (*). A hyphen (-) indicates no available data for that field.*

Order	Family	Species	Accession number
Alcyonacea	Alcyoniidae	*Sarcophyton glaucum* *	KC864836.1
		Sinularia compressa *	KC864856.1
	Xeniidae	*Anthelia* sp.	KC864826.1
			KC864812.1
			KC864830.1
			KC864819.1
			KC864815.1
		Heteroxenia fuscescens	KC864814.1
			KC864806.1
			KC864810.1
		Ovabunda ainex	KY442690.1
			KY442672.1
			KY442673.1
			KY442689.1
		Ovabunda arabica	KY442686.1
			KY442685.1
		Ovabunda biseriata	KY442677.1
			KY442693.1
			KY442692.1
		Ovabunda faraunensis	KY442667.1
			KY442666.1
			KY442697.1
			KY442696.1
		Ovabunda impulsatilla	KY442684.1
			KY442683.1
			KY442682.1
			KY442681.1
		Ovabunda macrospiculata	KY442678.1
			KY442675.1
		Ovabunda verseveldti	KY442662.1
			KY442661.1
		Xenia actuosa	KC864805.1
		Xenia ainex	KC864811.1
			KC864809.1
			KC864797.1
		Xenia sp.	KY442629.1

			KC864803.1
			KC864852.1
			KC864801.1
			KC864822.1
			KY442657.1
			KY442658.1
		Xenia umbellata	KC864857.1
			KY442653.1
			KC864848.1

APPENDIX B: LIVE ROCK ASSOCIATED TAXA DNA SEQUENCE IDENTIFICATIONS

Table 6.10: Morphological and molecular identifications of marine invertebrates associated with Kenyan and Indonesian live rock in South Africa. *A final identification was assigned based on a combination of the individual morphological and molecular identifications, with use of discretion where appropriate. Species are arranged according to their taxonomic rank. The number after the live rock source indicates the replicate number. Colour-coding indicates the outcome of sequencing per sample for the mitochondrial cytochrome c oxidase I (COI) and ribosomal internal transcribed spacer (ITS) coding regions: grey = successfully sequenced and subsequently used in phylogenetic analysis; blue = successfully sequenced but no informative hits generated and therefore not used in further phylogenetic analysis; yellow = sequenced with no hits generated; red = non-target DNA amplfified; X = not sequenced. Abbreviations: NA = not available; SF = subfamily; F = family; O = order; SCL = subclass.*

Phylum	Class	Order	Family	Morphological identification	Molecular identification	Final identification	Sample ID	Live rock source	COI	ITS
Annelida	Polychaeta	Amphinomida	Amphinomidae	*Eurythoe* sp.	*Eurythoe complanata*	*Eurythoe complanata*	72	Kenya 1		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	74	Kenya 1		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	75	Kenya 1		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	76	Kenya 1		x
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	77	Kenya 1		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	78	Kenya 1		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	79	Kenya 1		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	80	Kenya 1		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	81	Kenya 1		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	82	Kenya 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	83	Kenya 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	84	Kenya 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	85	Kenya 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	88	Kenya 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	90	Kenya 2		x
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	91	Kenya 2		x
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	92	Kenya 2		x
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	103	Indonesia 2		x
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	104	Indonesia 2		x
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	105	Indonesia 2		x
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	106	Indonesia 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	122	Indonesia 2		

Phylum	Class	Order	Family	Morphological identification	Molecular identification	Final identification	Sample ID	Live rock source	COI	ITS
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	123	Indonesia 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	124	Indonesia 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	125	Indonesia 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	126	Indonesia 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	127	Indonesia 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	128	Indonesia 2		
				Eurythoe sp.	*Eurythoe complanata*	*Eurythoe complanata*	161	Indonesia 1		x
		Eunicida	Eunicidae	*Palola siciliensis*	*Eunice harassii*	*Eunice harassii*	95	Kenya 2		x
				Eunice petersi	*Eunice petersi*	*Eunice petersi*	73	Kenya 1		
				Eunice sp.	*Eunice* sp.	*Eunice* sp.	71	Kenya 1		
				Lysidice ninetta	*Lysidice ninetta*	*Lysidice ninetta*	86	Kenya 3		
				Lysidice ninetta	*Lysidice ninetta*	*Lysidice ninetta*	87	Kenya 2		
		Incertae sedis	Spintheridae	Spintheridae (F)	NA	Spintheridae (F)	97	Kenya 2		x
		Phyllodocida	Chrysopetalidae	*Bhawania* sp.	*Bhawania cryptocephala*	*Bhawania cryptocephala*	89	Kenya 2		
			Nereididae	*Perinereis* sp.	*Perinereis* sp.	*Perinereis* sp.	68	Indonesia 1		x
				Perinereis sp.	*Perinereis* sp.	*Perinereis* sp.	70	Indonesia 1		
			Syllidae	Syllidae (F)	Syllinae (SF)	Syllinae (SF)	93	Kenya 2		
				Syllidae (F)	Syllinae (SF)	Syllinae (SF)	96	Kenya 2		x
		Sabellida	Sabellidae	*Bispira* sp.	*Bispira* sp.	*Bispira* sp.	107	Indonesia 2	x	x
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	108	Indonesia 2		x
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	109	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	110	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	111	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	112	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	113	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	114	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	115	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	116	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	117	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	118	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	119	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	120	Indonesia 2		
				Bispira sp.	*Bispira* sp.	*Bispira* sp.	121	Indonesia 2		
		NA	NA	Polychaeta (CL)	NA	Polychaeta (CL)	69	Indonesia 1		x

Phylum	Class	Order	Family	Morphological identification	Molecular identification	Final identification	Sample ID	Live rock source	COI	ITS
		NA	NA	Polychaeta (CL)	NA	Polychaeta (CL)	94	Kenya 2		x
		NA	NA	Polychaeta (CL)	NA	Polychaeta (CL)	98	Kenya 1		x
		NA	NA	Polychaeta (CL)	NA	Polychaeta (CL)	99	Indonesia 1		x
		NA	NA	Polychaeta (CL)	NA	Polychaeta (CL)	100	Indonesia 1		x
		NA	NA	Polychaeta (CL)	NA	Polychaeta (CL)	101	Indonesia 1		x
		NA	NA	Polychaeta (CL)	NA	Polychaeta (CL)	102	Indonesia 1	x	x
Arthropoda	Malacostraca	Amphipoda	Hyalidae	Amphipoda (O)	Hyalidae (F)	Hyalidae (F)	162	Kenya 1		
				Amphipoda (O)	Hyalidae (F)	Hyalidae (F)	163	Kenya 3		
				Amphipoda (O)	Hyalidae (F)	Hyalidae (F)	169	Indonesia 2		
		Decapoda	Porcellanidae	*Petrolisthes* sp.	NA	*Petrolisthes* sp.	167	Indonesia 2		x
			Pilumnidae	*Heteropanope* sp.	NA	*Heteropanope* sp.	168	Indonesia 2		
		Isopoda	Idoteidae	Idoteidae (F)	NA	Idoteidae (F)	160	Indonesia 1		x
			NA	Isopoda (O)	NA	Isopoda (O)	164	Kenya 3		x
			NA	Isopoda (O)	NA	Isopoda (O)	165	Kenya 1		x
			NA	Isopoda (O)	NA	Isopoda (O)	166	Kenya 1		x
Cnidaria	Anthozoa	Actiniaria	Aiptasiidae	*Aiptasia* sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	51	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	52	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	53	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	54	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	55	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	56	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	57	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	58	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	59	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	60	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	61	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	62	Indonesia 3	x	
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	63	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	64	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	65	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	66	Indonesia 3		
				Aiptasia sp.	*Aiptasia pulchella*	*Aiptasia pulchella*	67	Indonesia 3		
		Alcyonacea	Xeniidae	*Xenia* sp.	*Xenia umbellata*	*Xenia umbellata*	146	Kenya 1	x	
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	147	Kenya 1		

Phylum	Class	Order	Family	Morphological identification	Molecular identification	Final identification	Sample ID	Live rock source	COI	ITS
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	148	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	149	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	150	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	151	Kenya 1	x	
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	152	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	153	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	154	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	155	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	156	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	157	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	158	Kenya 1		
				Xenia sp.	*Xenia umbellata*	*Xenia umbellata*	159	Kenya 1		
				Asterinidae (F)	*Asterina* sp.	*Asterina* sp.	44	Indonesia 3		x
				Asterinidae (F)	*Asterina* sp.	*Asterina* sp.	45	Indonesia 3		x
				Asterinidae (F)	*Asterina* sp.	*Asterina* sp.	46	Indonesia 3		x
			Asterinidae	Asterinidae (F)	*Asterina* sp.	*Asterina* sp.	47	Indonesia 3		x
				Asterinidae (F)	*Asterina* sp.	*Asterina* sp.	48	Indonesia 3		x
				Asterinidae (F)	*Asterina* sp.	*Asterina* sp.	49	Indonesia 3		
				Asterinidae (F)	*Asterina* sp.	*Asterina* sp.	50	Indonesia 3		
	Asteroidea	Valvatida		Valvatida (O)	NA	Valvatida (O)	27	Kenya 1	x	x
				Valvatida (O)	NA	Valvatida (O)	28	Kenya 1	x	x
				Valvatida (O)	NA	Valvatida (O)	29	Kenya 1	x	x
Echinodermata			NA	Valvatida (O)	NA	Valvatida (O)	30	Kenya 2	x	x
				Valvatida (O)	NA	Valvatida (O)	31	Kenya 3	x	x
				Valvatida (O)	NA	Valvatida (O)	32	Kenya 3	x	x
				Valvatida (O)	NA	Valvatida (O)	33	Indonesia 2	x	x
				Valvatida (O)	NA	Valvatida (O)	34	Indonesia 2	x	x
				Ophiocomella sp.	NA	*Ophiocomella* sp.	1	Kenya 1	x	x
				Ophiocomella sp.	NA	*Ophiocomella* sp.	2	Kenya 1	x	x
				Ophiocomella sp.	NA	*Ophiocomella* sp.	3	Kenya 1	x	x
	Ophiuroidea	Ophiurida	Ophiocomidae	*Ophiocomella* sp.	NA	*Ophiocomella* sp.	4	Kenya 1	x	x
				Ophiocomella sp.	NA	*Ophiocomella* sp.	5	Kenya 1	x	x
				Ophiocomella sp.	NA	*Ophiocomella* sp.	6	Kenya 1	x	x
				Ophiocomella sp.	NA	*Ophiocomella* sp.	7	Kenya 1	x	x

Phylum	Class	Order	Family	Morphological identification	Molecular identification	Final identification	Sample ID	Live rock source	COI	ITS
				Ophiocomella sp.	NA	*Ophiocomella* sp.	8	Kenya 1	x	x
				Ophiocomella sp.	NA	*Ophiocomella* sp.	9	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	10	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	11	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	12	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	13	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	14	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	15	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	16	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	17	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	18	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	19	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	20	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	21	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	22	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	23	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	24	Kenya 1	x	
				Ophiocomella sp.	NA	*Ophiocomella* sp.	25	Kenya 1	x	x
				Ophiocomella sp.	NA	*Ophiocomella* sp.	26	Kenya 1	x	x
			NA	Ophiurida (O)	NA	Ophiurida (O)	35	Indonesia 2	x	x
				Ophiurida (O)	NA	Ophiurida (O)	36	Indonesia 2	x	x
				Ophiurida (O)	NA	Ophiurida (O)	37	Indonesia 2	x	x
				Ophiurida (O)	NA	Ophiurida (O)	38	Indonesia 2	x	x
				Ophiurida (O)	NA	Ophiurida (O)	39	Indonesia 2	x	x
				Ophiurida (O)	NA	Ophiurida (O)	40	Indonesia 2	x	x
				Ophiurida (O)	NA	Ophiurida (O)	41	Indonesia 2	x	x
				Ophiurida (O)	NA	Ophiurida (O)	42	Indonesia 2	x	x
				Ophiurida (O)	NA	Ophiurida (O)	43	Indonesia 2	x	x
Mollusca	Bivalvia	Mytilida	Mytilidae	Mytilidae (F)	NA	Mytilidae (F)	135	Indonesia 1	x	x
				Mytilidae (F)	NA	Mytilidae (F)	136	Indonesia 1	x	x
				Mytilidae (F)	NA	Mytilidae (F)	137	Kenya 2	x	
				Mytilidae (F)	NA	Mytilidae (F)	138	Kenya 2	x	x
				Mytilidae (F)	NA	Mytilidae (F)	139	Kenya 2	x	
				Mytilidae (F)	NA	Mytilidae (F)	140	Kenya 2	x	x

