THE TAXONOMY, BIODIVERSITY, AND EVOLUTIONARY HISTORY OF BLACK CORALS (ORDER ANTIPATHARIA)

THESIS BY
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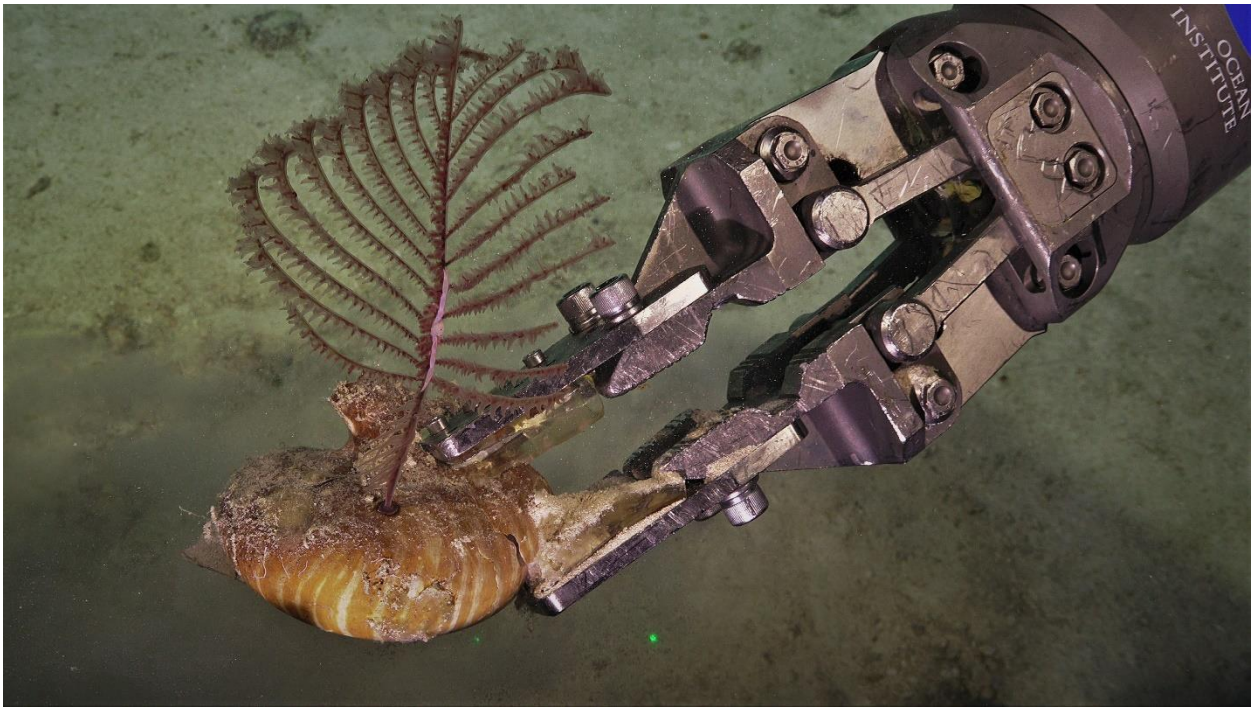

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This thesis contains images in the form of in-situ, lab, and scanning electron microscopy. Some images were taken by Erika Gress, Aabha Sant and Savannah Hesidence and their permission was provided for their images to be used in this thesis. Dr. Robin Beaman produced the map presented in Chapter 4, Figure 4. Lauriane Baraf drew Figure 2 in Chapter 1. Every reasonable effort has been made to obtain permission acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has omitted or was incorrectly acknowledged.

Abstract

Anthropogenic activities are threatening biodiversity on a global scale. Mitigating these threats is difficult because we lack fundamental knowledge on the number of valid species, their distributions, and systematic relationships between species. Overcoming these knowledge gaps requires identification of the ecological and evolutionary processes that produce and maintain biodiversity patterns, which can ultimately inform conservation actions that protect these processes. Emerging technologies are providing biodiversity information through an improved ability to collect specimens in remote areas (e.g., remotely operated vehicles to sample the deep sea), image specimens (e.g., deep sea cameras and advances in Scanning Electron Microscopy), and depict molecular relationships between closely related species (e.g., genome-scale next-generation sequencing), thus providing an opportunity to update the taxonomy of life on earth. Most of our understanding of marine biodiversity is derived from more accessible habitats within 20 meters of the ocean surface. This is problematic because the first 20 meters of depth represents ~1% of the marine environment. Therefore, more research is required to understand biodiversity groups that occur in the other 99% of the world's oceans.

The order Antipatharia (Cnidaria: Anthozoa), also known as black corals, is an anthozoan lineage in the Hexacorallia that consists of > 75% of species occurring below 50 meters depth. Black corals have ecological importance because they provide habitat to diverse invertebrates from just below the surface to over 8,000 meters depth. Black corals also have cultural and economic importance because they are thought to ward off evil spirits and disease, and therefore are harvested and sold around the world. The group is understudied due to a limited number of taxonomic experts, lack of informative morphological features to delineate species, genera, and families, and a lack of phylogenetic resolution among commonly used molecular markers. Black corals are especially understudied in the Great Barrier Reef (GBR) and Coral Sea, simply because most black coral taxonomists live and work in Europe and the United States. As a result, few species are recorded as occurring in Australia in global biodiversity databases (e.g., Atlas of Living Australia) despite hundreds of black coral specimens in the collections of Australian Museums. The next step is to build upon and make use of these collections to improve our understanding of black corals. My PhD aimed to address the knowledge gaps pertaining to

the taxonomy, taxonomic diversity, molecular systematics, and evolutionary history of black corals. Specifically, I collected and sequenced black corals to better understand how many black coral species occur in the Great Barrier Reef and Coral Sea, which represents the largest coral reef system in the world and where the taxonomic diversity of black corals is generally unknown, and used morphological and molecular data to revise the taxonomy of black corals and to better understand their evolutionary history.

The descriptions of species, genera, and families within this thesis are **informal**, and holotype designations and etymologies are purposefully **excluded** to avoid *nomen nudum* designations, as per the International Code of Zoological Nomenclature, Article 13 (Ferraris & Eschmeyer 2000). The chapters are not verbatim reproduced versions of papers that have been published, or are currently in review for publication that include formal descriptions and/or amendments to the taxonomy of the Antipatharia.

This thesis consists of four data chapters (Chapters 2–5) that follow a natural progression of basic scientific discovery. In **Chapter 2**, I thoroughly examined the largest unidentified collection of black corals from the deep Coral Sea to address taxonomic and biodiversity knowledge gaps in black corals in the region. The specimens examined were collected during the CIDARIS project between 1986 and 1992 and accessioned into the collections of the Museum of Tropical Queensland (MTQ). Specifically, I examined the morphological characteristics of 21 specimens that were collected at depths between ~1,000 and ~2,500 m, compared these features to the literature to identify the specimens to the species level, and created a growth profile for 13 specimens that represented two closely related accepted species (*Bathypathes patula* Brook, 1889 and *B. seculata* Opresko, 2005). I identified five species from five genera and increased the number of known genera in the region below 700 meters from one to six. Additionally, the growth profile between the two species led to the discovery that *B. seculata* is the juvenile stage of *B. patula*, leading to the synonymization of *B. seculata*.

In addition to examining material from the MTQ, I visited and collected tissue samples and contacted museum curators to arrange for the donation of over 200 tissue samples from museums both in Australia (Australian Museum, South Australian Museum, Queensland Museum), and internationally (Royal Belgian Institute of Natural Sciences,

Belgium; National Institute of Water and Atmospheric Research, New Zealand; Smithsonian Institution and California Academy of Sciences, USA; University of Genova, Italy). In addition, I collected over 50 black corals on SCUBA diving expeditions along the length of the GBR and at one site in Kimbe Bay, Papua New Guinea, and collected over 50 black corals from remotely operated vehicle expeditions in the deep GBR and Coral Sea. Many of these ~300 samples are analysed in Chapters 3, 4, and 5.

In Chapter 3, I used Papua New Guinea and Great Barrier Reef black coral specimens and targeted enrichment of Ultraconserved element (hereafter "UCEs") and exonic loci sequence data to address knowledge gaps pertaining to the taxonomy and phylogenetics of black corals. UCEs are 100s to 1,000s of regions of the genome that evolve so slowly over evolutionary timescales that matching UCE regions can be aligned across highly divergent taxa. Specifically, 31 specimens were sequenced using target capture protocols. These 31 samples represent five out of the seven valid black coral families, including specimens that I collected from the Great Barrier Reef and Papua New Guinea. This chapter provided morphological and molecular (conserved element and exonic loci) evidence to support the description of *Blastopathes medusa*, a new species and genus. I also compared targeted capture sequencing methods with commonly used mitochondrial intergenic region nad5-IGR-nad1 to demonstrate that UCEs and exons provide more informative phylogenies than mitochondrial markers, which is required to address knowledge gaps outlined in my thesis (Chapter 1).

In **Chapter 4,** I used morphological and genetic data of 34 black corals from the GBR and Coral Sea and 46 black corals from elsewhere in the world to address knowledge gaps related to the taxonomy and biodiversity of GBR and Coral Sea black corals. Specifically, GBR and Coral Sea specimens were described morphologically and identified to the species level to increase the number of species known from the region from seven to 24. These specimens were compared using an integrated approach using both morphology and molecular phylogenomics based on a maximum likelihood UCE/exon phylogeny of all 80 black corals samples, which will be used as the basis for the largest taxonomic revision for the Order in over 15 years. Based on my results, I provide evidence to support the description of five new species, two new genera and two new families, increasing the total

number of families in the Antipatharia from seven to nine. While the data to support these decisions is presented and discussed, as with previous chapters, this thesis chapter does not include the formal descriptions of the new species, genera or families due to complications that could arise by placing these descriptions in a thesis rather than a taxonomic journal with respect to the rules outlined in the International Code on Zoological Nomenclature (ICZN). The manuscript, which will formalize these descriptions in accordance with the rules of ICZN will be submitted shortly.

While in **Chapters 2–4** I address taxonomic and biodiversity knowledge gaps, the evolutionary history of black corals is generally unknown. Therefore, In **Chapter 5,** I combined a time-calibrated phylogeny, morphological, and bathymetric data in a comparative framework to establish and explore the evolutionary history of black corals from their origins 443 million years ago. The results highlight the events that led to the wide bathymetric range of black corals, beginning with an invasion from continental slope depths (250 to 1,999 m) onto the continental shelf (1 to 249 m) in the aftermath of the Permian-Triassic mass extinction event. All subsequent transitions are offshore, including four independent slope lineages in the last 30 My that have invaded abyssal depths (2,000 to >8,000 m). This very recent expansion into the abyss follows the evolution of pinnules that likely aided these lineages to persist when nutrition levels were low and invade deeper habitats with lower nutrition availability.

This thesis demonstrates that morphological and next-generation molecular sequencing approaches provide multiple lines of evidence to address long-standing and fundamental knowledge gaps pertaining to the taxonomy, biodiversity, and evolutionary history of black corals. I have discovered that the order Antipatharia contains greater taxonomic diversity than previously thought, particularly in the Great Barrier Reef and Coral Sea where the number of species known to occur in the region is increased from one to the 21 species that I identified in this thesis. I also provide evidence for the description of six new species, three new genera and two new families. This demonstrated the breath of systematic revisions that are required to update the taxonomy of the Order Antipatharia. Using advanced phylogenomic techniques and comparative methods I highlight the evolutionary origins and bathymetric evolution of the group.

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Chapter 1 General introduction

1.1 Biodiversity Shortfalls

Anthropogenic activities are threatening biodiversity on a global scale (Pelletier & Coltman 2018). This is true even in the most distant and remote habitats on earth, including the deepest oceans (Jamieson *et al.* 2017) and the tallest mountain ranges (Zhang *et al.* 2018b). Effects of anthropogenic activities are expected to increase with increasing human population size (McKee *et al.* 2004), warranting intervention (e.g., establishment of marine reserves and laws pertaining to conservation of biodiversity) in threatened habitats both accessible and remote. Unfortunately, conservation resources such as time, money, and personnel are limited. To protect biodiversity most effectively, conservation resources need to be allocated towards the right types of interventions in the right locations, thereby making the greatest possible difference to conservation outcomes (Horowitz *et al.* 2018b; Pressey *et al.* 2015). Our ability to make informed conservation decisions is underpinned by our knowledge of different facets of biodiversity (Margules *et al.* 2002).

Biodiversity can be considered in many ways; however, to depict the full breadth of biodiversity, features from genes to communities should be considered (Margules *et al.* 2002), including species with deficient data, and undescribed species (Trindade-Filho *et al.* 2012). This includes knowledge about regional species richness, species distributions, and molecular relationships between species. There are three "biodiversity shortfalls" that are detailed in the literature relating to the features described above:

1) The '*Linnean shortfall*' that refers to our lack of knowledge pertaining to how many species currently exist on Earth (Brown & Lomolino 1998);

2) The '*Wallacean shortfall*' that refers to our lack of knowledge about the distributions of most species (Lomolino 2011); and,

3) The '*Darwinian shortfall*' that refers to our lack of knowledge about species' evolutionary histories and molecular relationships between them (Díaz *et al.* 2013).

Numerous comprehensive reviews (Cardoso *et al.* 2011; Hortal *et al.* 2015) demonstrate that these three biodiversity shortfalls are interconnected and need to be dealt with in conjunction. For example, the Darwinian shortfall is related to the Linnean

shortfall because if some species are unknown and excluded from genetic analyses, a complete and accurate phylogeny cannot be reconstructed (Hortal *et al.* 2015).

Biodiversity knowledge is incomplete because we have not yet sufficiently surveyed many of Earth's habitats. This is especially true for the marine realm where sampling effort is biased towards accessible and shallow habitats with charismatic, coral reef-associated species (Grand *et al.* 2007; Hortal *et al.* 2015; Ribeiro *et al.* 2016; Triantis *et al.* 2012; Vale & Jenkins 2012). This bias can be attributed to lower costs, simpler logistics, and because these types of surveys garner greater interest among the general public (Brandt *et al.* 2016; Bridge *et al.* 2013; Hendriks *et al.* 2006). However, the first 20 meters of depth represents only 1% of the seafloor (Figure 1.1). Many marine species occur in deeper habitats (Bridge *et al.* 2013) that fill different and important ecological roles that contribute to sustaining overall biodiversity (Cathalot *et al.* 2015; Hourigan *et al.* 2017). Consequently, we still lack knowledge of the taxonomy and biodiversity of many marine groups, especially those that are most taxonomically diverse in deeper habitats, like black corals.

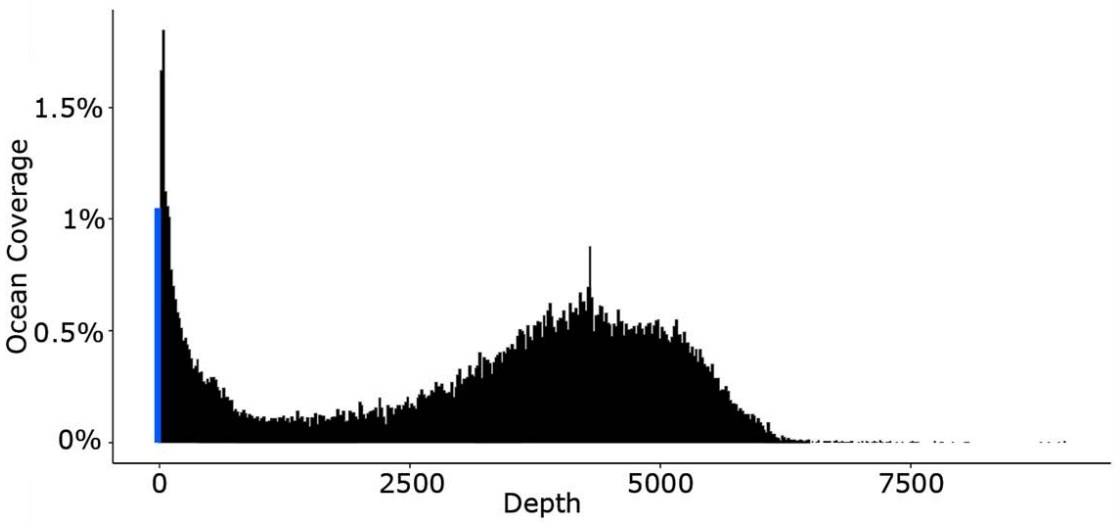


Figure 1.1 Proportion of benthic ocean habitats per depth. Each bar represents 20 meters. Blue bar represents 0 to 20 meters depth.

Black corals (Order Antipatharia) are a group of hexacoral anthozoans in the phylum Cnidaria found in all oceans, latitudes, and most depths from 2 m to 8,600 m (Wagner *et al.* 2012). Black corals have skeletons made up of chitin and scleroproteins, have polyps (feeding mouths) with six tentacles, and small (< 1 mm tall) spines that occur along the

skeleton (Bo *et al.* 2012b). Black corals are important to understand and protect because they provide habitat for other fauna (Bo *et al.* 2012b; Sánchez 1999; Wagner *et al.* 2012). For example, an individual black coral colony at 105 m depth in Southern California was found to host 2,554 invertebrates (Love *et al.* 2007). Associated fauna, including damselfish and gobies, lay their eggs between skeletal spines of black corals, making them an important resource for fish reproduction (Suarez *et al.* 2015; Wagner *et al.* 2012). Black corals also have economic and cultural importance because they are thought to combat disease and evil spirits, and are consequently harvested as jewellery all over the world (Wagner *et al.* 2012). Little is known about black corals because most of the 45 genera and ~296 currently accepted and nominal species (Molodtsova & Opresko 2022) occur in depths greater than 50 meters (Cairns 2007; Opresko 2019; Wagner *et al.* 2012) and are therefore logistically challenging to collect. There are also a limited number of taxonomic experts that work on black corals and a lack of informative morphological features to differentiate species, while many commonly used molecular markers (e.g. COX3, COX1, ND4) are not capable of resolving genus and species-level taxonomic relationships (Brugler *et al.* 2013; Horowitz *et al.* 2020 [Chapter 3 in this thesis]). These factors have resulted in the group being relatively understudied, especially when compared to their close relatives- shallow water hard corals (Scleractinia). These challenges confound knowledge pertaining to the taxonomy and biodiversity of the group (Linnean shortfall), geographic ranges of species (Wallacean shortfall), and molecular relationships between species and their evolutionary history (Darwinian shortfall). This PhD thesis aimed to overcome these shortfalls in parallel to provide much needed biodiversity data for conservation efforts.

1.2 History of black coral taxonomy

Black coral taxonomy has traditionally been based on morphology: considering a species as a group of individuals that have at least one homologous morphological feature that unites them, and differences within a morphological feature to define boundaries between species, genera, or families. This taxonomic approach (the phenetic species concept) was adopted because for much of the past 250 years since the publication of Linneaus' *Systema Naturae* (1758), morphology was the only line of evidence available to make taxonomic decisions. In other coral groups, reproduction has been used as a line of

evidence (Ramírez-Portilla *et al.* 2022); however, there was, and still is little known about black coral reproduction. Additionally, most black coral species were described before the advent of DNA sequencing and molecular phylogenetics, which are requisite for making substantial taxonomic decisions. The morphological approach to taxonomy is retrospectively problematic because it is not possible to differentiate homologous traits that were passed down from a common ancestor (a feature that should define taxonomic groups given that taxonomy should reflect systematic relationships) from analogous traits that result from convergent evolution and do not reflect systematic relationships. For example, three genera have been created for unbranched "whip-like" species: *Stichopathes* Brook, 1889, *Cirrhipathes* de Blainville 1830, and *Pseudocirrhipathes* Bo et al., 2009. The informative features that separate the genera are the polyp characteristics (*Stichopathes* has one row of polyps, *Cirrhipathes* as multiple rows of polyps, and *Pseudocirrhipathes* has irregularly arranged polyps on one side of the stem) (Bo *et al.* 2009). However, based on numerous molecular studies, the unbranched morphology has independently evolved more than three times over evolutionary history, with *Stichopathes* and *Cirrhipathes* being polyphyletic (Barrett *et al.* 2020; Bo *et al.* 2012a; Brugler *et al.* 2013; Lapian *et al.* 2007; Terrana *et al.* 2021). Polyphyly is also an issue for six out of the seven valid black coral families (Bo *et al.* 2012a; Gress *et al.* 2020; Horowitz *et al.* 2020 [Chapter 3 in this thesis]; Macisaac *et al.* 2013; Opresko *et al.* 2020), which suggests that taxonomic reviews and associated revisions are required.

In the last 10 years, with the incorporation of molecular evidence, the approach to black coral taxonomy has adapted from the phenetic species concept to a unified species concept. The unified species concept dictates that species are separately evolving metapopulation lineages and considers properties from previous species concepts (e.g., morphologically distinguishable, or ecologically divergent) as conditional (de Queiroz 2007). Studies over the past 10 years have repeatedly demonstrated that the taxonomy needs to be revised with molecular considerations (Bo *et al.* 2012a; Gress *et al.* 2020; Horowitz *et al.* 2020 [Chapter 3 in this thesis]; Macisaac *et al.* 2013; Opresko *et al.* 2020); however, a formal taxonomic review of black corals is not possible with present-day evidence for the following reasons:

1) Many species remain undiscovered (Danovaro *et al.* 2010) and many species have not yet been sequenced. This is because many habitats and regions around the world have yet to be surveyed with a focus on black corals and extracting DNA is challenging for black corals due to inhibitors that interfere with downstream PCR reactions (Quattrini *et al.* 2020).

2) It is difficult, expensive, and time-consuming to arrange deep-sea expeditions with the explicit purpose of collecting black coral species (most of which occur in the deep) for DNA sequencing, because the ranges of most species are unknown (Molodtsova *et al.* 2022).

3) The commonly used and readily available molecular markers for black corals are mitochondrial genes that have 2.3 times slower evolutionary rates compared to octocorals, and many other anthozoans (Brugler *et al.* 2013). Some potential reasons for low evolution rates include low mutation rates and high levels of mtDNA repair, molecular convergence, and historical bottlenecks (McFadden *et al.* 2021; Shearer *et al.* 2002); however, these theories remain to be tested. Slow evolution results in species with different morphological features after vicariance events; however, their mtDNA has not yet evolved for some closely related species, resulting in undifferentiated relationships in phylogenetic trees (Bo *et al.* 2012a; Brugler *et al.* 2013; Horowitz *et al.* 2020 [Chapter 3 in this thesis]).

Deep-sea expeditions (dredging, trawling, or use of remotely operated vehicles) are required to collect deep-sea specimens. However, these expeditions take years to plan, require many personnel to oversee the ship and research operations, and are expensive to conduct. An alternative to deep-sea expeditions is to utilize already collected specimens housed in museum collections (Ponder *et al.* 2001). Museum collections have been used to describe new species (Healy 2021), update knowledge about species ranges (Drinkrow & Cherry 1995; Skelton *et al.* 1995; Väisänen *et al.* 1994), and to inform conservation actions (Prendergast *et al.* 1993; Vane-Wright *et al.* 1994). Museum collections can also be used as a "checklist" of species that occur in a region. For example, the National Institute of Water and Atmospheric Research in New Zealand contains the largest and most taxonomically

diverse collection of black coral specimens from New Zealand waters (Tracey & Hjorvarsdottir 2019). Therefore, identifying specimens from museum collections can be used to calculate regional biodiversity. If a museum has low representation of a certain coral group from a region, or if the collection consists of specimens that were not preserved with downstream DNA extractions in mind, systematic expeditions can be planned to build up the collection to produce these regional checklists.