Phylum	Class	Order	Family	Morphological identification	Molecular identification	Final identification	Sample ID	Live rock source	COI	ITS
				Mytilidae (F)	NA	Mytilidae (F)	141	Kenya 2	x	
				Mytilidae (F)	NA	Mytilidae (F)	142	Kenya 2	x	x
				Mytilidae (F)	NA	Mytilidae (F)	143	Indonesia 1	x	x
				Septifer bilocularis	NA	*Septifer bilocularis*	144	Indonesia 2	x	x
				Septifer bilocularis	NA	*Septifer bilocularis*	145	Indonesia 2	x	
	Prosobranchia (SCL)	NA	NA	Prosobranchia (SCL)	NA	Prosobranchia (SCL)	170	Indonesia 3		
				Prosobranchia (SCL)	NA	Prosobranchia (SCL)	171	Indonesia 3	x	
				Prosobranchia (SCL)	NA	Prosobranchia (SCL)	172	Indonesia 3	x	x
				Prosobranchia (SCL)	NA	Prosobranchia (SCL)	173	Indonesia 3	x	
				Prosobranchia (SCL)	NA	Prosobranchia (SCL)	174	Indonesia 3	x	
Platyhelminthes	Incertae sedis	Acoela	Convolutidae	Convolutidae (F)	NA	Convolutidae (F)	129	Indonesia 3	x	
				Convolutidae (F)	NA	Convolutidae (F)	130	Indonesia 3	x	x
				Convolutidae (F)	NA	Convolutidae (F)	131	Indonesia 3	x	x
				Convolutidae (F)	NA	Convolutidae (F)	132	Indonesia 3	x	x
				Convolutidae (F)	NA	Convolutidae (F)	133	Indonesia 3	x	x
				Convolutidae (F)	NA	Convolutidae (F)	134	Indonesia 3	x	x

Table 6.11: Species assignment of live rock associated taxa based on the cytochrome *c* oxidase I (COI) barcoding region. *The number after the live rock source indicates the replicate number. The best hits obtained from both BLAST and BOLD searches are listed for each sample. Identifications were inferred based on the phylogenetic reconstruction of COI sequence data and are listed accordingly. A hyphen indicates that no identification was assigned. Abbreviations: ID = identification; O = order; F = family; SF = subfamily.*

Sample ID	Live rock source	Taxon	Morphological ID	BLAST				BOLD		Phylogenetic inference	
				E-value	Identity (%)	Accession number	Species	Similarity (%)	Species	Species	Figure
COI 44	Indonesia 3	Starfish	*Asterina* sp.	0.0	99	KP768168.1	*Asterina* sp.	93.76	*Asterina gibbosa*	*Asterina* sp.	3.18
COI 45	Indonesia 3	Starfish	*Asterina* sp.	0.0	99	KP768168.1	*Asterina* sp.	93.3	*Asterina gibbosa*	*Asterina* sp.	3.18
COI 46	Indonesia 3	Starfish	*Asterina* sp.	0.0	99	KP768168.1	*Asterina* sp.	93.73	*Asterina gibbosa*	*Asterina* sp.	3.18
COI 47	Indonesia 3	Starfish	*Asterina* sp.	0.0	99	KP768168.1	*Asterina* sp.	93.98	*Asterina gibbosa*	*Asterina* sp.	3.18
COI 48	Indonesia 3	Starfish	*Asterina* sp.	0.0	99	KP768168.1	*Asterina* sp.	93.17	*Asterina gibbosa*	*Asterina* sp.	3.18
COI 49	Indonesia 3	Starfish	*Asterina* sp.	0.0	99	KP768168.1	*Asterina* sp.	93.99	*Asterina gibbosa*	*Asterina* sp.	3.18
COI 50	Indonesia 3	Starfish	*Asterina* sp.	0.0	99	KP768168.1	*Asterina* sp.	93.74	*Asterina gibbosa*	*Asterina* sp.	3.18
COI 51	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 52	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 53	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 54	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 55	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 56	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 57	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 58	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 59	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 60	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 61	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 63	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 64	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 65	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 66	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	93	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 67	Indonesia 3	Sea anemone	*Aiptasia* sp.	0.0	99	HG423148.1	*Aiptasia pulchella*	100	*Aiptasia pulchella*	*Aiptasia pulchella*	3.11
COI 68	Indonesia 1	Polychaete	*Perinereis* sp.	8,00E-100	82	JX661455.1	*Nereis aibuhitensis*	84.73	*Nereis* sp.	Nereididae (F)	3.13
COI 70	Indonesia 1	Polychaete	*Perinereis* sp.	1,00E-173	84	AB603862.1	*Hediste atoka*	93.87	*Nereis* sp.	Nereididae (F)	3.13
COI 71	Kenya 1	Polychaete	*Eunice* sp.	7,00E-121	80	DQ317813.1	*Palola* sp.	82.4	*Eunice* sp.	*Eunice* sp.	3.15

Sample ID	Live rock source	Taxon	Morphological ID	BLAST				BOLD		Phylogenetic inference	
				E-value	Identity (%)	Accession number	Species	Similarity (%)	Species	Species	Figure
COI 72	Kenya 1	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 73	Kenya 1	Polychaete	*Eunice petersi*	4,00E-98	78	KT124732.1	*Palola* sp.	80.58	*Eunice* sp.	*Eunice* sp.	3.15
COI 74	Kenya 1	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	98.89	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 75	Kenya 1	Polychaete	*Eurythoe* sp.	0.0	99	KY972418.1	*Eurythoe complanata*	99.53	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 76	Kenya 1	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 77	Kenya 1	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 78	Kenya 1	Polychaete	*Eurythoe* sp.	0.0	99	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 79	Kenya 1	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 80	Kenya 1	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.69	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 81	Kenya 1	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 82	Kenya 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 83	Kenya 2	Polychaete	*Eurythoe* sp.	0.0	99	KY972418.1	*Eurythoe complanata*	99.37	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 84	Kenya 2	Polychaete	*Eurythoe* sp.	0.0	99	KY972418.1	*Eurythoe complanata*	99.37	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 85	Kenya 2	Polychaete	*Eurythoe* sp.	0.0	99	KY972418.1	*Eurythoe complanata*	99.21	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 86	Kenya 3	Polychaete	*Lysidice ninetta*	0.0	100	KR916860.1	*Lysidice ninetta*	100	*Lysidice ninetta*	*Lysidice ninetta*	3.15
COI 87	Kenya 2	Polychaete	*Lysidice ninetta*	0.0	100	KR916860.1	*Lysidice ninetta*	100	*Lysidice ninetta*	*Lysidice ninetta*	3.15
COI 88	Kenya 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 89	Kenya 2	Polychaete	*Bhawania* sp.	0.0	99	KX525480.1	*Bhawania cryptocephala*	98.34	*Bhawania cryptocephala*	*Bhawania* sp.	3.13
COI 90	Kenya 2	Polychaete	*Eurythoe* sp.	0.0	98,36	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 91	Kenya 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 92	Kenya 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	100	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 93	Kenya 2	Polychaete	Syllidae (F)	0.0	100	KY263090.1	Syllidae (F)	100	Syllidae (F)	Syllinae (SF)	3.13
COI 95	Kenya 2	Polychaete	*Eunice siciliensis*	0.0	99	GQ497535.1	*Eunice harassii*	99.54	*Eunice harassii*	*Eunice harassii*	3.15
COI 96	Kenya 2	Polychaete	Syllidae (F)	0.0	100	KY263090.1	Syllidae (F)	100	Syllidae (F)	Syllinae (SF)	3.13
COI 103	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.69	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 104	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.53	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 105	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	99	KY972418.1	*Eurythoe complanata*	99.54	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 106	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	99	KY972418.1	*Eurythoe complanata*	99.24	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 108	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 109	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 110	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 111	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 112	Indonesia 2	Fanworm	*Bispira* sp.	1,00E-142	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12

Sample ID	Live rock source	Taxon	Morphological ID	BLAST				BOLD		Phylogenetic inference	
				E-value	Identity (%)	Accession number	Species	Similarity (%)	Species	Species	Figure
COI 113	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 114	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 115	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 116	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 117	Indonesia 2	Fanworm	*Bispira* sp.	1,00E-142	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 118	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 119	Indonesia 2	Fanworm	*Bispira* sp.	6,00E-141	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 120	Indonesia 2	Fanworm	*Bispira* sp.	1,00E-142	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 121	Indonesia 2	Fanworm	*Bispira* sp.	1,00E-142	82	LT717721.1	*Bispira* sp.	87.27	Sabellida (O)	*Bispira* sp.	3.12
COI 122	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.84	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 123	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.84	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 124	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.84	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 125	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.84	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 126	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.84	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 127	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.84	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 128	Indonesia 2	Polychaete	*Eurythoe* sp.	0.0	98	KY972418.1	*Eurythoe complanata*	99.84	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 160	Indonesia 1	Isopod	Isopoda (O)	0.0	85	MH242804.1	*Idotea urotoma*	84.58	*Idotea urotoma*	Idoteidae (F)	3.16
COI 161	Indonesia 1	Polychaete	*Eurythoe* sp.	0.0	99	KY972418.1	*Eurythoe complanata*	99.69	*Eurythoe complanata*	*Eurythoe complanata*	3.14
COI 162	Kenya 1	Amphipod	Amphipoda (O)	6,00E-146	81	KX054867.1	*Talitridae* sp.	83.31	Amphipoda (O)	Hyalidae (F)	3.17
COI 163	Kenya 3	Amphipod	Amphipoda (O)	0.0	85	HM465005.1	*Amphipoda* sp.	84.63	Amphipoda (O)	Hyalidae (F)	3.17
COI 168	Indonesia 2	Crab	*Heteropanope* sp.	5,00E-117	80	HM750947.1	*Actaeodes tomentosus*	79.77	*Actaeodes tomentosus*	-	-
COI 169	Indonesia 2	Amphipod	Amphipoda (O)	0.0	85	HM465005.1	*Amphipoda* sp.	85.16	Amphipoda (O)	Hyalidae (F)	3.17

Table 6.12: Species assignment of live rock associated taxa based on the nuclear ribosomal internal transcribed spacer (ITS) coding region. *The number after the live rock source indicates the replicate number. The best hits obtained from BLAST searches are listed for each sample. Highlighted sequences were used in phylogenetic analyses. Abbreviations: ID = identification; SCL = subclass; O = order; F = family.*

Sample ID	Live rock source	Taxon	Morphological ID	BLAST			
				E-value	Identity (%)	Accession number	Species
ITS 9	Kenya 1	Brittle star	*Ophiocomella* sp.	4,00E-46	88.89%	AF212169.2	*Asterias forbesii*
ITS 10	Kenya 1	Brittle star	*Ophiocomella* sp.	3,00E-47	89%	AF212169.2	*Asterias forbesii*
ITS 11	Kenya 1	Brittle star	*Ophiocomella* sp.	6,00E-49	90%	AF212169.2	*Asterias forbesii*
ITS 12	Kenya 1	Brittle star	*Ophiocomella* sp.	6,00E-49	90%	AF212169.2	*Asterias forbesii*
ITS 13	Kenya 1	Brittle star	*Ophiocomella* sp.	6,00E-49	90%	AF212169.2	*Asterias forbesii*
ITS 18	Kenya 1	Brittle star	*Ophiocomella* sp.	6,00E-49	90%	AF212169.2	*Asterias forbesii*
ITS 19	Kenya 1	Brittle star	*Ophiocomella* sp.	3,00E-47	89%	AF212169.2	*Asterias forbesii*
ITS 20	Kenya 1	Brittle star	*Ophiocomella* sp.	6,00E-49	90%	AF212169.2	*Asterias forbesii*
ITS 21	Kenya 1	Brittle star	*Ophiocomella* sp.	3,00E-47	89%	AF212169.2	*Asterias forbesii*
ITS 23	Kenya 1	Brittle star	*Ophiocomella* sp.	6,00E-49	90%	AF212169.2	*Asterias forbesii*
ITS 49	Indonesia 3	Starfish	*Asterina* sp.	1,00E-75	96%	KX592567.1	*Asterias amurensis*
ITS 50	Indonesia 3	Starfish	*Asterina* sp.	1,00E-75	96%	KX592567.1	*Asterias amurensis*
ITS 51	Indonesia 3	Sea anemone	*Aiptasia* sp.	5,00E-92	78%	KT852235.1	*Sagartia troglodytes*
ITS 52	Indonesia 3	Sea anemone	*Aiptasia* sp.	1,00E-103	79%	KT852235.1	*Sagartia troglodytes*
ITS 53	Indonesia 3	Sea anemone	*Aiptasia* sp.	2,00E-90	78%	KT852235.1	*Sagartia troglodytes*
ITS 54	Indonesia 3	Sea anemone	*Aiptasia* sp.	1,00E-97	78%	KT852235.1	*Sagartia troglodytes*
ITS 55	Indonesia 3	Sea anemone	*Aiptasia* sp.	4,00E-108	79%	KT852235.1	*Sagartia troglodytes*
ITS 56	Indonesia 3	Sea anemone	*Aiptasia* sp.	4,00E-83	77%	KT852235.1	*Sagartia troglodytes*
ITS 57	Indonesia 3	Sea anemone	*Aiptasia* sp.	6,00E-81	82%	KT852235.1	*Sagartia troglodytes*
ITS 58	Indonesia 3	Sea anemone	*Aiptasia* sp.	8,00E-35	82%	EU591569.1	*Parazoanthus* sp.
ITS 59	Indonesia 3	Sea anemone	*Aiptasia* sp.	4,00E-103	79%	KT852235.1	*Sagartia troglodytes*
ITS 60	Indonesia 3	Sea anemone	*Aiptasia* sp.	3,00E-109	80%	KT852235.1	*Sagartia troglodytes*
ITS 61	Indonesia 3	Sea anemone	*Aiptasia* sp.	4,00E-98	78%	KT852235.1	*Sagartia troglodytes*
ITS 62	Indonesia 3	Sea anemone	*Aiptasia* sp.	1,00E-68	92%	EU591575.1	*Parazoanthus* sp.
ITS 70	Indonesia 1	Polychaete	*Aiptasia* sp.	3,00E-95	80.56%	AF332156.1	*Perinereis floridana*
ITS 72	Kenya 1	Polychaete	*Eurythoe* sp.	1,00E-54	91%	KY982576.1	*Smithsonidrilus hummelincki*
ITS 73	Kenya 1	Polychaete	*Eunice petersi*	7,00E-82	94%	FJ872121.1	*Americonuphis reesei*
ITS 74	Kenya 1	Polychaete	*Eurythoe* sp.	3,00E-61	90%	KX985919.1	*Cernosvitoviella farkasi*