1.3 Black coral evolutionary history

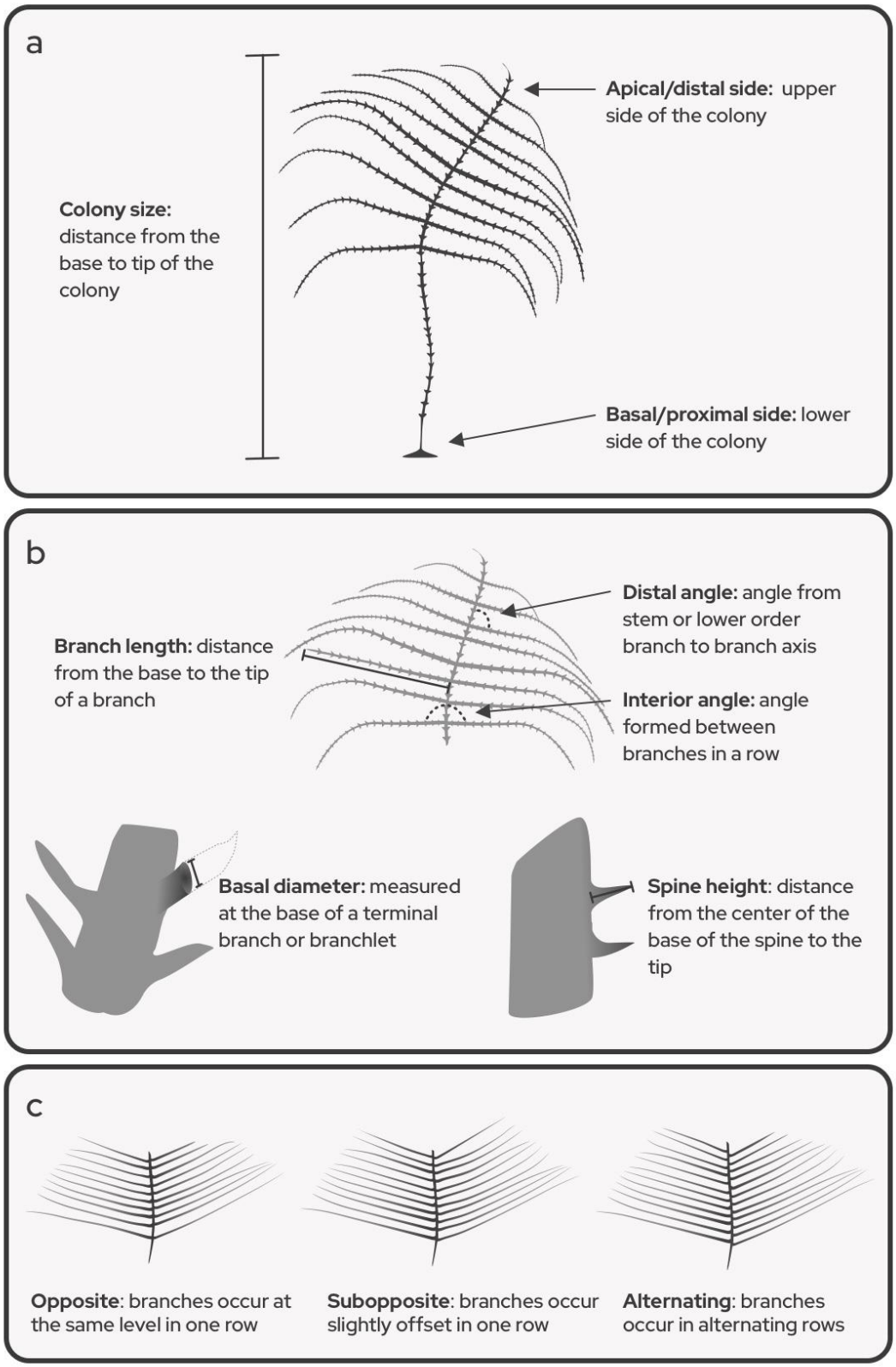
Black corals are a bathymetrically (to at least 8,600 m) and geographically cosmopolitan group, and they have diverse morphological features (unbranched or branched, sparsely or complexly branched, wide array of spine and polyp characteristics) (Figure 1.2). Black corals are therefore a model taxon for understanding how corals have evolved through deep time because understanding the evolutionary history of black corals can provide insight into the drivers that have shaped similarly widespread and morphologically diverse groups.

Unfortunately, many anthozoan groups have skeletons composed of proteins that are not well preserved in the fossil record (Gupta & Briggs 2010; Williams 2020). For this reason, the evolutionary history of black corals is poorly understood. The only fossils identified as a black coral were found in southern China and date to the Ordovician (Balinski *et al.* 2012; Baliński & Sun 2017); however, the fossil's identity was questioned because of morphological differences between the fossils and extant species (Brugler *et al.* 2013). Without a fossil record, it is difficult to determine how morphological features have evolved through time without knowledge of ancestral states and the first appearance of particular morphological characters. However, phylogenetic techniques that enable ancestral reconstruction of character states from extant species can estimate likely ancestral morphological characteristics from extant morphologies (e.g., stochastic character mapping) (Huelsenbeck *et al.* 2003). Similar methods (e.g., dispersal-extinction-cladogenesis models) can estimate ancestral ranges based on extant species' ranges (Höhna *et al.* 2016), and although most regions of the world have not been surveyed with a focus on black corals, species' bathymetric extents are generally known (Molodtsova *et al.* 2022; Molodtsova & Opresko 2017). While fossil data are not available for black corals, insight can be gathered

from secondary calibration using dates of black coral lineages and closely related taxa estimated from other studies (Quattrini *et al.* 2020).

The prevalent hypothesis for metazoan evolution from a morphological standpoint is that species with specialized morphological features selected to perform specific functions come from simpler species without specialized features (Deline *et al.* 2018; Valentine *et al.* 1994). Many studies have focused on the evolution of branching pattern, notably the appearance of pinnules, which are specialized branches arranged in equally distant rows with equal or increasing or decreasing lengths along a lower-order branch or stem (Opresko 2002; Opresko *et al.* 2016) (Figure 1.2C). Pinnules are found on some black coral species, but pinnules are also found on other organisms in both marine (e.g., arms of crinoids and branches of some soft corals and sea pens) and terrestrial (e.g., flowering plants like acacias and non-flowering plants like ferns) habitats. The evolutionary history of pinnules has provoked curiosity among scientists for over 100 years (Bather 1890) because the feature often evolves from simpler, non-pinnulate ancestors (Boyce 2005) and can evolve multiple times independently within a group across divergent lineages (Ausich & Kammer 2001). To date, no studies have investigated the evolutionary history of pinnulation in the Anthozoa or how these adaptations have affected their ability to invade new environments that can aid range expansion through deep time.

Jablonski et. al. (1983) first proposed that deep-sea fauna have shallow-water ancestors. This was discovered by comparing shallow/onshore (~0-250 m depth) and deep/offshore (>250 m depth) fossil records over the last 500 My to determine that benthic fossil shelf communities were more taxonomically similar with Cretaceous slope fauna than Cambrian-Ordovician slope fauna. This hypothesis has been tested on, and proven true for, some marine groups (Anon 1880; Chan *et al.* 2021; Jacobs & Lindberg 1998; Smith & Stockley 2005), but not all (Lindner *et al.* 2008; Pante *et al.* 2012; Stolarski *et al.* 2011; Thuy 2013), suggesting that the drivers that shape species' ranges through depth are complex. To date, little research has investigated how and why bathymetric extents of corals have evolved through deep time, which would improve our understanding of what drives and maintains biodiversity patterns.


a
Colony size: distance from the base to tip of the colony
Apical/distal side: upper side of the colony
Basal/proximal side: lower side of the colony
b
Branch length: distance from the base to the tip of a branch
Distal angle: angle from stem or lower order branch to branch axis
Interior angle: angle formed between branches in a row
Basal diameter: measured at the base of a terminal branch or branchlet
Spine height: distance from the center of the base of the spine to the tip
c
Opposite: branches occur at the same level in one row
Subopposite: branches occur slightly offset in one row
Alternating: branches occur in alternating rows

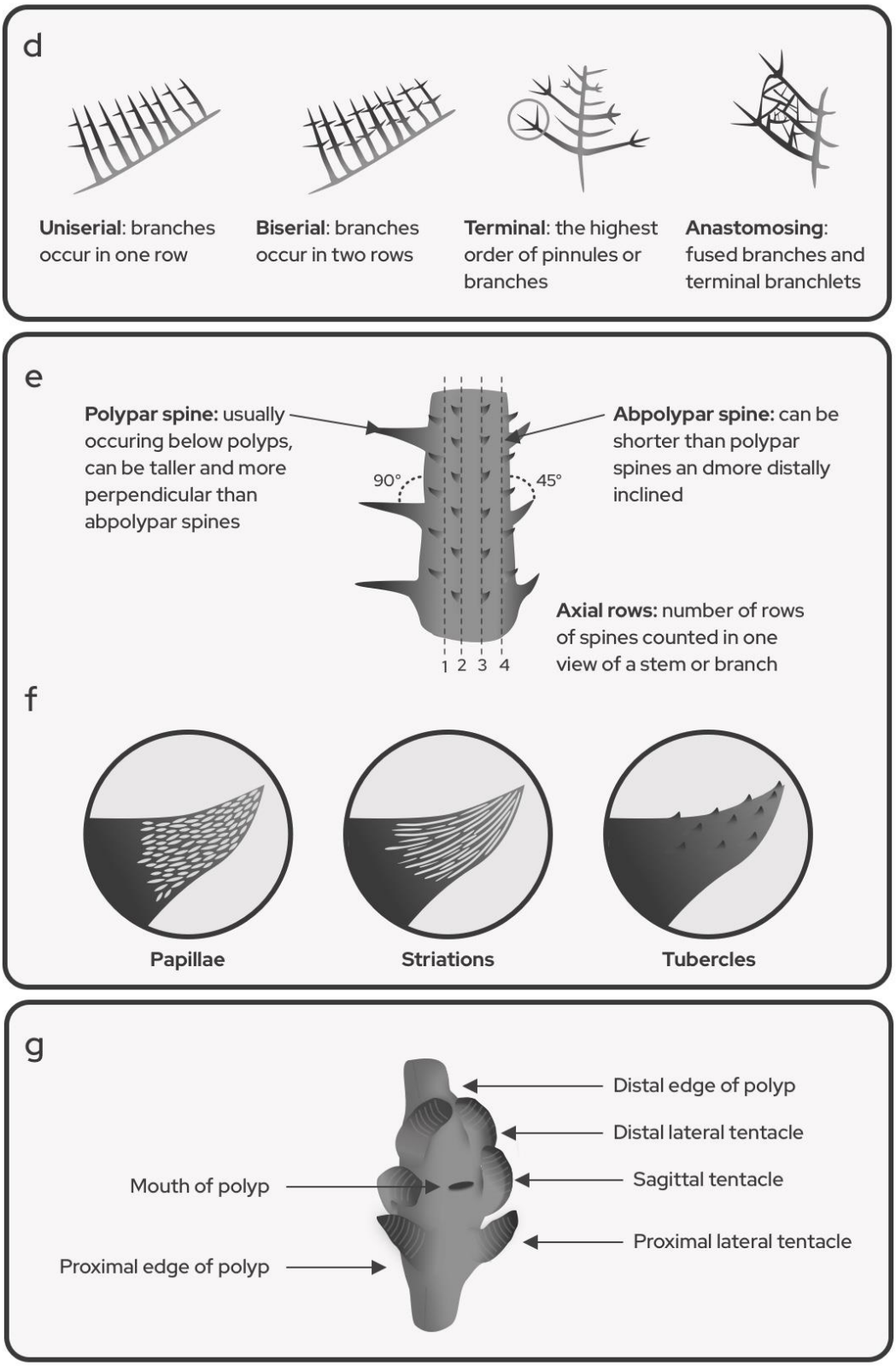


Figure 1.2 Descriptions of morphological features of black corals and how they are measured. a, measuring colony height, and names for the top and bottom of the colony; b, measuring branch length, diameter, and angles, and spine heights; c–d, types of branching patterns; e, types of spines and how they are measured; f, types of spine ornamentation; g, parts of a polyp.