Sample ID	Live rock source	Taxon	Morphological ID	BLAST			
				E-value	Identity (%)	Accession number	Species
ITS 75	Kenya 1	Polychaete	*Eurythoe* sp.	3,00E-61	90%	KX985919.1	*Cernosvitoviella farkasi*
ITS 77	Kenya 1	Polychaete	*Eurythoe* sp.	8,00E-57	93%	MG252211.1	*Enchytronia christenseni*
ITS 78	Kenya 1	Polychaete	*Eurythoe* sp.	3,00E-36	87%	MF802174.1	*Globulidrilus riparius*
ITS 79	Kenya 1	Polychaete	*Eurythoe* sp.	2,00E-58	93%	MG252211.1	*Enchytronia christenseni*
ITS 81	Kenya 1	Polychaete	*Eurythoe* sp.	4,00E-60	94%	MG252211.1	*Enchytronia christenseni*
ITS 82	Kenya 2	Polychaete	*Eurythoe* sp.	4,00E-60	94%	MG252211.1	*Enchytronia christenseni*
ITS 83	Kenya 2	Polychaete	*Eurythoe* sp.	4,00E-60	94%	MG252211.1	*Enchytronia christenseni*
ITS 84	Kenya 2	Polychaete	*Eurythoe* sp.	4,00E-60	94%	MG252211.1	*Enchytronia christenseni*
ITS 85	Kenya 2	Polychaete	*Eurythoe* sp.	9,00E-57	93%	MG252211.1	*Enchytronia christenseni*
ITS 86	Kenya 3	Polychaete	*Lysidice ninetta*	5,00E-128	89%	DQ470257.1	*Lysidice ninetta*
ITS 87	Kenya 2	Polychaete	*Lysidice ninetta*	4,00E-55	92%	MG252211.1	*Enchytronia christenseni*
ITS 88	Kenya 2	Polychaete	*Eurythoe* sp.	7,00E-72	95%	KU894350.1	*Lumbricillus cf. helgolandicus*
ITS 89	Kenya 2	Polychaete	*Bhawania* sp.	1,00E-59	93%	KY982576.1	*Smithsonidrilus hummelincki*
ITS 106	Indonesia 2	Polychaete	*Eurythoe* sp.	4,00E-60	94%	MG252211.1	*Enchytronia christenseni*
ITS 109	Indonesia 2	Fanworm	*Bispira* sp.	0.0	89%	KX894910.1	*Bispira manicata*
ITS 110	Indonesia 2	Fanworm	*Bispira* sp.	0.0	89%	KX894910.1	*Bispira manicata*
ITS 111	Indonesia 2	Fanworm	*Bispira* sp.	0.0	89%	KX894910.1	*Bispira manicata*
ITS 112	Indonesia 2	Fanworm	*Bispira* sp.	0.0	89%	KX894910.1	*Bispira manicata*
ITS 113	Indonesia 2	Fanworm	*Bispira* sp.	7,00E-177	88%	KX894910.1	*Bispira manicata*
ITS 114	Indonesia 2	Fanworm	*Bispira* sp.	3,00E-175	88%	KX894910.1	*Bispira manicata*
ITS 115	Indonesia 2	Fanworm	*Bispira* sp.	3,00E-180	89%	KX894910.1	*Bispira manicata*
ITS 116	Indonesia 2	Fanworm	*Bispira* sp.	3,00E-165	87%	KX894910.1	*Bispira manicata*
ITS 117	Indonesia 2	Fanworm	*Bispira* sp.	1,00E-164	89%	KX894910.1	*Bispira manicata*
ITS 118	Indonesia 2	Fanworm	*Bispira* sp.	7,00E-177	88%	KX894910.1	*Bispira manicata*
ITS 119	Indonesia 2	Fanworm	*Bispira* sp.	3,00E-180	89%	KX894910.1	*Bispira manicata*
ITS 120	Indonesia 2	Fanworm	*Bispira* sp.	7,00E-177	88%	KX894910.1	*Bispira manicata*
ITS 121	Indonesia 2	Fanworm	*Bispira* sp.	1,00E-173	88%	KX894910.1	*Bispira manicata*
ITS 122	Indonesia 2	Polychaete	*Eurythoe* sp.	3,00E-61	90%	KX985919.1	*Cernosvitoviella farkasi*
ITS 123	Indonesia 2	Polychaete	*Eurythoe* sp.	3,00E-61	90%	KX985919.1	*Cernosvitoviella farkasi*
ITS 124	Indonesia 2	Polychaete	*Eurythoe* sp.	3,00E-61	90%	KX985919.1	*Cernosvitoviella farkasi*
ITS 125	Indonesia 2	Polychaete	*Eurythoe* sp.	3,00E-61	90%	KX985919.1	*Cernosvitoviella farkasi*
ITS 126	Indonesia 2	Polychaete	*Eurythoe* sp.	3,00E-61	90%	KX985919.1	*Cernosvitoviella farkasi*
ITS 127	Indonesia 2	Polychaete	*Eurythoe* sp.	3,00E-61	90%	KX985919.1	*Cernosvitoviella farkasi*

Sample ID	Live rock source	Taxon	Morphological ID	BLAST			
				E-value	Identity (%)	Accession number	Species
ITS 128	Indonesia 2	Polychaete	*Eurythoe* sp.	3,00E-61	90%	KX985919.1	*Cernosvitoviella farkasi*
ITS 146	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 147	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 148	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 149	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 150	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 151	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 152	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 153	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 154	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 155	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 156	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 157	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 158	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 159	**Kenya 1**	**Soft coral**	***Xenia umbellata***	**0.0**	**99%**	**KY442653.1**	***Xenia umbellata***
ITS 162	Kenya 1	Amphipod	Amphipoda (O)	2,00E-43	90%	FJ422963.1	*Gammarus wilkitzkii*
ITS 163	Kenya 3	Amphipod	Amphipoda (O)	2,00E-43	90%	FJ422963.1	*Gammarus wilkitzkii*
ITS 168	Indonesia 2	Crab	*Heteropanope* sp.	1,00E-80	85%	AF316385.1	*Eriocheir leptognathus*
ITS 169	Indonesia 2	Amphipod	Amphipoda (O)	2,00E-43	90%	FJ422963.1	*Gammarus wilkitzkii*
ITS 170	Indonesia 3	Gastropod	Prosobranchia (SCL)	2,00E-122	84%	AF296849.1	*Megathura crenulata*
ITS 171	Indonesia 3	Gastropod	Prosobranchia (SCL)	2,00E-122	84%	AF296849.1	*Megathura crenulata*
ITS 173	Indonesia 3	Gastropod	Prosobranchia (SCL)	2,00E-122	84%	AF296849.1	*Megathura crenulata*
ITS 174	Indonesia 3	Gastropod	Prosobranchia (SCL)	2,00E-122	84%	AF296849.1	*Megathura crenulata*

Table 6.13: Non-target DNA amplifications. *The best hits obtained from both BLAST and BOLD analyses are listed for each sample. The number after the live rock source indicates the replicate number. The 'targeted taxon' column indicates the live rock associated taxon of intended DNA amplification. A hyphen indicates that no hit resulted for that sequence. Highlighted hits are known to be of pathogenic nature.*

Sample ID	Source	Marker	Taxon	BLAST				BOLD		Targeted taxon
				E-value	Identity (%)	Accession no.	Species	Similarity (%)	Species	
63	Indonesia 3	ITS	Dinoflagellates	0.0	99%	AF184940.1	*Symbiodinium* sp.	100	*Symbiodinium* sp.	Actiniaria
64	Indonesia 3	ITS	Dinoflagellates	0.0	89%	EU074875.1	*Symbiodinium* sp.	-	-	Actiniaria
65	Indonesia 3	ITS	Dinoflagellates	0.0	99%	AF184940.1	*Symbiodinium* sp.	100	*Symbiodinium* sp.	Actiniaria
66	Indonesia 3	ITS	Dinoflagellates	0.0	88%	EU074875.1	*Symbiodinium* sp.	-	-	Actiniaria
67	Indonesia 3	ITS	Dinoflagellates	0.0	99%	AF184940.1	*Symbiodinium* sp.	100	*Symbiodinium* sp.	Actiniaria
69	Indonesia 1	COI	Proteobacteria	**3,00E-55**	**73.85%**	**CP020562.1**	***Halomonas* sp.**	82.42	*Bermanella marisrubri*	Polychaeta
94	Kenya 2	COI	Proteobacteria	**1,00E-173**	**84.38%**	**CP009355.1**	***Vibrio tubiashii***	**80.73**	***Vibrio tubiashii***	Polychaeta
96	Kenya 2	COI	Proteobacteria	**0.0**	**86%**	**CP018681.2**	***Vibrio harveyi***	**84.19**	***Vibrio* sp.**	Polychaeta
97	Kenya 2	COI	Proteobacteria	2,00E-16	95.24%	CP021330.1	*Maritalea myrionectae*	-	-	Polychaeta
98	Kenya 1	COI	Proteobacteria	**1,00E-178**	**84.67%**	**CP009355.1**	***Vibrio tubiashii***	**84.39**	***Vibrio tubiashii***	Polychaeta
99	Indonesia 1	COI	Proteobacteria	**5,00E-162**	**83.08%**	**CP033135.1**	***Vibrio campbellii***	**80.96**	***Vibrio coralliilyticus***	Polychaeta
100	Indonesia 1	COI	Proteobacteria	5,00E-112	78.42%	CP035457.1	*Photobacterium damselae*	-	-	Polychaeta
101	Indonesia 1	COI	Proteobacteria	**0.0**	**95.26%**	**CP033135.1**	***Vibrio campbellii***	**89.18**	***Vibrio campbellii***	Polychaeta
129	Indonesia 3	ITS	Dinoflagellates	0.0	99%	KY697974.1	*Amphidinium gibbosum*	72.36	*Amphidinium massartii*	Platyhelminthes
141	Kenya 2	ITS	Chromista	0.0	99%	KM222058	*Amphileptus marinus*	83.11	*Collema curtisporum*	Bivalvia
147	Kenya 1	COI	Proteobacteria	**7,00E-81**	**75.79%**	**CP036200.1**	***Shewanella* sp.**	90.62	*Bermanella marisrubri*	Alcyonacea
148	Kenya 1	COI	Mollusca	1,00E-108	82.96%	HQ258872.1	*Arcopsis* sp.	82.65	*Arcopsis* sp.	Alcyonacea
149	Kenya 1	COI	Proteobacteria	**8,00E-165**	**83.31%**	**CP009355.1**	***Vibrio tubiashii***	81.48	*Bermanella marisrubri*	Alcyonacea
150	Kenya 1	COI	Proteobacteria	2,00E-106	78.32%	AP019537.1	*Marinobacter hydrocarbonoclasticus*	89.46	*Bermanella marisrubri*	Alcyonacea
152	Kenya 1	COI	Proteobacteria	5,00E-82	76.02%	AP018725.1	*Sulfuriflexus mobilis*	89.34	*Bermanella marisrubri*	Alcyonacea
153	Kenya 1	COI	Proteobacteria	**7,00E-86**	**76.72%**	**CP026675.1**	***Pseudomonas* sp.**	93.41	*Bermanella marisrubri*	Alcyonacea
154	Kenya 1	COI	Proteobacteria	**3,00E-89**	**77.05%**	**CP026675.1**	***Pseudomonas* sp.**	93.02	*Bermanella marisrubri*	Alcyonacea
155	Kenya 1	COI	Proteobacteria	**5,00E-147**	**81.61%**	**CP031473.1**	***Vibrio coralliilyticus***	**80.73**	***Vibrio tubiashii***	Alcyonacea
156	Kenya 1	COI	Proteobacteria	**0.0**	**85.18%**	**CP009355.1**	***Vibrio tubiashii***	**80.26**	***Vibrio tubiashii***	Alcyonacea
157	Kenya 1	COI	Proteobacteria	**2,00E-91**	**76.95%**	**CP015876.1**	***Pseudomonas putida***	93.22	*Bermanella marisrubri*	Alcyonacea