1.4 Thesis aims and outline

Black corals dominate marine habitats and have important ecological roles in the worlds' oceans, yet significant and fundamental knowledge gaps remain regarding the taxonomy, systematics, and evolutionary history of the group. Overcoming these knowledge gaps can provide information to better understand and conserve biodiversity and its processes (Cowling *et al.* 1999; Margules *et al.* 2002; Margules & Pressey 2000; Pressey 2004; Rondinini *et al.* 2006). To overcome these knowledge gaps, I examined museum collections from around the world and collected new specimens on numerous expeditions from just below the surface to over 4,000 meters depth in the Coral Sea, Great Barrier Reef, and Papua New Guinea. I used an integrated approach combining traditional morphological analysis (descriptions of morphological features measured are summarized in Figure 1.2) with novel genomic-scale molecular methods. In **chapter 2**, I produced the first formal checklist of black corals from the deep Coral Sea, Australia, and provided evidence to synonymize a species. I did this by curating the Museum of Tropical Queensland black coral collection and morphologically examined 21 specimens collected during the CIDARIS project from 1986-1992, but which had remained unexamined. This collection represents the largest collection of black corals from the Coral Sea. In **chapter 3**, I sequenced 32 black corals with targeted capture methods to test the usefulness of resulting phylogenies against traditional mitochondrial molecular markers, and I described a new species and genus that I discovered and collected from Kimbe Bay, Papua New Guinea. I did this by collecting tissue samples from specimens in museums from all over the world and from samples of the new species from Kimbe Bay. In **chapter 4**, I updated the number of black coral species that are known to occur from the Great Barrier Reef and Coral Sea and I provided evidence to make the first substantial taxonomic revisions on the coral group in over 15 years. These revisions include the establishment of two families, two genera, and descriptions of five species. I did this by comparing morphological and targeted capture sequence data from 80 black corals to determine how many species occur in the region and identifying polyphyletic relationships among currently accepted genera and families, and then modified the taxonomy delineations by following the processes outlined in the International Code on Zoological Nomenclature (Ferraris & Eschmeyer 2000). In **chapter 5**, I explored the evolutionary history of black corals in terms of their bathymetric evolution and branch

evolution through deep time. I did this by sequencing 42 unique species from different families representing over 50% of valid genera sampled across different depths. I reconstructed a phylogeny from these specimens and time-calibrated the phylogeny based on secondary calibrations from dates estimated in Quattrini *et al.* (2020). I combined phylogenetic, morphological, and bathymetric data in a comparative framework to estimate ancestral states of depth and pinnulation across the evolutionary history of the group.

This thesis integrates all available museum specimens and newly collected specimens, and robust morphological and molecular data to address the overarching aim, which is to describe new species and revise the taxonomy, improve knowledge about regional black coral biodiversity in the Great Barrier Reef and Coral Sea, and illuminate the evolutionary history of this understudied anthozoan group.

Chapter 2 Black corals (Anthozoa: Antipatharia) from the deep (916 m–2542 m) Coral Sea, north-eastern Australia

The proposed revisions of the taxonomy of black corals presented in this chapter are **informal** to avoid *nomen nudum* designations, as per the International Code of Zoological Nomenclature, Article 13 (Ferraris & Eschmeyer 2000). Consequently, this chapter is not a verbatim reproduction of the published version of this paper.

This chapter is published as:
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2.1 Abstract

Black corals (Anthozoa: Antipatharia) occur in all the world's oceans in a wide range of habitats from shallow-water coral reefs to the deep-sea. However, the taxonomy of black corals is poorly known compared to many other anthozoan groups. This knowledge gap is particularly acute for the deep-sea, where collecting specimens is logistically difficult and costly. Here, I identify 21 black coral specimens collected from the western Coral Sea adjacent to north-east Australia. The specimens represent five nominal species from five genera and two families. All species represent new records for the region, including the first record for the family Cladopathidae Brook, 1889. I describe the morphology of these specimens, note geographic and bathymetric range expansions, and provide evidence to support the hypothesis that *Bathypathes seculata* Opresko, 2005 is the juvenile stage of *Bathypathes patula* Brook, 1889, thus warranting synonymization. My findings demonstrate that deep-sea antipatharians in this region are much more diverse than previously reported. Furthermore, this study highlights the importance of museum collections in terms of increasing our understanding of taxonomy and patterns of biodiversity, particularly for poorly studied habitats such as the deep-sea.

2.2 Introduction

Deep-sea corals (>200 m deep) play important ecological roles by providing three-dimensionality to deep-sea habitats (Roberts & Cairns 2014) and preserving diversity during

extreme temperature and sea level changes (Paulay 1990; Pellissier *et al.* 2014). However, deep-sea diversity and ecosystem functions are threatened by trawling (Pusceddu *et al.* 2014), mining (Sharma 2015), pollution (van Cauwenberghe *et al.* 2013), ocean warming (Roberts & Cairns 2014), and acidification (Roberts & Cairns 2014), and recovery from disturbance is slow (Althaus *et al.* 2009). Deep-sea corals therefore require adequate protection, but knowledge gaps regarding the distribution of species in deep-sea ecosystems (Danovaro *et al.* 2010; Woolley *et al.* 2016) can inhibit effective conservation actions (Bridge *et al.* 2016; Mace 2004; Ponder *et al.* 2001).

Black corals are found in every ocean and in all marine habitat types at depths from 2 m to 8,600 m (Wagner *et al.* 2012). Black corals provide important three-dimensional habitat structure and host a rich associated fauna, and are therefore an important component of many benthic ecosystems (Bo *et al.* 2012a; Sánchez 1999; Wagner *et al.* 2012). However, over 75% of known black coral species occur deeper than 50 m (Cairns 2007), resulting in this group being relatively understudied (Brugler *et al.* 2013). Acquiring new information to better understand deep-sea black corals is difficult due to depth limitations associated with SCUBA (Bridge *et al.* 2013), and because expeditions that use unmanned survey technology (e.g., remotely operated vehicles or autonomous underwater vehicles) take years to organize and are expensive to execute (Brandt *et al.* 2016). Specimens housed in museum collections provide an inexpensive alternative to in-situ surveys of deep-sea habitats, and can provide a valuable source of information on global biodiversity, particularly for poorly-known invertebrate taxa (Ponder *et al.* 2001). Museum collections have been utilized to examine species geographic distributions (Skelton *et al.* 1995; Väisänen *et al.* 1994), including species range expansions (Drinkrow & Cherry 1995; Wernberg *et al.* 2011), and to inform conservation actions (Prendergast *et al.* 1993; Vane-Wright *et al.* 1994). Given the logistical difficulties associated with studying black corals in the deep-sea, museum records present a unique opportunity to provide information on biodiversity at depth.

The western Coral Sea is a global hotspot for marine biodiversity (Tittensor *et al.* 2010). While the diversity of the continental shelf off north-east Australia, particularly the shallow reefs of the Great Barrier Reef (GBR) is well established, the vast majority of the

Coral Sea consists of deep-sea habitats in depths of 1,000 m–5,000 m. The biodiversity associated with deep-sea habitats in the western Coral Sea remain poorly known compared to the continental shelf (Beaman *et al.* 2016). For example, only one antipatharian species, *Abyssopathes lyra* (Brook, 1889), has been recorded from the western Coral Sea below 700 m depth (OBIS 2017). This is surprising given that the region lies within the Indo-West Pacific hotspot of marine biodiversity and is known to support a diverse fauna of shallow-water anthozoans, including antipatharians.

The CIDARIS project, conducted by the Queensland Museum from 1986 to 1992, collected specimens from the western Coral Sea, and remains one of the few studies of the deep-sea benthos in the region (Davie 2006). Specimens collected during this project led to numerous new species and range expansions of known species (Ahyong 2012; Baba 1994; Crowther *et al.* 2011). However, the black corals collected during these expeditions were never identified beyond the order level due to a lack of appropriate taxonomic expertise.

To address gaps in knowledge about the diversity of deep-sea antipatharians in the region, I used morphological characteristics to identify 21 antipatharian colonies collected during the CIDARIS expeditions from depths of 916 m to 2,542 m. I identified and expanded the ranges of three nominal species including Bathypathes patula Brook, 1889, *Schizopathes affinis* Brook, 1889, and *Abyssopathes lyriformis* Opresko, 2002. I also identified two additional nominal species *Parantipathes* cf. *hirondelle* Molodtsova (2006), and *Heteropathes* cf. *americana* (Opresko, 2003), although these identifications could not be confirmed because the specimen's lacked polyps and/or stems required for species-level identification. Assuming these identifications are correct, these records also represent range expansions for *P. hirondelle* and *H. americana*. In addition, I developed a growth profile for specimens identified as *B. seculata* Opresko, 2005 and *B. patula* Brook, 1889 to investigate the boundary between these two species.

2.3 Materials and methods

2.3.1 Species identification

I examined 21 specimens collected on CIDARIS expedition's I–III between 1986 and 1992, which are housed in the collections of the Museum of Tropical Queensland (MTQ) (Supplementary Table 2.1). Each specimen was identified to the species level based on

corallum and skeletal spine attributes as defined in the literature. Descriptions and measurement methods of morphological features of black corals are described in Figure 1.2. Corallum attributes examined were branching pattern, stem length, pinnule and subpinnule length and arrangement; and skeletal spine attributes were size, density, morphology, and ornamentation (Brook 1889; de Matos *et al.* 2014; Molodtsova 2006; Opresko 2002). Morphological information regarding polyps was included wherever possible, but in most cases, specimens were lacking soft tissue. Skeletal images of specimens were taken using JEOL 5410 LV and Hitachi TM1000 scanning electron microscopes. To prepare specimens for imaging, ~5 cm fragments were cut from the pinnules and/or subpinnules of each specimen. Fragments were immersed in bleach for one to ten minutes (depending on the amount of tissue) to remove the soft tissue and expose the skeleton. Fragments were periodically immersed in 90% ethanol towards the end of the bleaching process to inspect the skeleton, determine if all the tissue was removed, and ensure that the bleach did not erode the skeleton. Specimens were then dried for 24 hours before being coated with gold. When using the Hitachi TM1000, specimens were imaged without being coated due to time limitations associated with the microscope. Lower magnification images were taken to measure spine density, and higher magnification images were taken to measure spine size, morphology, and ornamentation.

2.3.2 Species ranges

Known species ranges were calculated based on occurrence records in the Ocean Biogeographic Information System (OBIS 2017). I then compared the geographic location (latitude/longitude) of each specimen to all other occurrence records in OBIS to identify range extensions using ArcGIS™.

2.3.3 Growth profiles for *Bathypathes patula* and *Bathypathes seculata*.

There has been uncertainty surrounding the taxonomic status of *B. seculata* and its boundary with the morphologically similar *B. patula*. *B. patula* was first described by Brook, 1889, and the species is known from many specimens (Brook 1889; Cooper 1909; Pasternak 1977; Pesch, A.J. 1914; Roule 1905). *B. seculata* was described, and is only known, from one specimen with similar morphological features to *B. patula* (see Opresko 2005). They are currently differentiated based on the ratio of the longest pinnule compared to the total

colony height (Opresko 2005); however, the low number of occurrences of *B. seculata* compared to *B. patula* and the many similarities between the two species descriptions led us to question whether *B. seculata* might be a juvenile form of *B. patula*. I created growth profiles of specimens from the collection identified as *B. seculata* (n=11) and *B. patula* (n=2) based on ratio differences between the species described by Opresko (Opresko 2005), which allowed us to address the issue for the first time. In addition to these 13 specimens, I also included the holotypes of *B. seculata* and *B. patula* (Brook 1889; Opresko 2005), and eight *B. patula* specimens from the literature (Cooper 1909; Pasternak 1977; Pesch, A.J. 1914). Growth profiles consisted of the following measurements: (1) total colony size (cm); (2) length of the longest pinnule (cm); (3) length of the pinnulated portion of the stem (cm); (4) ratio of the length of the longest pinnule to the length of the pinnulated stem; (5) position of the longest pinnule; (6) pinnule density per 3 cm per side; (7) length of striatum on the stem (cm); (8) number of ridges on striatum from one view; (9) striatum description; (10) spine density per one view; (11) spine height (nm); (12) spine shape; (13) spine surface features; and, (14) spine orientation. I compared morphological characters among the specimens, the type specimens, and B. patula specimens from the literature to investigate whether the criteria that define the taxonomic boundary between the two species can be explained by colony size.

2.4 Results

The 21 specimens examined represented five nominal species (*Bathypathes patula, Schizopathes affinis, Abyssopathes lyriformis, Parantipathes* cf. *hirondelle*, and *Heteropathes* cf. *americana*) from five genera and two families (Supplementary Table 2.1). All five species represented new species ranges for the western Coral Sea (Figure 2.1). The occurrences of these species expand their known geographic ranges from 1,000 km to > 15,000 km, and the known bathymetric ranges for some species by up to ~1,000 m (Figure 2.1). Morphometric analysis of growth profiles for *B. seculata* and *B. patula* revealed that the main taxonomic boundary can be explained by colony size, and therefore *B. seculata* represents a juvenile form of *B. patula*, and the two species should be synonymized. A summary of the specimens examined is presented below.

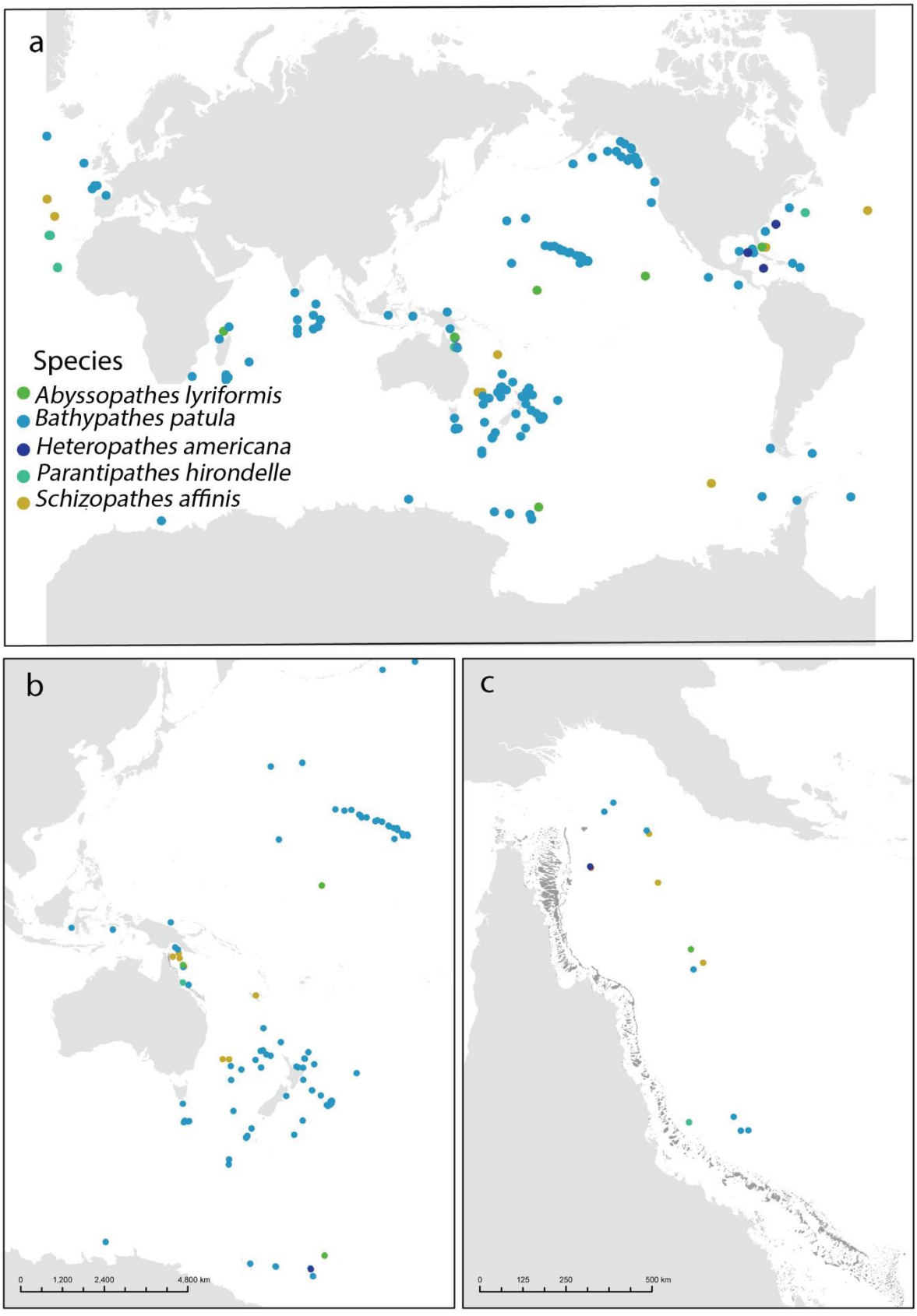

Figure 2.1 *Map of collection sites for the specimens in this study. a, global map of occurrences of species included in the study; b, view of the Central and Eastern Indo-Pacific region; c, new black coral records from the Coral Sea.*

Order: Antipatharia

Family: Schizopathidae Brook, 1889

Genus: *Abyssopathes* Brook, 1889

Species*: Abyssopathes lyriformis*, Opresko, 2002

(Figures 2.1 and 2.2) *Abyssopathes lyriformis*, Opresko (2002): 421; Molodtsova & Opresko (2017): 355.

Material examined: MTQ material (preserved in ethyl alcohol): G62078 (Station data in Supplementary Table 2.1). Diagnosis (after Opresko 2002): Corallum monopodial and pinnulate. Pinnules arranged in two lateral or anterolateral rows and one multiple anterior row containing two to three times the number of pinnules in either lateral row. Lateral pinnules simple; anterior pinnules usually with one, rarely two, secondary pinnules. Tertiary pinnules very rarely present on some secondary anterior pinnules. Basal lateral pinnules curved posteriorly (away from side with anterior pinnules), with distal ends of those in one row directed towards those in opposite row, thus forming a somewhat open funnel-like structure. Distal lateral pinnules only slightly curved or straight. Pinnulated section of corallum inclined to substrate due to ~45° bend in stem near base. Lateral pinnules 4 mm to 5 mm apart, resulting in 14 pinnules total per 3 cm. Density of anterior pinnules up to 13 per 2 cm. Spines on the lateral pinnules conical, compressed, 0.02 mm to 0.04 mm long; those on anterior pinnules and subpinnules up to 0.06 mm long and often inclined distally. Three to four rows of spines seen in lateral view; with three to 11 spines per mm in each row. Polyps unknown.

Description of specimen: The CIDARIS specimen is like the type in both the ~45° angle made between the pinnulated section of corallum and the substrate (Figure 2.2a) and in the arrangement of and distance between primary pinnules. For example, the lowest set of lateral pinnules are nearly opposite with the rest alternating up the stem with distances of ~4 mm between each on one side of the stem, resulting in two to three pinnules per cm (Figure 2.2b). Lateral pinnule density is like the type in that 14 pinnules can be counted per 3 cm proximally to 12 per 3 cm apically (counting pinnules on both sides of the stem) (Figure 2.2b). Three rows of spines on lateral pinnules can be seen from one aspect (Figure 2.2c) with about three to four spines per mm in each row. Spines are triangular or rounded,

compressed, and 0.01 mm to 0.03 mm tall (Figure 2.2c). Anterior pinnules are present in association with more than half of the lateral pinnules, range between 0.05 mm and 3 mm in length, and have either none or one secondary pinnule. The maximum density of the anterior pinnules is seven per 2 cm which is less than the 13 per 2 cm reported for the type (Figure 2.2a). Spines on anterior pinnules are irregularly angled, with most spines elongated and inclined distally and some less inclined/ triangular, all of which are 0.03 mm to 0.04 mm tall (Figure 2.2d).

Discussion: *A. lyriformis* is closely related to *A. lyra*, however *A. lyriformis* has secondary pinnules while *A. lyra* does not (or only rarely). *A. lyriformis* also has: 1) a higher density of lateral pinnules (14 per 3 cm versus eight per 3 cm; 2) a higher density of anterior pinnules (13 per 2 cm versus <8 per 2 cm); and, 3) larger spines on the anterior pinnules than on lateral pinnules, whereas *A. lyra* has subequal spine sizes on lateral and anterior pinnules. Lateral pinnule density of the CIDARIS specimen is like *A. lyriformis*, however the anterior pinnule density is lower than the type (seven pinnules per 2 cm). A lower density of anterior pinnules was also found in a specimen identified as A. lyriformis described in Molodtsova & Opresko (2017) (eight to 12 per 2 cm). Also, like the CIDARIS specimen, Molodtsova & Opresko (2017) reported anterior pinnules with smaller spines than the type. This either reveals plasticity among these features or potentially two different species, warranting genetic tests for confirmation.

Distribution: This species has been reported from the Central Pacific, Southern, West Indian, and West Atlantic Oceans from depths of 3,475 m to 4,892 m, and this study expands the distribution to the Coral Sea, Australia at 2,542 m (Figure 2.1c), a range expansion of > 4,000 km and depth range expansion of > 1,000 m.

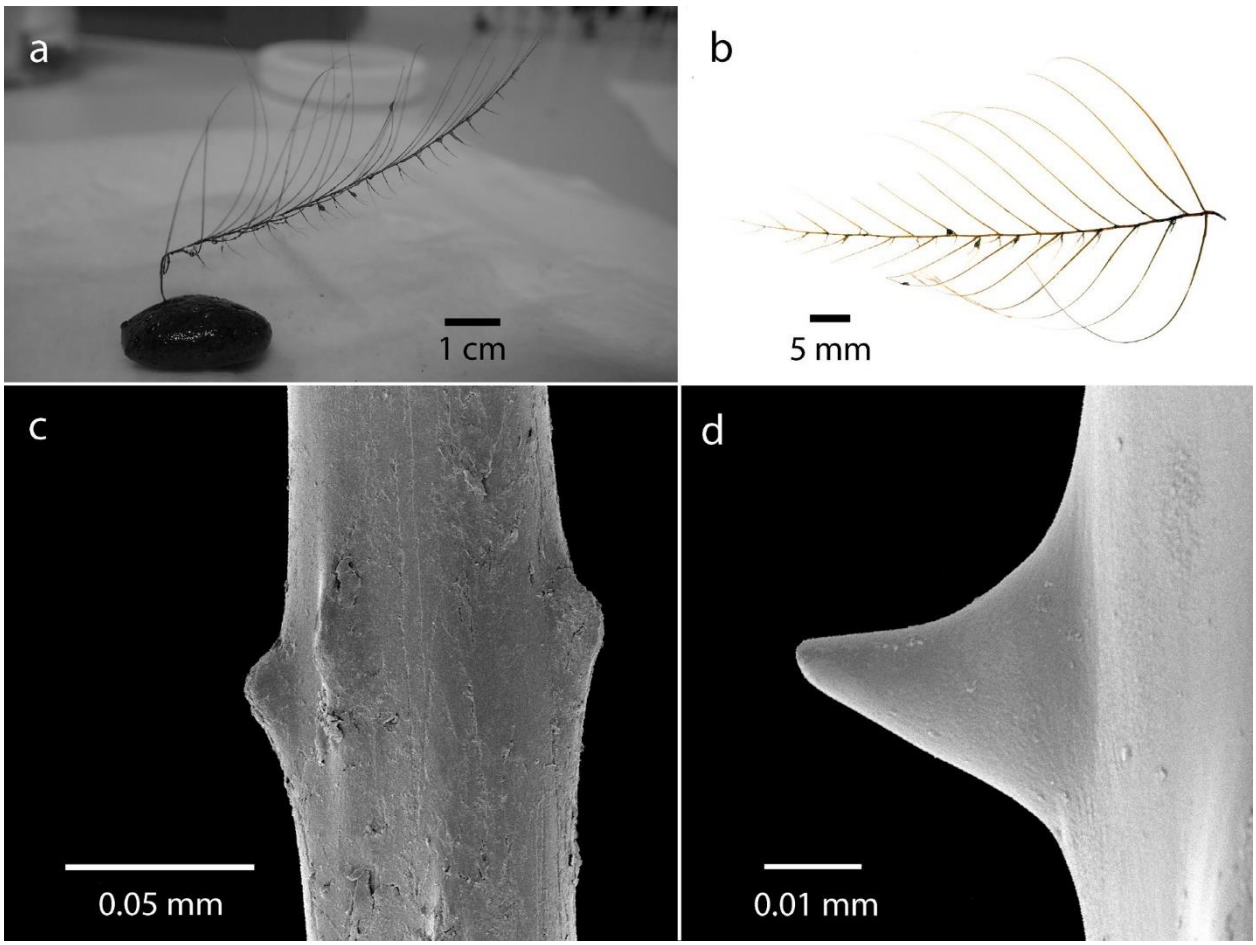

Figure 2.2 *Abyssopathes lyriformis:* a, G62078, lateral view of corallum showing 45° bend in stem near base and density and size of anterior pinnules; b, G62078, top view showing pinnule density; c, G62078, spines on a section of a lateral pinnule; d, G62078, single spine on anterior pinnule.