158	Kenya 1	COI	Proteobacteria	**4,00E-118**	**79.10%**	**CP036200.1**	***Shewanella* sp.**	84.78	*Bermanella marisrubri*	Alcyonacea
159	Kenya 1	COI	Proteobacteria	**8,00E-115**	**78.85%**	**CP036200.1**	***Shewanella* sp.**	87.4	*Bermanella marisrubri*	Alcyonacea
164	Kenya 3	COI	Proteobacteria	**0.0**	**87.38%**	**CP025797.1**	***Vibrio owensii***	**85.06**	***Vibrio campbellii***	Arthropoda
165	Kenya 1	COI	Proteobacteria	**2,00E-137**	**80.74%**	**CP025795.1**	***Vibrio hyugaensis***	**80.14**	***Vibrio tubiashii***	Arthropoda
166	Kenya 1	COI	Proteobacteria	**2,00E-126**	**80.32%**	**CP009355.1**	***Vibrio tubiashii***	**78.59**	***Vibrio tubiashii***	Arthropoda
167	Indonesia 2	COI	Proteobacteria	**7,00E-101**	**77.96%**	**CP025795.1**	***Vibrio hyugaensis***	**76.43**	***Vibrio parahaemolyticus***	Arthropoda
170	Indonesia 3	COI	Proteobacteria	**0.0**	**85.96%**	**CP033578.1**	***Vibrio mediterranei***	**85.02**	***Vibrio shilonii***	Gastropoda

APPENDIX C: MARINE AQUARIUM HOBBYIST SURVEY

1. Which South African province do you live in? *

(a) KwaZulu-Natal

(b) Gauteng

(c) Mpumalanga

(d) Limpopo

(e) Northern Cape

(f) Eastern Cape

(g) Western Cape

(h) Free State

(i) North West

2. How long have you kept a marine aquarium for? *

(a) 0 - 1 year (I am still a beginner)

(b) 2 - 5 years

(c) 5 - 10 years

(d) 10 years +

3. Do you trade fish/ coral frags/ invertebrates/ unwanted organisms with fellow aquarium hobbyists? *

(a) Yes, I trade

(b) No, I do not trade

(c) I would prefer not to say

4. If you keep live rock in your aquarium, do you know what type it is? If applicable, please select more than one option. *

(a) Indonesian

(b) Kenyan

(c) Fiji

(d) Locally cultured rock

(e) Rock from a 'broken down' aquarium

(f) I do not know where it comes from

5. Do you quarantine your live rock before you put it into your tank? If applicable, please select more than one option. *

(a) I dip it

(b) I remove unwanted 'hitch-hikers' by hand

(c) I let it 'grow out' in a separate tank

(d) No, I like all the organisms that come with the rock

(e) No, I trust that the pet shop did it for me

6. What types of invertebrates have you noticed come off of your live rock? These could be any that you have seen on the rock before putting it into the aquarium, those that have appeared after the live rock has settled in the aquarium, or even those that have miraculously appeared out of nowhere after some time. If applicable, please select more than one option. *

(a) Polychaetes/worms

(b) Crabs/shrimp

(c) Gastropods/snails

(d) Starfish/brittle stars

(e) Sea anemone

(f) Hard coral/Soft coral

(g) Sponges

(h) Bivalves/mussels

(i) Flatworms

(j) Fanworms

(k) Algae/seaweed

(l) None

7. Please select which organisms have become a pest or a nuisance in your aquarium. Please select more than one if applicable. *

(a) Polychaetes/worms

(b) Crabs/shrimp

(c) Gastropods/snails

(d) Starfish/brittle stars

(e) Sea anemone

(f) Hard coral/Soft coral

(g) Sponges

(h) Bivalves/mussels

(i) Flatworms

(j) Fanworms

(k) Algae/seaweed

(l) None

8. If you have had any pesty invertebrates in your aquarium, how do you manage to control them? If applicable, please select more than one option. *

(a) I remove them by hand and dispose of them

(b) Chemicals

(c) Biological control (I introduced a new fish/invertebrate to prey on the pest)

(d) Inject coral/sea anemones with chemical or liquid solution

(e) No, I have never had a problem with pesty organisms

9. If you have had to remove any invertebrate/fish/algae from your tank, where do you dispose of it? If applicable, please select more than one option. *

(a) Out into the garden

(b) Down the toilet/drain

(c) Release it into the ocean

(d) Thrown it into the rubbish bin

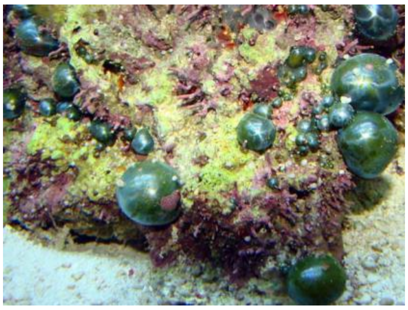
(e) I have never had to remove anything from my aquarium

10. Do you recognize any of the following pests?

(a) *Aiptasia*/glass anemone

Credit: Narrissa Spies

(b) Green bubble algae



Credit: FishLore.com

(c) *Asterina* sp. (tiny white starfish on the glass of an aquarium)

Credit: Paul Whitby, Reefkeeping magazine (reefkeeping.com)

(d) Tiny banded brittle stars

Credit: jbsmarines.co.uk

(e) Brown/red coloured flatworms usually attached to coral

Credit: Gitte Wehr

11. Do you think that the aquarium pet trade has the potential to move pest organisms into and around South Africa? *

(a) Yes

(b) No

(c) Maybe

(d) I do not know enough about this issue

* This question requires an answer in order to submit the questionnaire

APPENDIX D: MARINE AQUARIUM HOBBYIST SURVEY RESULTS

Table 6.14: Provinces of residence of the surveyed marine aquarium hobbyists in South Africa.

Province	Frequency	Percentage (%)
KwaZulu-Natal	25	39.7
Gauteng	25	39.7
Mpumalanga	1	1.6
Eastern Cape	2	3.2
Western Cape	9	14.3
Free State	1	1.6
Total	63	100.0

Table 6.15: Expertise level of the surveyed marine aquarium hobbyists in South Africa.

Response	Frequency	Percentage (%)
0 - 1 year (beginner)	10	15.9
2 - 5 years	25	39.7
5 - 10 years	16	25.4
10 years +	12	19.0
Total	63	100.0

Table 6.16: Proportion of the surveyed marine aquarium hobbyists who do/do not take part in the trading of fish, corals, invertebrates, and/or unwanted organisms with fellow aquarium hobbyists.

Response	Frequency	Percentage (%)
Yes	43	68.3
No	18	28.6
I would prefer not to say	2	3.2
Total	63	100.0

Table 6.17: Types of live rock kept by the surveyed marine aquarium hobbyists in South Africa.

Live rock type	Responses		Percentage of cases (%)*
	N	Percentage (%)	
Indonesian	9	9.8	14.3
Kenyan	10	10.9	15.9
Fiji	16	17.4	25.4
Locally cultured rock	7	7.6	11.1
Rock from an old 'broken down' aquarium	30	32.6	47.6
I do not know where it comes from	20	21.7	31.7
Total	92	100.0	146.0

* Questionnaire respondents were allowed to check all that applied.

Table 6.18: Methods used by the surveyed marine aquarium hobbyists to get rid of organisms associated with live rock before addition of the live rock to their aquarium.

Method	Responses		Percentage of cases (%)*
	N	Percentage (%)	
I dip it	9	12.3	14.3
I remove unwanted 'hitch-hikers' by hand	8	11.0	12.7
I let it 'grow out' in a separate tank	7	9.6	11.1
No, I like all the organisms that come with the rock	39	53.4	61.9
No, I trust that the pet shop did it for me	10	13.7	15.9
Total	73	100.0	115.9

* Questionnaire respondents were allowed to check all that applied.

Table 6.19: Live rock associated taxa observed by the surveyed marine aquarium hobbyists.

Taxon	Responses		Percentage of cases (%)*
	N	Percentage (%)	
Polychaetes/worms	49	16.3	77.8
Crabs/shrimp	24	8.0	38.1
Gastropods/snails	34	11.3	54.0
Starfish/brittle stars	46	15.3	73.0
Sea anemone	18	6.0	28.6
Hard coral/Soft coral	13	4.3	20.6
Sponges	37	12.3	58.7
Bivalves/mussels	13	4.3	20.6
Flatworms	18	6.0	28.6
Fanworms	17	5.6	27.0
Algae/seaweed	31	10.3	49.2
None	1	0.3	1.6
Total	301	100.0	477.8

* Questionnaire respondents were allowed to check all that applied.

Table 6.20: Live rock associated taxa that have become a pest in the surveyed hobbyist's marine aquaria.

Taxon	Responses		Percentage of cases (%)*
	N	Percentage (%)	
Polychaetes/worms	13	10.8	20.6
Crabs/shrimp	8	6.7	12.7
Gastropods/snails	9	7.5	14.3
Starfish/brittle stars	6	5.0	9.5
Sea anemone	17	14.2	27.0
Hard coral/Soft coral	2	1.7	3.2
Sponges	6	5.0	9.5
Bivalves/mussels	1	0.8	1.6
Flatworms	17	14.2	27.0
Fanworms	4	3.3	6.3
Algae/seaweed	18	15.0	28.6
None	19	15.8	30.2
Total	120	100.0	190.5

* Questionnaire respondents were allowed to check all that applied.

Table 6.21: Methods used to get rid of pest taxa associated with live rock by the surveyed marine aquarium hobbyists.

Response	Responses		Percentage of cases (%)*
	N	Percentage (%)	
I remove them by hand and dispose of them	34	32.7	54.0
Chemicals	12	11.5	19.0
Biological control	26	25.0	41.3
Inject coral/sea anemones with chemical or liquid solution	24	23.1	38.1
No, I have never had a problem with pesty organisms	8	7.7	12.7
Total	104	100.0	165.1

* Questionnaire respondents were allowed to check all that applied.

Table 6.22: Methods of disposal of pest taxa associated with live rock used by the surveyed marine aquarium hobbyists.

Response	Responses		Percentage of cases (%)*
	N	Percentage (%)	
Out into the garden	21	25.0	33.3
Down the toilet/drain	19	22.6	30.2
Release it into the ocean	8	9.5	12.7
Thrown it into the rubbish bin	26	31.0	41.3
I have never had to remove anything from my aquarium	10	11.9	15.9
Total	84	100.0	133.3

* Questionnaire respondents were allowed to check all that applied.

Table 6.23: Commonly-observed pest taxa associated with live rock identified by the surveyed marine aquarium hobbyists.

Taxon	Responses		Percentage of cases (%)*
	N	Percentage (%)	
Aiptasia/glass anemone	59	27.4	96.7
Green bubble algae	39	18.1	63.9
Asterina	47	21.9	77.0
Tiny banded brittle stars	44	20.5	72.1
Brown/red coloured flatworms	26	12.1	42.6
Total	215	100.0	352.5

* Questionnaire respondents were allowed to check all that applied.

Table 6.24: Proportion of the surveyed marine aquarium hobbyists' responses to the question: Do you think that the aquarium pet trade has the potential to move pest organisms into and around South Africa?

Response	Frequency	Percentage (%)
Yes	26	41.3
No	10	15.9
Maybe	17	27.0
I do not know enough about this issue	10	15.9
Total	63	100.0

APPENDIX E: PET STORE STOCK INVENTORIES

Table 6.25: Stock inventories of marine aquarium fish traded in pet stores in South Africa. *Letters A, B, or C represent the pet stores within Cape Town (CPT), Durban (DBN), or Johannesburg/Pretoria (JHB/PA), and each visit per store is allocated a roman numeral (i, ii, iii). Common family names are in parentheses next to the scientific family name. Numbers within the table indicate the total number of live specimens counted per store. A dash (-) is used in lieu of a zero (0) for visual ease.*

Class	Order	Family	Species	DBN									JHB/PTA									CPT								
				A			B			C			A			B			C			A			B			C		
				i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
Actinopterygii	Anguilliformes	Congridae (Garden eels)	*Heteroconger hassi*	-	-	-	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Muraenidae (Moray eels)	*Echidna nebulosa*	-	-	-	-	1	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
			Echidna polyzona	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
			Gymnomuraena zebra	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Gymnothorax favagineus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
			Gymnothorax steindachneri	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Lophiiformes	Antennariidae (Frogfish)	*Antennarius commerson*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Perciformes	Acanthuridae (Surgeonfish, tangs, unicornfish)	*Acanthurus achilles*	-	1	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Acanthurus blochii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
			Acanthurus chirurgus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
			Acanthurus coerulus	-	-	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Acanthurus gahhm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
			Acanthurus japonicus	-	-	-	-	-	-	-	1	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	1	-	-	-
			Acanthurus leucosternon	-	-	1	-	1	-	1	-	-	1	10	11	-	3	2	-	-	-	4	2	-	3	1	-	2	3	-
			Acanthurus lineatus	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	3	1	-	-	-	1	-	-	-
			Acanthurus nigricauda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-
			Acanthurus nigrofuscus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	1
			Acanthurus olivaceus	-	-	1	2	-	1	1	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
			Acanthurus pyroferus	-	-	-	-	-	-	1	1	-	-	-	-	-	-	2	-	-	-	5	-	-	-	-	-	-	-	-
			Acanthurus tennenti	-	1	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	2	-	-	-
			Acanthurus thompsoni	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
			Acanthurus triostegus	-	-	-	1	-	-	-	-	-	-	-	1	-	1	-	-	-	1	5	-	-	-	2	2	2	-	-
			Acanthurus xanthopterus	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-
			Ctenochaetus binotatus	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
			Ctenochaetus cyanocheilus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-

Class	Order	Family	Species	DBN									JHB/PTA									CPT								
				A			B			C			A			B			C			A			B			C		
				i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
			Ctenochaetus hawaiiensis	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Ctenochaetus striatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	1
			Ctenochaetus strigosus	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-
			Naso brevirostris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-
			Naso caesius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	-	-	-	-	-	-	-	-	-	-
			Naso elegans	-	-	-	-	-	-	-	-	-	-	2	-	-	1	-	-	4	-	-	-	-	-	-	-	-	-	-
			Naso lituratus	-	1	-	1	1	-	-	1	-	-	-	-	-	2	3	-	-	-	-	-	-	-	-	1	-	-	-
			Naso unicornis	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
			Naso vlamingii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-	-	-	-	-
			Paracanthurus hepatus	1	1	-	2	-	-	-	1	-	-	-	15	-	10	8	8	-	-	-	1	1	-	-	-	-	-	-
			Zebrasoma desjardinii	-		1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-
			Zebrasoma flavescens	1	3	1	3	-	-	6	1	-	4	4	9	3	12	4	6	-	-	-	-	-	-	-	-	-	-	-
			Zebrasoma scopas	-	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	1	-	-	7	-	1	-	-	-	1
			Zebrasoma veliferum	-	1	-	-	-	1	-	-	-	-	-	2	1	6	3	-	1	-	-	-	3	-	-	-	-	-	-
			Zebrasoma xanthurum	1	-	-	-	-	-	-	-	-	-	1	-	1	1	1	-	-	-	-	-	-	-	-	-	-	1	-
		Apogonidae (Cardinalfish)	*Apogon compressus*	-	-	-	-	-	-	-	-	-	1	-	-	-	10	-	-	-	9	-	-	-	-	-	-	-	-	-
			Pterapogon kauderni	8	5	-	-	-	-	-	-	-	2	-	23	6	18	-	-	19	18	-	-	6	-	-	-	-	-	-
			Sphaeramia nematoptera	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	19	14	7	-	-	8	-	-	5	-	-	8
			Zoramia leptacantha	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Balistidae (Triggerfish)	*Balistes capriscus*	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Balistes punctatus	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Balistoides conspicillum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	1	-	-	-	-	-	-
			Melichthys vidua	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
			Odonus niger	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-
			Rhinecanthus aculeatus	-	-	-	-	-	-	-	-	-	1	-	-	2	-	2	-	-	-	-	1	-	-	-	-	-	-	-
			Rhinecanthus rectangulus	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Sufflamen chrysopterum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
			Xanthichthys auromarginatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
		Blenniidae (Blennies)	*Atrosalarias fuscus*	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
			Ecsenius bicolor	-	1	-	4	-	-	-	2	3	2	-	-	-	-	-	-	-	1	-	1	-	1	1	1	-	-	-
			Ecsenius lineatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
			Ecsenius lividanalis	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Ecsenius midas	-	1	1	6	1	-	2	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-

Class	Order	Family	Species	DBN									JHB/PTA									CPT								
				A			B			C			A			B			C			A			B			C		
				i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
			Meiacanthus atrodorsalis	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Meiacanthus mossambicus	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Meiacanthus smithii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
			Salarias fasciatus	-	-	-	-	-	-	4	2	-	-	-	-	1	1	-	3	1	-	-	1	7	-	-	1	-	1	-
		Callionymidae (Dragonets)	*Synchiropus marmoratus*	-	-	-	4	1	-	-	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-
			Synchiropus ocellatus	-	-	-	-	-	-	-	1	-	-	-	-	3	1	-	-	-	-	-	-	-	-	4	-	-	-	-
			Synchiropus picturatus	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
			Synchiropus splendidus	-	1	1	-	-	-	-	1	-	-	-	-	2	2	1	-	2	3	-	-	5	-	-	-	-	-	-
			Synchiropus stellatus	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Synchiropus sycorax	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
		Chaetodontidae (Butterflyfish)	*Chaetodon auriga*	-	1	-	-	1	-	-	-	-	-	-	-	-	-	1	-	3	-	-	-	-	1	-	-	-	-	-
			Chaetodon chrysurus	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Chaetodon guttatissimus	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
			Chaetodon kleinii	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1	-	-	-	-	-
			Chaetodon lunula	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
			Chaetodon mertensii	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Chaetodontoplus mesoleucus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1	-	-	-
			Chaetodon meyeri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
			Chaetodon miliaris	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Chaetodon plebius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
			Chaetodon unimaculatus	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Chaetodon vagabundus	-	-	-	-	1	-	-	-	-	2	1	-	-	-	-	-	4	-	-	-	-	3	1	-	1	-	-
			Chaetodon xanthocephalus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
			Chaetodon xanthurus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
			Chelmon rostratus	2	-	1	2	-	-	4	-	-	2	-	-	-	3	1	-	7	3	-	-	1	-	-	2	-	-	-
			Forcipiger flavissimus	2	-	-	2	1	1	-	-	-	1	2	1	-	-	1	-	4	-	-	-	-	-	1	-	-	-	-
			Hemitaurichthys polylepis	-	-	-	-	-	-	-	-	-	2	1	-	-	3	-	-	-	-	-	1	-	-	-	-	-	-	-
			Hemitaurichthys zoster	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
			Heniochus acuminatus	-	-	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Cirrhitidae (Hawkfish)	*Cirrhitops fasciatus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
			Neocirrhites armatus	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Oxycirrhites typus	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
		Ephippidae (Batfish)	*Platax teira*	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Class	Order	Family	Species	DBN									JHB/PTA									CPT								
				A			B			C			A			B			C			A			B			C		
				i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
		Gobiidae (Gobies)	*Amblygobius decussatus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
			Amblyeleotris diagonalis	-	-	-	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Amblyeleotris yanoi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
			Cryptocentrus cinctus	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-
			Fusigobius neophytus	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Gobiodon okinawae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-
			Koumansetta rainfordi	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Nemateleotris decora	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	3	-	-	2	-	-	-	-	-	-
			Nemateleotris exquisita	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Nemateleotris magnifica	-	-	1	-	-	-	5	5	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-
			Pomatoschistus minutus	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	2	-	-	-	1	1	-	1	-	-
			Priolepis nocturna	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Signigobius biocellatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
			Stonogobiops xanthorhinica	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Valenciennea helsdingeni	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-
			Valenciennea puellaris	-	-	-	4	6	-	-	1	8	3	-	-	-	1	-	-	-	-	9	1	8	-	-	-	-	-	2
			Valenciennea sexguttata	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	1	-	2	-
			Valenciennea strigata	-	2	-	2	-	-	-	1	-	-	1	-	-	-	-	-	3	-	-	-	-	-	-	-	-	2	-
		Grammatidae (Basslets)	*Gramma loreto*	-	-	2	2	-	-	1	3	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Labridae (Wrasse)	*Cirrhilabrus aurantidorsalis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
			Cirrhilabrus exquisitus	-	-	-	-	-	-	-	-	-	-	-	1	3	-	1	-	1	-	-	2	-	-	-	-	-	1	-
			Cirrhilabrus lubbocki	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	2
			Cirrhilabrus rubriventralis	-	1	-	8	-	-	-	-	2	-	-	-	-	-	-	-	-	6	-	-	-	4	-	-	-	-	-
			Coris formosa	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Halichoeres chloropterus	-	-	-	-	-	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-
			Halichoeres chrysus	-	2	2	-	-	-	-	-	-	2	-	-	4	1	1	-	-	-	-	-	7	-	-	-	1	-	-
			Halichoeres cosmetus	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	2	-
			Halichoeres hortulanus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
			Halichoeres marginatus	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-	-	-	2
			Halichoeres timorensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
			Halichoeres trispilus	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Labroides dimidiatus	4	-	-	4	-	2	-	4	3	-	3	4	6	26	8	-	7	-	5	-	7	3	3	2	-	1	-
			Macropharyngodon bipartitus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-

Class	Order	Family	Species	DBN									JHB/PTA									CPT								
				A			B			C			A			B			C			A			B			C		
				i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
			Macropharyngodon negrosensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
			Macropharyngodon ornatus	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Novaculichthys taeniourus	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
			Paracheilinus carpenteri	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
			Paracheilinus cyaneus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
			Paracheilinus filamentosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
			Paracheilinus lineopunctatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
			Pseudocheilinus hexataenia	-	-	-	-	-	-	-	1	-	-	-	2	-	2	2	-	-	4	-	-	7	-	-	-	-	-	-
			Thalassoma hardwicke	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Thalassoma lunare	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	1	-	-	-	-	-	-
			Wetmorella albofasciata	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Lutjanidae (Snappers)	*Lutjanus sebae*	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Microdesmidae (Dartfish)	*Ptereleotris evides*	-	-	-	-	-	2	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	2
			Ptereleotris heteroptera	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Ptereleotris monoptera	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Monodactylidae (Moonyfishes)	*Monodactylus argenteus*	-	-	-	-	-	-	-	-	-	5	5	-	3	2	-	-	-	-	-	-	-	-	-	-	2	-	-
		Mullidae (Goatfish)	*Parupeneus barberinus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
		Opistognathidae (Jawfish)	*Opistognathus aurifrons*	-	-	2	1	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Pholidichthyidae (Ray-finned fish)	*Pholidichthys leucotaenia*	-	-	-	-	-	-	-	-	-	-	-	-	10	9	-	-	-	-	-	-	-	-	-	-	-	-	-
		Plesiopidae (Longfins)	*Calloplesiops altivelis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
		Pomacanthidae (Angelfish)	*Apolemichthys trimaculatus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
			Apolemichthys xanthurus	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-
			Centropyge acanthops	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
			Centropyge argi	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Centropyge aurantia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
			Centropyge bicolor	-	-	-	-	-	-	-	1	-	-	2	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	1
			Centropyge bispinosa	-	1	1	-	-	-	1	-	-	-	-	4	2	1	4	-	-	2	-	-	6	-	-	-	-	-	-
			Centropyge eibli	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	2	2	1	-	-	1	-	-	1	-	-	1
			Centropyge ferrugata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
			Centropyge flavicauda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-
			Centropyge flavipectoralis	-	-	-	-	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	2	2	-	-	-	-
			Centropyge flavissima	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-