Genus: *Bathypathes* Brook, 1889

Species*:* *Bathypathes patula* Brook, 1889

(Figures 2.1, 2.3–2.4) *Bathypathes patula*, Molodtsova, 2006: 141–142 (complete synonymy). *Bathypathes seculata* Opresko, 2005: 130–133.

Material examined: MTQ material (preserved in ethyl alcohol): G35428, G35430, G61917, G73228, G61948, G61966, G61967, G61977, G61978, G61979, G61980, G61981, and G62049 (station data in Supplementary Table 2.1).

Diagnosis: (after Molodtsova 2006- emended): Colony monopodial, pinnulate; sickle-shaped stems. Pinnules simple; arranged along the stem in two lateral or anterolateral rows, ranging from 7 mm to 9 mm apart in each row. Pinnules increase in length from the lowest pair of pinnules, reach maximum length at about the mid-pair to just above the mid-pair, and then decrease in length towards the apex.

Spines simple, smooth with blunt tips, triangular or slightly compressed or elongated, 0.03 mm to 0.075 mm tall, either at 90° to the surface of the pinnule or slightly slanted distally, with bifurcation of a few spines; three to five rows visible in lateral view with about three spines per mm. Polyps up to 9 mm in transverse diameter, arranged in a single row.

In addition to the above diagnosis, I add the following: ratio of the length of the longest pinnule to length of the pinnulated portion of the stem largest among young colonies (1.5 to 2 for colonies with pinnulated portion of stem lengths less than 10 cm) and decreasing with age (<1 for colonies with pinnulated portions of the stem greater than 15 cm). The equation relating this ratio (y) to length of the pinnulated portion of the stem in cm [x] is y= 2.081 - 0.059x.

Description of the specimens: Two colonies resemble *B. patula* and 11 colonies resemble *B. seculata* based on the description of the holotypes. These specimens have curved to sickle-shaped stems when viewed from the side (Figures 2.3a–b) that range from 3.3 cm to 24 cm in total length. The length of the longest pinnule ranges from 1.6 cm to 10.5 cm and this pinnule is positioned at, or just above, the mid pair of pinnules (Figures 2.3a–b). The length of the unpinnulated stem ranges from 2.2 cm to 7.9 cm and the length of the

pinnulated stem ranges from 1.1 cm to 16.1 cm. The ratio of the length of the longest pinnule to the total colony size ranges between 0.32 and 1.15. Two colonies have ratios close to, or less than, 0.5 and 11 colonies have ratios close to, or greater than, 0.9, identifying them as either *B. patula* or *B. seculata*, respectively. The ratio of the length of the longest pinnule to the length of the pinnulated portion of the stem ranges between 0.48 and 1.94. The pinnule density on each side of the stem ranges between three and five pinnules per 3 cm; however, this range might be as much as six pinnules per 3 cm as one specimen has a pinnulated portion 2 cm in length with four pinnules on each side of the stem. Stem thickness just above the basal plate ranges between 0.1 mm and 0.9 mm. The striatum on the stem varies in position and length, starting anywhere between the lowest point of the stem to midway up the unpinnulated portion of the stem, and ending anywhere from midway up the unpinnulated portion of the stem to the apex of the colony. The number of ridges on the striatum ranges between two and six from one view, and the distinctiveness of the striatum ranges from not distinct to very distinct. Three to seven rows of spines can be counted in one view on the pinnules, and the spines are arranged irregularly in either spirals or in axial rows (Figures 2.3c–d). The difference between polypar and abpolypar spines are visible in some specimens, where polypar spines are ~0.07 mm and abpolypar spines are ~0.03 mm (Figure 2.3d). Spines are smooth with blunt tips, elongated, and are either at 90° to the surface of the pinnule or slightly slanted distally (Figure 2.3e), with one specimen showing some bifurcation of the spines (Figure 2.3f). Among the few specimens that have well preserved polyps, the transverse diameter is ~6 mm, the tentacles reach a maximum length of 3 mm, and the interpolypar space is ~2 mm. Polyp density is ~1.5 per cm (Figure 2.3g).

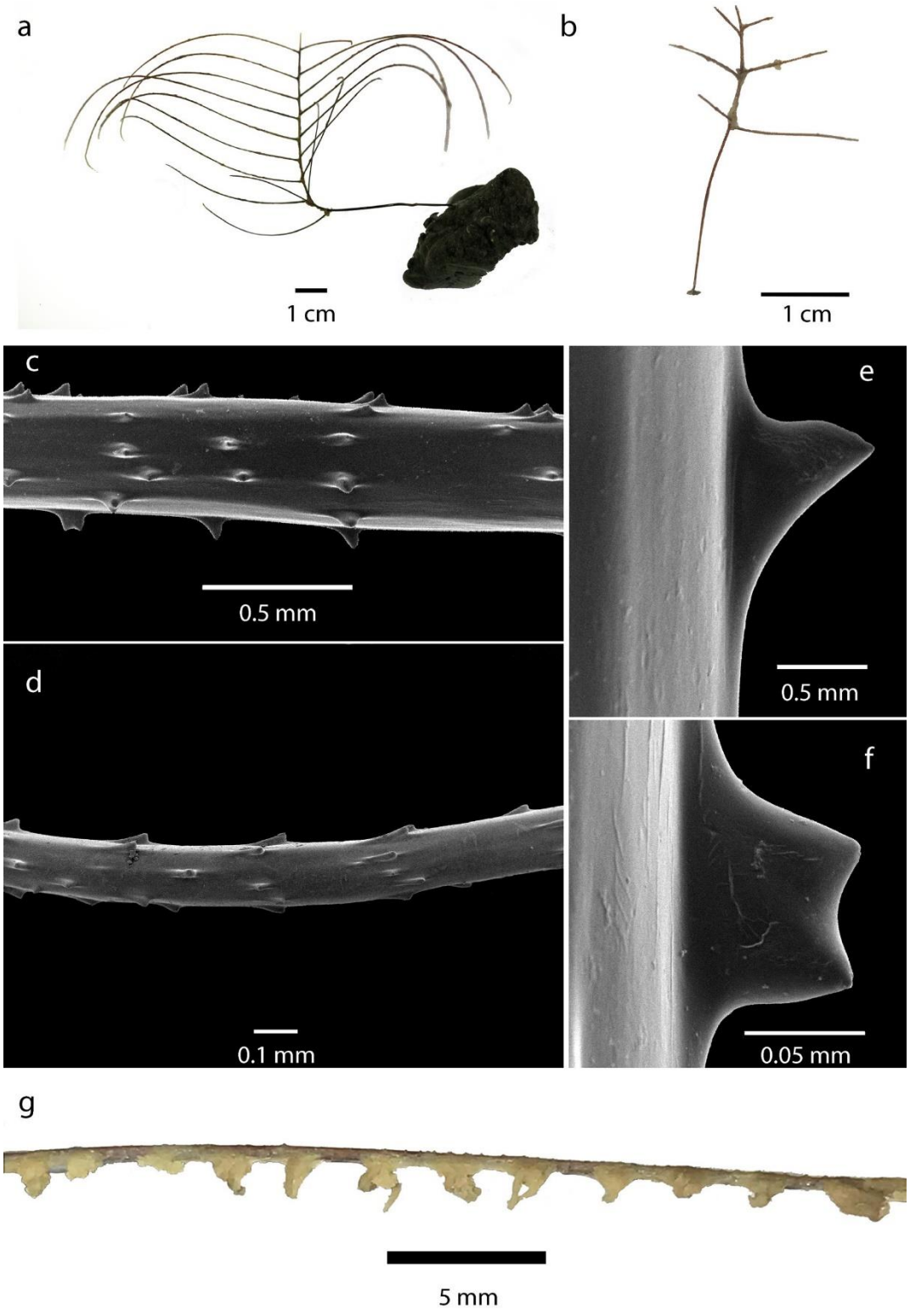

Figure 2.3 *Bathypathes patula*: a, G61948; b, G35430, anterolateral views of corallums showing curvature of stem and pinnule lengths; c, G61917; d, G61978, spines on sections of pinnules where c shows longer polypar spines (top side of pinnule in image) than abpolypar spines (bottom side of pinnule in image); e, G61978, single spine on pinnule; f, G61917, single spine showing bifurcation; g, G61978, polyps on section of pinnule.

Discussion: The discovery of 11 specimens identified as *B. seculata* based on similarities with the type specimen allows us to investigate whether the primary morphometric character that defines the taxonomic boundary between *B. seculata* and *B. patula* (ratio of pinnule length to stem length) can be explained by colony age, using length of the pinnulated portion of the stem as a proxy for age. Prior to this publication, *B. seculata* was defined as having a length of longest pinnule to stem length ratio of 0.9, and B. patula ~ 0.3 to 0.5. When comparing the length of the longest pinnule to the pinnulated portion of the stem (this is a modified ratio to account for variations in the length of the unpinnulated portion of the stem) among the 11 *B. seculata* and two *B. patula* specimens in the collection, the *B. seculata* and *B. patula* holotype specimens, and eight *B. patula* specimens described in the literature, results show a significant relationship between this ratio and colony size ($F_{1,21} = 51.87$, $P<0.001$). The equation relating ratio (y) to colony size (i.e., total length of the pinnulated portion of the stem) in cm [x] is $y= 2.081 - 0.059x$. This equation successfully explains approximately 70% of the total variation in the pinnule to colony size ratio. The type specimen of *B. seculata* has a total colony size of 9 cm and a ratio of 1.5, and *B. patula* specimens in the literature have total colony sizes that range from 6 cm to 25 cm and ratios ranging from 1.6 to 0.48. Prior to this study, the different ratios and colony sizes between the *B. seculata* type specimen and *B. patula* specimens suggested potentially different species. However, when considering specimens from the collection (Supplementary Table 2.2), there is a strong negative correlation between ratio and colony size (Figure 2.4), which means that the ratio decreases with increasing colony size and *B. seculata* is therefore a juvenile *B. patula*. I propose that the negative correlation between ratio and colony size is due to pinnules having slower growth rates than the stem, which is why as colony size gets larger, the ratio of the pinnule to colony size decreases. Allometric growth among corals has been shown to maximize their chances of survival and reproduction (Dornelas *et al.* 2017), and has previously been documented in the black coral *Leiopathes* (Antipatharia: Leiopathidae) (Lartaud *et al.* 2020). The advantage that the *Bathypathes* specimens have by growing allometrically can relate to a law of modular organisms, where proportional growth rates decrease with size due to geometric, structural, and energetic constraints (Dornelas *et al.* 2017).