Class	Order	Family	Species	DBN									JHB/PTA									CPT								
				A			B			C			A			B			C			A			B			C		
				i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
			Centropyge heraldi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
			Centropyge loricula	-	2	-	-	-	-	3	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Centropyge multispinis	-	-	-	-	1	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	1	-	-	-	-
			Centropyge tibicen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
			Centropyge vroliki	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	2	-	1	2
			Genicanthus lamarck	-	-	-	-	-	-	-	-	-	-	-	3	2	2	-	1	-	-	-	-	-	-	-	-	-	-	-
			Genicanthus melanospilos	-	-	-	-	-	-	-	-	-	2	-	-	1	-	1	-	-	-	-	-	3	-	-	-	-	-	-
			Genicanthus semifasciatus	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
			Genicanthus watanabei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
			Holacanthus ciliaris	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
			Holacanthus passer	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Holacanthus tricolor	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Pomacanthus asfur	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Pomacanthus imperator	-	-	-	3	3		-	-	3	-	3	-	-	2	2	-	2		4	1	1	-	-	-	-	-	-
			Pomacanthus navarchus	-	-	-	-	-	-	-	-	-	1	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-
			Pomacanthus semicirculatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
			Pomacanthus xanthometopon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-
			Pygoplites diacanthus	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Pomacentridae (Clownfish & Damselfish)	*Amphiprion barberi*	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-	-	-
			Amphiprion bicinctus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
			Amphiprion clarkii	20	-	2	-	-	-	-	-	-	5	5	-	-	-	-	-	-	-	-	8	4	-	-	-	-	-	-
			Amphiprion frenatus	-	-	-	-	-	-	-	-	-	-	-	-	5	14	3	24	-	-	-	-	-	-	-	-	-	-	-
			Amphiprion melanopus	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Amphiprion nigripes	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-
			Amphiprion ocellaris	34	5	-	1	-	2	-	14	-	12	1	-	-	-	-	30	30	6	-	-	7	-	-	-	-	-	-
			Amphiprion percula	-	-	-	1	-	-	2	-	-	-	-	-	-	-	6	13	-	2	-	-	-	-	-	-	-	-	5
			Amphiprion perideraion	20	6	2	-	-	-	-	-	-	-	-	-	2	6	2	-	-	-	-	-	-	-	-	-	-	-	-
			Amphiprion polymnus	18	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Premnas biaculeatus	-	-	-	-	-	-	-	-	-	1	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-
			Abudefduf saxatilis	-	-	-	-	1	-	-	-	-	-	-	-	1	6	-	-	-	-	-	-	-	-	-	-	-	-	-
			Chromis dimidiata	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	6	-	-	-	-	-	-
			Chromis viridis	-	3	-	-	-	3	-	5	-	-	-	28	-	-	9	-	6	10	-	38	16	-	-	7	11	4	3
			Chrysiptera hemicyanea	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	-	4	-	-	-	-	-	-	2

Class	Order	Family	Species	DBN									JHB/PTA									CPT								
				A			B			C			A			B			C			A			B			C		
				i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
			Chrysiptera parasema	2	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Chrysiptera springeri	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-
			Dascyllus carneus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-
			Dascyllus melanurus	-	-	-	-	-	-	-	-	-	-	-	-	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-
			Dascyllus trimaculatus	-	-	-	-	4	-	-	-	-	-	1	-	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-
			Neoglyphidodon oxyodon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-
			Neopomacentrus azysron	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Neopomacentrus cyanomos	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Pomacentrus auriventris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-
			Pomacentrus caeruleus	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
			Pomacentrus similis	-	-	-	-	-	-	-	-	-	-	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Pseudochromidae (Dottybacks)	*Pictichromis diadema*	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-
			Pictichromis dinar	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Pictichromis paccagnellae	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
		Scaridae (Parrotfish)	*Cetoscarus bicolor*	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Scarus taeniopterus	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Serranidae (Anthias & Bass)	*Nemanthias carberryi*	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Pseudanthias bicolor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-
			Pseudanthias dispar	-	6	-	-	-	-	-	4	5	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Pseudanthias kashiwae	1	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Pseudanthias squamipinnis	-	-	9	-	-	-	-	6	-	11	5	12	-	-	-	-	-	-	-	19	-	-	-	-	-	-	-
			Serranus tortugarum	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Siganidae (Foxfaces)	*Siganus magnificus*	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Siganus virgatus	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	6	-	-	-	-	-	-	-	-	-	-
			Siganus vulpinus	1	-	-	-	-	-	-	-	-	-	-	5	1	-	2	-	-	-	-	1	5	-	-	-	-	-	-
			Siganus uspi	-	-	-	-	-	-	-	-	-	-	1	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
		Zanclidae (Moorish idol)	*Zanclus cornutus*	2	-	-	-	3	-	-	1	-	-	7	-	-	-	1	-	-	2	7	-	-	-	-	-	-	-	-
	Scorpaeniformes	Dactylopteridae (Flying gurnards)	*Dactyloptena orientalis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
		Scorpaenidae (Scorpionfish)	*Dendrochirus biocellatus*	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Pterois antennata	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	2	1	-
	Syngnathiformes	Syngnathidae (Pipefish & Seahorses)	*Doryrhamphus excisus*	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Hippocampus sp.	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Tetraodontiformes	Monacanthidae	*Acreichthys tomentosus*	-	-	-	3	-	-	6	1	-	-	-	-	-	13	1	-	-	-	-	-	-	-	-	-	-	-	-

Class	Order	Family	Species	DBN									JHB/PTA									CPT								
				A			B			C			A			B			C			A			B			C		
				i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
		(Filefish)	*Chaetodermis penicilligerus*	-	-	-	-	-	-	-	-	-	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
		Tetraodontidae (Pufferfish)	*Arothron hispidus*	-	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Arothron nigropunctatus	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Canthigaster solandri	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
			Canthigaster valentini	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
Elasmobranchii	Myliobatiformes	Dasyatidae (Whiptail stingrays)	*Taeniura lymma*	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6.26: Total and average number of marine fish (across all species) stocked per store visit and per store per major trading hub, respectively. *The standard deviation (SD) is presented for the average number of fish stocked per store per major trading hub (i.e. mean ± SD).*

Major trading hub	Pet store	Visit number	Total number of fish per store visit	Average number of fish stocked per store per major trading hub
Durban	A	i	149	62 ± 41,8
		ii	48	
		iii	35	
	B	i	97	
		ii	50	
		iii	14	
	C	i	45	
		ii	85	
		iii	30	
Johannesburg/ Pretoria	A	i	96	129 ± 40,9
		ii	102	
		iii	158	
	B	i	98	
		ii	198	
		iii	101	
	C	i	120	
		ii	188	
		iii	104	
Cape Town	A	i	52	55 ± 38.4
		ii	94	
		iii	141	
	B	i	26	
		ii	35	
		iii	38	
	C	i	30	
		ii	29	
		iii	48	

Table 6.27: The number of species per family, and the contribution of species per family (%), to the most dominant order (Perciformes) of marine fish traded in South African pet stores.

Family	Common family name	No. of species	Contribution of species per family (%)
Acanthuridae	Surgeonfish, Tangs, Unicornfish	33	15,8
Apogonidae	Cardinalfish	4	1,9
Balistidae	Triggerfish	9	4,3
Blenniidae	Blennies	9	4,3
Callionymidae	Dragonets	6	2,9
Chaetodontidae	Butterflyfish	19	9,1
Cirrhitidae	Hawkfish	3	1,4
Ephippidae	Batfish	1	0,5
Gobiidae	Gobies	18	8,6
Grammatidae	Basslets	1	0,5
Labridae	Wrasse	25	12,0
Lutjanidae	Snappers	1	0,5
Microdesmidae	Dartfish	3	1,4
Monodactylidae	Moonyfishes	1	0,5
Mullidae	Goatfish	1	0,5
Opistognathidae	Jawfish	1	0,5
Pholidichthyidae	Ray-finned fish	1	0,5
Plesiopidae	Longfins	1	0,5
Pomacanthidae	Angelfish	30	14,4
Pomacentridae	Clownfish & Damselfish	26	12,4
Pseudochromidae	Dottybacks	3	1,4
Scaridae	Parrotfish	2	1,0
Serranidae	Anthias & Bass	6	2,9
Siganidae	Foxfaces	4	1,9
Zanclidae	Moorish idol	1	0,5

Table 6.28: Marine aquarium fish traded in pet stores in South Africa showing their common names, distribution ranges, occurrence in South African waters, frequency of occurence (FOC) (%) in a pet store nationally, and the maximum number of stock per species counted. *Distribution ranges and information on whether a species is native to South Africa were derived from FishBase (Froese and Pauly 2002). Highlighted species are alien (i.e. not native) to South African waters. Common family names are in parentheses next to their respective scientific family name.*

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
Actinopterygii	Anguilliformes	Congridae (Garden eels)	*Heteroconger hassi*	Spotted garden eel	Indo-Pacific	No	7,4	3
		Muraenidae (Moray eels)	*Echidna nebulosa*	Snowflake eel, Starry eel	Indo-Pacific, Eastern Central Pacific	Yes	11,1	1
			Echidna polyzona	Barred moray eel	Indo-Pacific	Yes	7,4	1
			Gymnomuraena zebra	Zebra moray eel	Indo-Pacific, Eastern Central Pacific	Yes	3,7	1
			Gymnothorax favagineus	Laced eel, Tessalata eel	Indo-Pacific	Yes	3,7	2
			Gymnothorax steindachneri	Brown speckled eel, Steindachner's moray eel	Hawaii	No	7,4	1
	Lophiiformes	Antennariidae (Frogfish)	*Antennarius commerson*	Commerson's frogfish	Indo-Pacific, Eastern Pacific	Yes	3,7	1
	Perciformes	Acanthuridae (Surgeonfish, tangs, unicornfish)	*Acanthurus achilles*	Achilles tang	Western and Eastern Central Pacific	No	11,1	2
			Acanthurus blochii	Ringtail surgeonfish	Indo-Pacific	Yes	3,7	1
			Acanthurus chirurgus	**Doctorfish, Doctorfish tang**	Western and Eastern Atlantic	No	3,7	2
			Acanthurus coerulus	**Carribean yellow tang**	Western and Eastern Atlantic	No	7,4	2
			Acanthurus gahhm	Black surgeonfish	Red Sea, Gulf of Aden	No	3,7	1
			Acanthurus japonicus	Japanese surgeonfish, Powder brown tang	Indo-West Pacific	No	11,1	3
			Acanthurus leucosternon	Powder blue tang	Indian Ocean, Western Pacific	Yes	51,9	11
			Acanthurus lineatus	Striped surgeonfish, Clown surgeonfish, Pyjama tang	Indo-Pacific	Yes	18,5	3
			Acanthurus nigricauda	Epaulette surgeonfish	Indo-Pacific	Yes	7,4	1

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Acanthurus nigrofuscus	Brown tang, Spot-cheeked surgeonfish	Indo-Pacific	Yes	14,8	1
			Acanthurus olivaceus	Orange band surgeonfish, Orange shoulder surgeonfish	Pacific Ocean	No	25,9	2
			Acanthurus pyroferus	Mimic tang, Chocolate surgeonfish	Indo-Pacific	No	14,8	5
			Acanthurus tennenti	Doubleband surgeonfish, Lieutenant tang	Indian Ocean	Yes	18,5	2
			Acanthurus thompsoni	Thompson's surgeonfish	Indo-Pacific	Yes	7,4	1
			Acanthurus triostegus	Convict tang, Convict surgeonfish	Indo-Pacific, Eastern Pacific	Yes	29,6	5
			Acanthurus xanthopterus	Yellowfin surgeonfish, Cuvier's surgeonfish	Indo-Pacific, Eastern Pacific	Yes	7,4	1
			Ctenochaetus binotatus	Twospot surgeonfish, Blue eye tang	Indo-Pacific	Yes	11,1	1
			Ctenochaetus cyanocheilus	Shorttail bristletooth surgeonfish	Western Pacific	No	3,7	1
			Ctenochaetus hawaiiensis	Chevron tang	Pacific Ocean	No	3,7	1
			Ctenochaetus striatus	Striated surgeonfish, Striped bristletooth surgeonfish	Indo-Pacific	Yes	11,1	1
			Ctenochaetus strigosus	Kole tang	Hawaiian Islands, Johnston Island	No	7,4	3
			Naso brevirostris	Spotted unicornfish	Indo-Pacific, Galápagos Islands	Yes	3,7	3
			Naso caesius	Grey unicornfish	Pacific Ocean	No	3,7	9
			Naso elegans	Elegant unicornfish	Indian Ocean	Yes	11,1	4
			Naso lituratus	Orange spine surgeonfish, Lipstick tang, Barcheek unicornfish, Clown tang	Eastern Pacific	No	25,9	3
			Naso unicornis	Bluespine unicornfish, Shortnose unicornfish	Indo-Pacific	Yes	7,4	1
			Naso vlamingii	Bignose unicornfish, Scibbled unicornfish	Indo-pan-Pacific	Yes	7,4	2
			Paracanthurus hepatus	Regal tang, Palette surgeonfish, Hippo tang	Indo-Pacific	Yes	37,0	15
			Zebrasoma desjardinii	Red Sea sailfin tang, Desjardin's sailfin tang	Indian Ocean	Yes	11,1	2
			Zebrasoma flavescens	**Yellow tang**	Pacific Ocean	No	48,1	12