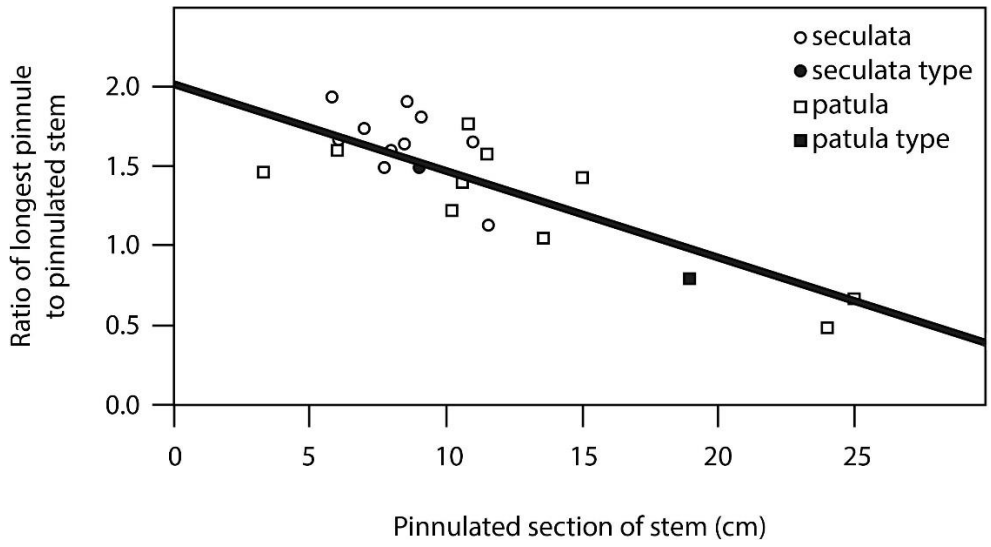

Figure 2.4 *B. patula* and *B. seculata* morphological comparison. Ratio of the longest pinnule and length of the pinnulated section of stem against length of pinnulated section of stem.

Another feature that was thought to be different between the two species is the position of the longest pinnule, where the longest pinnule for *B. patula* is positioned mid-way up the pinnulated portion of the stem (Brook 1889) while the longest pinnule for *B. seculata* is positioned at the apex (Opresko 2005). However, most specimens identified as *B. seculata* and *B. patula* have their longest pair of pinnules near the mid-pair or just above the mid-pair of pinnules. Other morphological features similar among these specimens include: pinnule density (three to five per 3 cm on one side of the stem), spine density (~3 spines per mm), spine height (0.03 mm to 0.075 mm), spine shape (blunt tip, elongated, and triangular or slanted), and spine arrangement (three to five axial rows per view, sometimes irregular or spiral) (Supplementary Table 2.2).

One difference between these 13 specimens that cannot be explained by colony size is the position, distinctiveness, and length of the striatum. For example, the smallest specimen (G35430) has an indistinct striatum that covers the whole stem, the second smallest specimen (G61917) has very distinct striatum that is about 1.6 cm in length and starts at the base and ends midway-up the unpinnulated portion of the stem, and a mid-sized specimen (G61948) has very distinct striatum 6 cm in length that starts about 0.5 cm from the base and extends to the first set of pinnules (Supplementary Table 2.2). Another unexplainable difference among these specimens is the length of the unpinnulated portion of the stem. Although there seems to be a general decrease in the ratio between the length of the unpinnulated portion of the stem and total stem length, where the smallest sized

specimen has a ratio of ~0.66, and the largest sized specimen (G61980) has a ratio of ~0.33, the change in ratio with colony size does not seem to be consistent among all the specimens. For example, specimens G61917, G62049, G61979, G61978, and G61948 have total stem lengths of 5.8 cm, 7.5 cm, 7.75 cm, 8.6 cm, and 11 cm, respectively, with ratios ranging from 0.46 to 0.41; however, one specimen from Siboga Station 74A (Pesch, A.J. 1914) and G61966 have total stem lengths of 6 cm and 7 cm and ratios of 0.58 and 0.35, respectively. Given that striatum characteristics and length of the unpinnulated portion of the stem seem to be highly variable characteristics, and no other distinct differences can be found between the *B. seculata* and *B. patula* specimens, the holotype specimens, and *B. patula* specimens described in the literature, I hereby synonymize *B. seculata* with *B. patula*.

Distribution: Specimens assigned to this species have been reported from the Pacific, Atlantic, and Indian Oceans from depths of 100 m to 5,500 m. This study expands the known distribution to the Coral Sea, Australia (Figure 2.1c). Despite the many records of *B. patula* listed in OBIS, most are based on in-situ observations without the specimens having been collected. Given that it is difficult to identify this species based on in situ observation alone, I am unable to confidently calculate the distance of the range expansion.

Genus: *Parantipathes* Brook, 1889

Species*:* *Parantipathes* cf. *hirondelle* Molodtsova, 2006

(Figures 2.1 and 2.5) *Parantipathes hirondelle* Molodtsova, 2006: 142–143 (complete synonymy).

Material examined: MTQ material (preserved in ethyl alcohol): G35429 and G62019 (Station data in Supplementary Table 2.1).

Diagnosis after Molodtsova, 2006: Corallum monopodial or very sparsely branched, pinnulate. Pinnules up to 2 cm in length, forming distinct bilateral and alternating semispiral groups, with three to four pinnules per group, 33 to 40 pinnules (total for all groups) per cm. Spines simple, smooth, rounded at the apex, triangular and compressed; 0.02 mm to 0.06 mm tall; arranged in longitudinal rows, four to five of which are visible in lateral view, with

six to seven spines per mm in each row. Polyps elongated, 0.9 mm to 1.7 mm in transverse diameter, with 6 to 6.5 per cm.

Description of the specimens: The CIDARIS specimens are like the type of *P. hirondelle* in both the density and orientation of pinnules where 38 pinnules (total for all groups) can be counted per cm compared with 33 to 40 per cm in the type, and in having, on average, six rows of pinnules that form distinct semispiral alternating groups (Figure 2.5a–5b). The number of pinnules per group increases from one and two pinnules per group (corresponding to a total of three rows) basally on the stem to three and five pinnules per group (total of eight rows) distally near the apex. In contrast, the type of *P. hirondelle* has no more than four pinnules per group. The CIDARIS specimens are like the type of *P. hirondelle* by also having longer posterior pinnules than anterior pinnules. For example, the length of the longest posterior pinnules for specimens G35429 and G62019 are ~2 cm and 0.5 cm, and the maximum length of anterior pinnules are ~1 cm and 0.3 cm, respectively (Figures 2.5a and 2.5c). The spines of the CIDARIS specimens are smooth, simple, triangular, sometimes inclined distally, with polypar spine heights being ~0.05 mm and abpolypar spine heights being ~0.03 mm from mid-base to tip (Figure 2.5d). Three to four axial rows of spines can be seen from one aspect (Figure 2.5d) with ~0.25 mm between adjacent spines, resulting in five to six spines per mm (Figure 2.5b).

Remarks: Currently there are nine nominal species in the genus *Parantipathes*: *P. larix* (Esper, 1790), *P. tetrasticha* (Pourtales, 1868), *P. laricides* van Pesch, 1914, *P. euantha* (Pasternak, 1958), *P. wolffi* Pasternak, 1977, *P. helicosticha* Opresko, 1999, *P. hirondelle* Molodtsova, 2006, *P. dodecasticha* Opresko, 2015, and *P. robusta* Opresko, 2015. The taxonomic characters of the first seven species have been thoroughly summarized by Molodtsova (2006), and the last two have been described and compared to the former by Opresko (Opresko 2015). G35429 and G62019 have pinnules that form distinct semispiral groups with their longest pinnules being 2 cm and 0.5 cm, respectively. The longest pinnule length is relatively small for *Parantipathes* spp. but could potentially be any of the nine species except for *P. larix* and *P. tetrasticha*, which have much larger maximum pinnule lengths (6 cm to 12 cm and 4 cm, respectively). However, the ratio of the longer posterior pinnules compared to anterior pinnules is like P*. hirondelle*, and when considering pinnule

arrangement, the remaining pinnule characteristics fit best with the description of *P. hirondelle* because the CIDARIS specimens form distinct semi spiral groups that do not arise from the same point for a given group as with *P. laricides* and *P. wolffi*. Five to six spines can be counted per mm (Figure 2.5b) and three to four rows of spines can be counted from one aspect (Figure 2.5d), which falls between *P. hirondelle* (six to seven spines per mm and four to five rows of spines per one aspect) and *P. helicosticha* (2 to 3.5 spines per mm and three to four rows of spines per one aspect). However, *P. helicosticha* is reported to have ~25 pinnules per cm (counting pinnules around the stem) while the CIDARIS specimens have ~38 pinnules, like the type of *P. hirondelle* (33-40 pinnules per cm). The spines in the CIDARIS specimens range from 0.03 mm to 0.05 mm in height from mid-base to tip (Figure 2.5d), which fits with *P. hirondelle* (0.02 mm to 0.06 mm) and excludes the CIDARIS specimens from being *P. dodecasticha* and *P. helicosticha* because the latter two have spine heights as large as 0.18 mm and 0.22 mm, respectively. Additionally, the CIDARIS specimens are unlikely to be *P. dodecasticha* because *P. dodecasticha* has about twice the number of pinnule rows as the CIDARIS specimens (12 in *P. dodecasticha* compared to six in the CIDARIS specimens), and a higher average number of pinnules per group (five to six pinnules per group in *P. dodecasticha* compared to three to four pinnules per group in the CIDARIS specimens). Lastly, the CIDARIS specimens are unlike the type of *P. robusta* because *P. robusta* characteristically has forked spines not found in the CIDARIS specimens.

Polyps are not present on either of the CIDARIS specimens, therefore, I cannot compare polyp size and density with these features in the other species.

Distribution: *P. hirondelle* has been reported from the North Atlantic Ocean from depths of 305 m to 1,401 m, and this study potentially expands the distribution to the Coral Sea, Australia (Figure 2.1c), a range expansion of > 15,000 km.

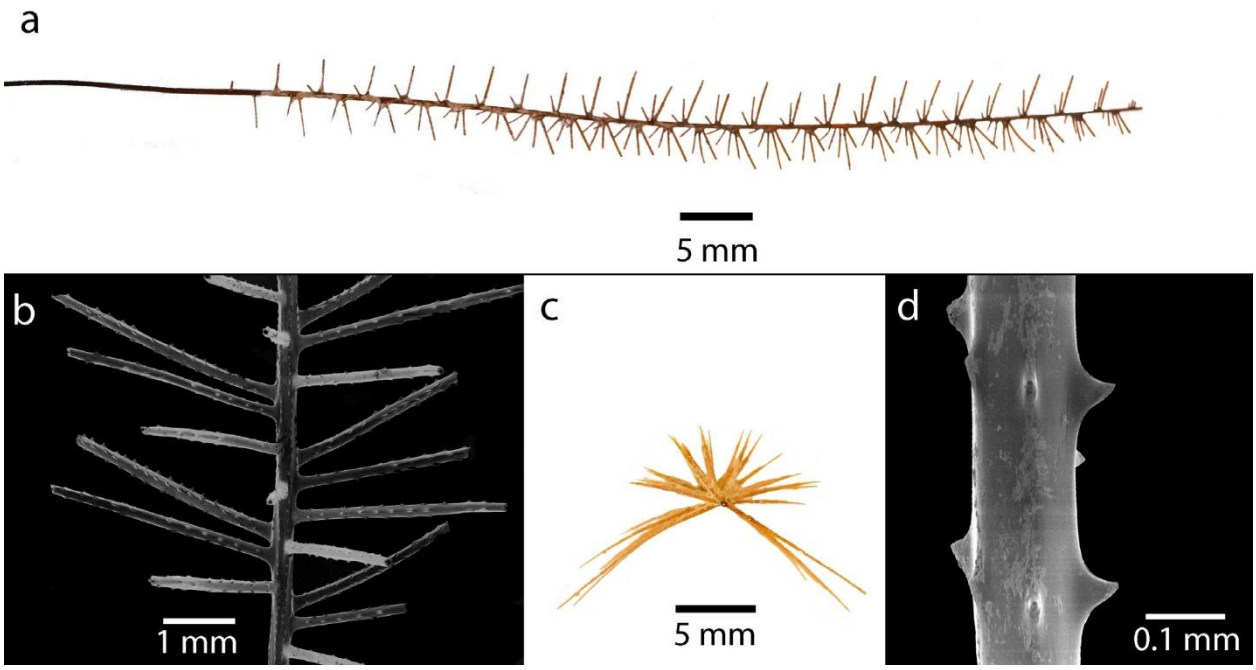

Figure 2.5 *Parantipathes* cf. *hirondelle:* a, G62019, side-view showing pinnule density along the stem; b, G35429, section of corallum showing pinnule orientation and density; c, G35429, cross-sectional view of the pinnules; d, G35429, spines on a section of pinnule.