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Zebrasoma scopas	Scopas tang, Brown tang, Two-tone tang	Indo-Pacific	Yes	22,2	7
			Zebrasoma veliferum	Sailfin tang	Western Indian Ocean, Pacific Ocean	No	29,6	6
			Zebrasoma xanthurum	**Purple tang, Yellowtail tang**	Western Indian Ocean	No	22,2	1
		Apogonidae (Cardinalfish)	*Apogon compressus*	Yellowstriped cardinalfish, Orangelined cardinalfish, Blue eye cardinal	Indo-West Pacific	No	11,1	1
			Pterapogon kauderni	Banggai cardinalfish	Banggai Islands, Indonesia	No	33,3	23
			Sphaeramia nematoptera	Pajama cardinalfish	Indo-Pacific	No	25,9	19
			Zoramia leptacantha	Threadfin cardinalfish, Bluestreak cardinalfish	Indo-Pacific	No	3,7	8
		Balistidae (Triggerfish)	*Balistes capriscus*	Grey triggerfish	Eastern Atlantic, Western Atlantic	No	3,7	1
			Balistes punctatus	Bluespotted triggerfish	Eastern Atlantic	No	3,7	1
			Balistoides conspicillum	Clown triggerfish	Indo-Pacific	Yes	7,4	3
			Melichthys vidua	Pinktail triggerfish	Indo-Pacific	Yes	3,7	1
			Odonus niger	Redtoothed triggerfish, Niger triggerfish	Indo-Pacific	Yes	14,8	1
			Rhinecanthus aculeatus	Picasso triggerfish, Lagoon triggerfish, Blackbar triggerfish	Indo-Pacific, Eastern Atlantic	Yes	14,8	2
			Rhinecanthus rectangulus	Reef triggerfish, Rectangular triggerfish	Indo-Pacific	Yes	3,7	1
			Sufflamen chrysopterum	Half-moon triggerfish, Flagtail triggerfish	Indo-West Pacific	Yes	3,7	1
			Xanthichthys auromarginatus	Gilded triggerfish, Bluethroated triggerfish	Indo-Pacific	Yes	3,7	2
		Blenniidae (Blennies)	*Atrosalarias fuscus*	Black sailfin blenny	Indo-Pacific	No	3,7	2
			Ecsenius bicolor	Bicolor blenny, Flame tail blenny, Red tail blenny	Indo-Pacific	No	37,0	4
			Ecsenius lineatus	Dotdash blenny, Linear blenny	Indo-West Pacific	No	3,7	1
			Ecsenius lividanalis	Blue-head comb-tooth blenny, Yellow-tail midas blenny	Western Pacific	No	3,7	1
			Ecsenius midas	Midas blenny, Lyretail blenny, Golden blenny	Indo-Pacific	Yes	29,6	6

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Meiacanthus atrodorsalis	Forktail blenny	Western Pacific	No	3,7	2
			Meiacanthus mossambicus	Mozambique fang blenny	Western Indian Ocean	No	3,7	1
			Meiacanthus smithii	White blenny, Smith's blenny, Disco blenny	Indo-West Pacific	No	3,7	1
			Salarias fasciatus	Lawnmower blenny, Sailfin blenny, Algae blenny, Jeweled blenny	Indo-Pacific	No	37,0	7
		Callionymidae (Dragonets)	*Synchiropus marmoratus*	Marbled dragonet, Scorpion blenny	Western Indian Ocean	No	18,5	4
			Synchiropus ocellatus	Scooter blenny, Ocellated dragonet, Scooter dragonet	Pacific Ocean	No	14,8	4
			Synchiropus picturatus	Picturesque dragonet, Spotted mandarin	Indo-West Pacific	No	11,1	4
			Synchiropus splendidus	Mandarinfish, Mandarin dragonet	Western Pacific	No	33,3	5
			Synchiropus stellatus	Starry dragonet	Indian Ocean, around oceanic islands	Yes	7,4	2
			Synchiropus sycorax	Red ruby dragonet	Philippines	No	3,7	1
		Chaetodontidae (Butterflyfish)	*Chaetodon auriga*	Threadfin butterflyfish	Indo-Pacific	Yes	18,5	3
			Chaetodon chrysurus	Pearl scale butterfly	Indian Ocean	Yes	3,7	1
			Chaetodon guttatissimus	Peppered butterflyfish	Indian Ocean, Indonesia	Yes	7,4	1
			Chaetodon kleinii	Sunburst butterflyfish	Indo-Pacific, Eastern Pacific	Yes	11,1	2
			Chaetodon lunula	Raccoon butterflyfish	Indo-Pacific, Southeast Atlantic	Yes	7,4	1
			Chaetodon mertensii	Atoll butterflyfish, Yellowback butterflyfish	Pacific Ocean	No	3,7	1
			Chaetodontoplus mesoleucus	Singapore butterflyfish, Vermiculated angelfish	Indo-West Pacific	No	7,4	2
			Chaetodon meyeri	Scrawled butterflyfish	Indo-Pacific	Yes	3,7	3
			Chaetodon miliaris	Millet butterflyfish	Eastern Central Pacific	No	7,4	1
			Chaetodon plebius	Bluespot butterflyfish	Western Pacific	No	3,7	2
			Chaetodon unimaculatus	Teardrop butterflyfish	Indo-Pacific, Micronesia	Yes	7,4	1
			Chaetodon vagabundus	Vagabond butterflyfish	Indo-Pacific	Yes	25,9	4

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Chaetodon xanthocephalus	Yellowhead butterflyfish	Western Indian Ocean, Sri Lanka, Maldives	Yes	3,7	1
			Chaetodon xanthurus	Crosshatch butterflyfish, Redcheckered butterflyfish	Western Pacific	No	3,7	3
			Chelmon rostratus	Copperband butterflyfish	Western Pacific	No	40,7	7
			Forcipiger flavissimus	Yellow longnose butterflyfish, Forceps butterflyfish	Indo-Pacific, Micronesia, Eastern Pacific	Yes	37,0	4
			Hemitaurichthys polylepis	Pyramid butterflyfish	Pacific Ocean	No	14,8	3
			Hemitaurichthys zoster	Brownandwhite butterflyfish, Black pyramid butterflyfish	Indian Ocean	Yes	3,7	3
			Heniochus acuminatus	**Pennant coralfish, Longfin bannerfish, Coachman**	Indo-Pacific, Micronesia	Yes	7,4	2
		Cirrhitidae (Hawkfish)	*Cirrhitops fasciatus*	Redbarred hawkfish, Red bar hawkfish	Indo-Pacific	No	3,7	2
			Neocirrhites armatus	Flame hawkfish	Pacific Ocean	No	7,4	2
			Oxycirrhites typus	Longnose hawkfish	Indo-Pacific, Eastern Pacific	Yes	7,4	2
		Ephippidae (Batfish)	*Platax teira*	Longfin batfish	Indo-West Pacific	Yes	3,7	3
		Gobiidae (Gobies)	*Amblygobius decussatus*	Orange crosshatch goby	Western Central Pacific	No	3,7	2
			Amblyeleotris diagonalis	Diagonal shrimp goby	Indo-Pacific	No	7,4	5
			Amblyeleotris yanoi	Flagtail shrimp goby	Indonesia	No	3,7	1
			Cryptocentrus cinctus	Yellow prawn goby	Western Pacific	No	11,1	2
			Fusigobius neophytus	Fusi goby	Indo-Pacific	Yes	3,7	2
			Gobiodon okinawae	Yellow clown goby, Yellow coral goby	Western Pacific	No	7,4	2
			Koumansetta rainfordi	Rainford's goby, Court jester goby	Western Pacific	No	3,7	1
			Nemateleotris decora	Elegant firefish, Purple firefish	Indo-Pacific	No	11,1	5
			Nemateleotris exquisita	Exquisite firefish	Indian Ocean	Yes	3,7	1
			Nemateleotris magnifica	Fire fish, Fire goby	Indo-Pacific, Micronesia	Yes	14,8	5
			Pomatoschistus minutus	Sand goby, Sand milk goby	Eastern Atlantic, Mediterranean, Black Sea	No	18,5	3
			Priolepis nocturna	Blackbarred reefgoby, Zebra goby	Indo-Pacific	No	3,7	1

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Signigobius biocellatus	Twospot goby, Crab-eyed goby	Western Pacific	No	3,7	1
			Stonogobiops xanthorhinica	Yellownoseprawn goby	Western Pacific	No	3,7	2
			Valenciennea helsdingeni	Sleeper railway glider goby, Two stripe goby	Indo-West Pacific	Yes	3,7	5
			Valenciennea puellaris	Diamond watchman goby, Orangespotted sleepergoby	Indo-Pacific	No	37,0	9
			Valenciennea sexguttata	Chalk goby, Six spotted sleeper goby, Sleeper blue dot goby	Indo-Pacific	No	11,1	3
			Valenciennea strigata	Bluestreak goby, Goldenhead sleeper goby, Pennant glider	Indo-Pacific	Yes	22,2	3
		Grammatidae (Basslets)	*Gramma loreto*	Royal gramma	Western Central Atlantic	No	18,5	3
		Labridae (Wrasse)	*Cirrhilabrus aurantidorsalis*	Orangeback fairy wrasse	Indonesia	No	3,7	4
			Cirrhilabrus exquisitus	Exquisite wrasse, Exquisite fairy wrasse	Indo-Pacific	Yes	22,2	3
			Cirrhilabrus lubbocki	Lubbock's fairy wrasse	Western Central Pacific	No	7,4	2
			Cirrhilabrus rubriventralis	Social wrasse, Social fairy wrasse	Western Indian Ocean	No	18,5	8
			Coris formosa	Queen coris, Red wrasse	Western Indian Ocean	Yes	3,7	1
			Halichoeres chloropterus	Pastelgreen wrasse, Green wrasse	IndoMalaysia	No	7,4	2
			Halichoeres chrysus	Canary wrasse, Golden wrasse, Yellow wrasse	Eastern Indian Ocean, Tonga	No	29,6	7
			Halichoeres cosmetus	Adorned wrasse	Western Indian Ocean	Yes	7,4	2
			Halichoeres hortulanus	Marble wrasse, Green marble wrasse	Indo-Pacific	Yes	3,7	1
			Halichoeres marginatus	Dusky wrasse	Indo-Pacific	No	11,1	2
			Halichoeres timorensis	Timor wrasse	Sri Lanka, Indonesia	No	3,7	2
			Halichoeres trispilus	Triplespot wrasse	Western Indian Ocean	Yes	3,7	3
			Labroides dimidiatus	Bluestreak cleaner wrasse	Indo-Pacific	Yes	63,0	26
			Macropharyngodon bipartitus	Divided leopard wrasse	Western Indian Ocean	Yes	3,7	2
			Macropharyngodon negrosensis	Black leopard wrasse, Yellowspotted wrasse	Eastern Indian Ocean, Western Pacific	No	3,7	2

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Macropharyngodon ornatus	Ornate leopard wrasse, False leopard	Indo-Pacific	No	3,7	2
			Novaculichthys taeniourus	Rockmover wrasse, Dragon wrasse	Indo-Pacific, Eastern Pacific	Yes	7,4	1
			Paracheilinus carpenteri	Carpenter's flasher wrasse	Western Pacific, Tonga	No	7,4	1
			Paracheilinus cyaneus	Blue flasher wrasse	Western Central Pacific	No	3,7	3
			Paracheilinus filamentosus	Filamented flasher wrasse	Indo-Pacific	No	3,7	3
			Paracheilinus lineopunctatus	Spotlined flasher	Philippines	No	3,7	3
			Pseudocheilinus hexataenia	Sixline wrasse	Indo-Pacific	Yes	22,2	7
			Thalassoma hardwicke	Sixbar wrasse, Sixbanded wrasse	Indo-Pacific	Yes	3,7	1
			Thalassoma lunare	Moon wrasse, Green moon wrasse, Lunar wrasse	Indo-Pacific	Yes	11,1	1
			Wetmorella albofasciata	White banded possum wrasse	Indo-Pacific	No	3,7	1
		Lutjanidae (Snappers)	*Lutjanus sebae*	Red emperor, Red snapper	Indo-West Pacific	Yes	3,7	1
		Microdesmidae (Dartfish)	*Ptereleotris evides*	Blackfin dartfish, Scissortail dartfish	Indo-Pacific	Yes	11,1	3
			Ptereleotris heteroptera	Blacktail goby, Indigo dartfish , Spot-tail dartfish	Indo-Pacific	Yes	3,7	3
			Ptereleotris monoptera	Lyretail dartgoby, Blue goby	Indo-Pacific	No	3,7	1
		Monodactylidae (Moonyfishes)	*Monodactylus argenteus*	Silver moonyfish, Silver moony, Butter bream, Diamondfish	Indo-West Pacific	Yes	18,5	5
		Mullidae (Goatfish)	*Parupeneus barberinus*	Dashanddot goatfish	Indo-Pacific	Yes	3,7	2
		Opistognathidae (Jawfish)	*Opistognathus aurifrons*	Yellowhead jawfish	Western Central Atlantic	No	14,8	2
		Pholidichthyidae (Ray-finned fish)	*Pholidichthys leucotaenia*	Engineer blenny, Convict blenny, Zebra blenny	Western Central Pacific	No	7,4	1
		Plesiopidae (Longfins)	*Calloplesiops altivelis*	Comet, Marine betta	Indo-Pacific	No	3,7	3
		Pomacanthidae (Angelfish)	*Apolemichthys trimaculatus*	Flagfin angelfish	Indo-West Pacific	Yes	3,7	2
			Apolemichthys xanthurus	Cream angelfish, Yellowtail angelfish	Western Indian Ocean	No	11,1	2
			Centropyge acanthops	African pygmy angelfish, African Cherubfish, Jumping bean	Western Indian Ocean	Yes	7,4	3

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Centropyge argi	Cherubfish, Cherub angel	Western Atlantic	No	3,7	2
			Centropyge aurantia	Golden pygmy angelfish, Velvet dwarf angel, Golden angelfish	Western Pacific	No	3,7	1
			Centropyge bicolor	Bicolor angelfish	Indo-Pacific, Micronesia	Yes	18,5	2
			Centropyge bispinosa	Coral beauty, Twospined angelfish	Indo-Pacific	Yes	33,3	6
			Centropyge eibli	Blacktail angelfish, Red stripe angelfish, Orangelined angelfish, Eibl's angelfish	Eastern Indian Ocean	No	25,9	2
			Centropyge ferrugata	Rusty angelfish	Western Pacific	No	3,7	2
			Centropyge flavicauda	Pygmy yellowtail angelfish, White tailed pygmy angelfish	Indo-West Pacific	Yes	7,4	1
			Centropyge flavipectoralis	Yellowfin angelfish, Moonbeam angelfish	Maldives, Sri Lanka	No	14,8	2
			Centropyge flavissima	Lemonpeel angelfish	Western Central Pacific	No	11,1	1
			Centropyge heraldi	False lemonpeel angelfish, Herald's angelfish, Yellow angelfish	Indo-Pacific Ocean	No	3,7	2
			Centropyge loricula	Flame angel	Pacific Ocean	No	14,8	3
			Centropyge multispinis	Bluefin dwarf, Brown pygmy angelfish, Dusky cherub, Multispined angelfish	Indo-West Pacific	Yes	14,8	2
			Centropyge tibicen	Keyhole angelfish, Puller angelfish	Western Pacific, Eastern Indian Ocean	No	3,7	1
			Centropyge vroliki	Pearlscale angelfish, Half black angelfish	Western Pacific, Eastern Indian Ocean	No	18,5	2
			Genicanthus lamarck	Blackstriped angelfish, Lamarck's angelfish	Indo-West Pacific	No	14,8	3
			Genicanthus melanospilos	Spotbreast angelfish, Swallowtail angelfish	Western Pacific, Eastern Indian Ocean	No	18,5	3
			Genicanthus semifasciatus	Masked swallowtail angelfish	Western Pacific	No	7,4	2
			Genicanthus watanabei	Blackedged angelfish, Watanabei angelfish	Pacific Ocean	No	3,7	1
			Holacanthus ciliaris	Queen angelfish	Western Atlantic, Eastern Central Atlantic	No	3,7	1
			Holacanthus passer	King angelfish, Passer angelfish	Eastern Pacific	No	7,4	2

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Holacanthus tricolor	Rock beauty	Western Atlantic	No	3,7	1
			Pomacanthus asfur	Arabian angelfish	Western Indian Ocean	No	3,7	1
			Pomacanthus imperator	Emperor angelfish	Indo-Pacific	Yes	37,0	4
			Pomacanthus navarchus	Bluegirdled angelfish, Majestic angelfish	Indo-Pacific	No	14,8	1
			Pomacanthus semicirculatus	Koran angelfish, Semicircle angelfish	Indo-West Pacific	Yes	3,7	1
			Pomacanthus xanthometopon	Blueface angelfish, Yellowface angelfish	Indo-Pacific	No	7,4	1
			Pygoplites diacanthus	Regal angelfish, Royal angelfish	Indo-Pacific	Yes	3,7	1
		Pomacentridae (Clownfish & Damselfish)	*Amphiprion barberi*	Barberi clownfish, Fiji clownfish	Fiji, Tonga, American Samoa	No	7,4	25
			Amphiprion bicinctus	Red sea clownfish, Twobanded anemonefish	Red Sea, Chagos Archipelago	No	3,7	1
			Amphiprion clarkii	Clark's anemonefish, Yellowtail clownfish	Indo-West Pacific	No	22,2	2
			Amphiprion frenatus	Tomato clownfish	Western Pacific	No	14,8	24
			Amphiprion melanopus	Cinnamon clownfish, Blackbacked anemonefish, Dusky anemonefish	Pacific Ocean	No	3,7	2
			Amphiprion nigripes	Rose skunk clownfish, Maldive anemonefish	Maldives, Sri Lanka	No	3,7	4
			Amphiprion ocellaris	Ocellaris clownfish, Common clownfish	Indo-West Pacific	No	40,7	34
			Amphiprion percula	Orange clownfish	Western Pacific	No	22,2	13
			Amphiprion perideraion	Pink skunk clownfish, Pink anemonefish	Western Pacific	No	22,2	2
			Amphiprion polymnus	Saddleback clownfish	Western Pacific	No	7,4	18
			Premnas biaculeatus	Maroon clownfish	Indo-West Pacific	No	7,4	4
			Abudefduf saxatilis	Sergeantmajor	Atlantic Ocean	No	11,1	6
			Chromis dimidiata	Chocolatedip chromis, Bicolor damselfish	Red Sea	No	11,1	6
			Chromis viridis	Bluegreen chromis	Indo-Pacific	No	48,1	38
			Chrysiptera hemicyanea	Azure damselfish, Halfblue demoiselle, Yellowdipped damsel	Indo-West Pacific	No	14,8	15

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Chrysiptera parasema	Yellowtail damselfish, Yellowtail blue damsel	Western Pacific	No	7,4	2
			Chrysiptera springeri	Blue sapphire damsel, Springer's demoiselle	Western Pacific	No	7,4	12
			Dascyllus carneus	Cloudy dascyllus, Indian dascyllus	Indian Ocean	Yes	3,7	7
			Dascyllus melanurus	Four stripe damselfish	Western Pacific	No	7,4	5
			Dascyllus trimaculatus	Threespot dascyllus, Domino damsel, Domino	Indo-Pacific	Yes	14,8	4
			Neoglyphidodon oxyodon	Neon velvet damselfish	Western Central Pacific	No	3,7	3
			Neopomacentrus azysron	Rocket tail damsel	Indo-West Pacific	No	3,7	16
			Neopomacentrus cyanomos	Regal demoiselle	Indo-West Pacific	Yes	3,7	2
			Pomacentrus auriventris	Yellowbelly damsel, Goldbelly damsel	Western Central Pacific	No	3,7	4
			Pomacentrus caeruleus	Blue damsel, Caerulean damsel	Western Indian Ocean	Yes	7,4	3
			Pomacentrus similis	Similar damsel	Indian Ocean	No	3,7	23
		Pseudochromidae (Dottybacks)	*Pictichromis diadema*	Bicolor dottyback, Diadem dottyback, Purpletop dottyback	Western Central Pacific	No	7,4	3
			Pictichromis dinar	Royal dinar dottyback, Blue eye royal dottyback	Western Pacific	No	3,7	8
			Pictichromis paccagnellae	Royal dottyback, Bicolor dottyback, False gramma	Western Pacific	No	7,4	2
		Scaridae (Parrotfish)	*Cetoscarus bicolor*	Bicolour parrotfish, Bumphead parrotfish	Western Indian Ocean	No	3,7	1
			Scarus taeniopterus	Princess parrotfish	Western Atlantic	No	3,7	1
		Serranidae (Anthias & Bass)	*Nemanthias carberryi*	Threadfin anthias	Western Indian Ocean	Yes	3,7	2
			Pseudanthias bicolor	Bicolor anthias	Indo-Pacific, Micronesia	No	3,7	1
			Pseudanthias dispar	Dispar anthias	Pacific Ocean	No	14,8	7
			Pseudanthias kashiwae	Red fairy anthias	Indo-Pacific	Yes	7,4	6
			Pseudanthias squamipinnis	Sea goldie, Lyretail coralfish	Indo-West Pacific	Yes	22,2	19
			Serranus tortugarum	Chalk bass	Western Atlantic	No	3,7	4
		Siganidae (Foxfaces)	*Siganus magnificus*	Magnificent rabbitfish, Magnificent foxface	Eastern Indian Ocean	No	3,7	3

Class	Order	Family	Species	Common name	Distribution	Native to South Africa?	FOC (%)	Maximum stock count
			Siganus virgatus	Two barred rabbitfish, Barhead spinefoot	Indo-West Pacific	No	7,4	6
			Siganus vulpinus	Foxface rabbitfish, Foxface lo	Western Pacific	No	22,2	5
			Siganus uspi	Bicolored foxface	Fiji	No	3,7	1
		Zanclidae (Moorish idol)	*Zanclus cornutus*	Moorish idol	Indo-Pacific, Eastern Pacific	Yes	25,9	7
	Scorpaeniformes	Dactylopteridae (Flying gurnards)	*Dactyloptena orientalis*	Oriental flying gurnard	Indo-Pacific	Yes	3,7	1
		Scorpaenidae (Scorpionfish)	*Dendrochirus biocellatus*	Twospot turkeyfish, Twinspot lionfish	Indo-Pacific, Japan	No	3,7	2
			Pterois antennata	Broadbared lionfish, Banded lionfish	Indo-Pacific	Yes	14,8	2
	Syngnathiformes	Syngnathidae (Pipefish & Seahorses)	*Doryrhamphus excisus*	Bluestriped pipefish	Indo-Pacific, Eastern Pacific	Yes	3,7	3
			Hippocampus sp.	Unidentified	-	-	3,7	3
	Tetraodontiformes	Monacanthidae (Filefish)	*Acreichthys tomentosus*	Aiptasiaeating file fish	Indo-West Pacific	No	18,5	13
			Chaetodermis penicilligerus	Prickly leatherjacket, Tasselled leatherjacket	Indo-West Pacific	No	11,1	1
		Tetraodontidae (Pufferfish)	*Arothron hispidus*	Whitespotted puffer	Indo-Pacific, Eastern Pacific	Yes	7,4	2
			Arothron nigropunctatus	Dogfaced puffer, Blackspotted puffer	Indo-Pacific	Yes	3,7	1
			Canthigaster solandri	Spotted sharpnose, Falseeye toby, Jewel pufferfish	Indo-Pacific, Micronesia	Yes	3,7	1
			Canthigaster valentini	Valentin's sharpnose puffer, Saddled puffer	Indo-Pacific	Yes	7,4	3
Elasmobranchii	Myliobatiformes	Dasyatidae (Whiptail stingrays)	*Taeniura lymma*	Bluespotted ribbontail ray	Indo-West Pacific	Yes	3,7	1