Genus: *Schizopathes* Brook, 1889
Species*:* *Schizopathes affinis* Brook, 1889

(Figures 2.1 and 2.6) *Schizopathes affinis*, Molodtsova & Opresko (2017): 360–361 (complete synonymy).

Material examined: MTQ material (preserved in ethyl alcohol): G61837, G61944, G61951, and G73230 (Station data in Supplementary Table 2.1).

Diagnosis: Colony monopodial, unbranched, pinnulate. Pinnules simple, arranged alternately in two lateral rows along stem; decreasing in length toward apex of corallum and inclined distally. Pinnules 8 mm to 10 mm apart proximally, 5 mm to 6 mm apart near the top of corallum (approximately seven pinnules total per 3 cm on lower part of corallum and about ten per 3 cm on upper part in holotype). Polypar spines small, triangular, and compressed; mostly 0.03 mm to 0.05 mm tall (up to 0.08 mm near distal end of pinnules); four to five rows visible in lateral view; with about six spines per mm. Abpolypar spines usually smaller than polypar spines, about 0.03 mm or absent. Polyps 3 mm to 4.5 mm in transverse diameter, with about three polyps per cm.

Description of the specimens: The CIDARIS specimens of *S. affinis* are similar to the type in both the density of the pinnules (increasing from about 4.5 pinnules in total per 3 cm and ~7 mm of distance between pinnules proximally to eight per 3 cm and ~5 mm of distance between pinnules towards the apex of the corallum) (Figure 2.6a), and the size of the polypar spines (~0.06 mm) being larger than abpolypar spines (~0.04 mm) (Figure 2.6b). The colony also resembles the type specimen by having a distinct triangular shape and ratios of the longest and lowermost pinnule compared to the pinnulated portion of the stem being between 1 to 1.3, like the type specimen with a ratio of ~0.95. (Figure 2.6a). Four rows of spines can be seen from one aspect (Figure 2.6b) and spines are mostly simple (Figure 2.6c), with two specimens showing bifurcation of some polypar spines (Figure 2.6b).

Distribution: This species has been reported from the North-western Atlantic, Western Pacific, and Indian Oceans, and South China Sea, from depths of 1,900 m to 8,460 m, and this study expands the distribution to the Coral Sea, Australia (Figure 2.1c) at 1,576 m (Figure 2.1c), a range expansion of > 1,000 km, and depth range expansion of 333 m.

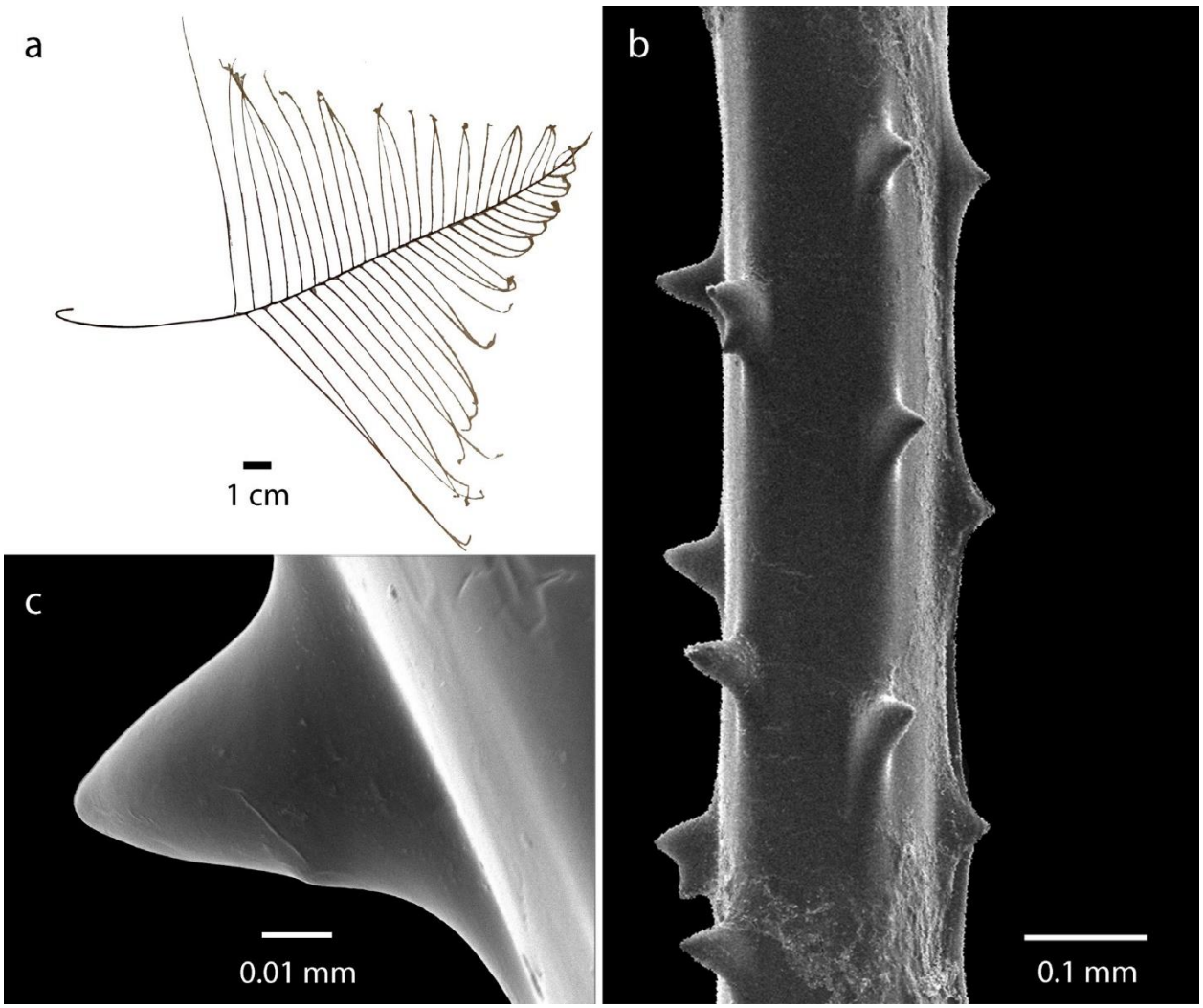

Figure 2.6 *Schizopthes affinis:* a, G61837, anterolateral view of corallum showing pinnule density, triangular corallum shape, and curved hook at basal end b, G61978, spines on a pinnule shows bifucation of some polypar spines (seen on left side of image) and larger polypar spines than abpolypar spines; c, G61837, spine on a section of a pinnule.

Family: Cladopathidae Brook, 1889

Genus: *Heteropathes* Opresko, 2011

Species*: Heteropathes cf. americana* (Opresko, 2003)

(Figures 2.1 and 2.7) *Heteropathes americana* (Opresko, 2003): 531–536

Material examined: MTQ material (preserved in ethyl alcohol): G73229 (Station data in Supplementary Table 2.1).

Diagnosis after (Opresko, 2003): Corallum monopodial and pinnulate; pinnules arranged in two lateral rows and one or two irregular anterior rows. Lateral pinnules simple, elongate, arranged alternately and inclined and curved distally and extending to the top of

the corallum; anterior pinnules short, subpinnulate, and extending out nearly perpendicular to the plane containing the stem and lateral pinnules. Anterior pinnules with one to three secondary pinnules, the lowermost two usually arranged bilaterally. Secondary pinnules may be subpinnulate, with the tertiary pinnules usually arising from upper and/or lower surface of secondaries. Spines on lateral pinnules small (about 0.05 mm or less), triangular, acute, compressed. Spines on anterior pinnules larger on one side of axis (up to 0.13 mm) and inclined distally. Polyps 5 mm to 6 mm in transverse diameter (from distal edge of distal lateral tentacles to the proximal edge of the proximal lateral tentacles).

Description of specimen: Monopodial with pinnules arranged in two lateral rows and one irregular row of anterior pinnules. Total stem length is 4 cm; however, the stem tip is broken. Lateral pinnules are simple, elongate, arranged alternately, except for the first set of lateral pinnules, which are nearly opposite, with 3 mm spaces between pinnules on one side of the stem, and are curved distally (Figure 2.7a). Anterior pinnules range between 0.8 cm and 1 cm length, are spaced 0.1 cm to 0.2 cm apart and have one to two orders of subpinnules (Figure 2.7b). Four to five rows of spines can be seen from one aspect on lateral pinnules (Figure 2.7c), and two rows of spines can be seen from one aspect on anterior pinnules (Figure 2.7d). Spines on lateral pinnules are smooth, simple, 0.045 mm in height, and are triangular, acute, and compressed (Figure 2.7c). Spines on anterior pinnules are less uniformly shaped, ranging from triangular to extremely distally inclined, varying in height from 0.04 mm to 0.08 mm, and are generally larger than spines on lateral pinnules, especially on the outer convex side (Figure 2.7e).

Discussion: Four nominal species are currently recognized in the genus: *H. heterorhodzos* (Forster Cooper, 1909), *H. americana* (Opresko, 2003), *H. pacifica* (Opresko, 2005), and *H. opreski* de Matos, 2014. Species in the genus *Heteropathes* are identified based on the relative size of spines on anterior pinnules and subpinnules and differences in the subpinnulation of the anterior pinnules. The CIDARIS specimen is least similar with *H. opreski* because *H. opreski* has shorter pinnules compared to the stem, has secondary pinnules on lateral primary pinnules, and much higher orders of subpinnulation on the anterior primary pinnules (“heavily subpinnulated” compared to one to two orders of subpinnulation for the CIDARIS specimen). The CIDARIS specimen is also unlike *H.*

heterorhodzos because *H. heterorhodzos* has five to six secondary pinnules arising from one point on anterior primary pinnules with no evidence of tertiary pinnules. Therefore, the CIDARIS specimen is likely to be either *H. americana* or *H. pacifica* because both species have one to two orders of anterior subpinnules, like G73229. These two species differ based on the length of the distal end of the stem above the pinnulated section where *H. americana* has a pinnulated section of ~1 cm followed distally by >7 cm without pinnulation while *H. pacifica* has pinnules extending to near the distal end of the stem. Although G73229 has long eyelash-shaped lateral pinnules, like *H. americana*, the stem tip of the MTQ specimen is broken which makes it difficult to identify the species based on the length of the uppermost unpinnulated section of the stem. These two species also differ in the relative size of spines between lateral and anterior pinnules where, like the CIDARIS specimen, *H. americana* colonies have significantly larger spines on outer convex sides of anterior pinnules while *H. pacifica* has generally equal spine heights between pinnule types. Although this specimen most resembles *H. americana*, the stem tip is broken, and the colony lacks polyps, which are features that would allow the species identification to be more definitive. Also, the CIDARIS specimen has four to five rows of spines visible in one view of lateral pinnules and two rows of spines visible in one view of anterior pinnules, which is more in line with *H. pacifica* (the *H. pacifica* type has four to five and three rows of spines in one view of lateral and anterior pinnules, respectively), but not too different than *H. americana* that it couldn't reveal plasticity for the characteristic (the *H. americana* type has five to eight and two to three rows of spines in one view of lateral and anterior pinnules, respectively).

Distribution: This species is known to occur in the Northwest Atlantic Ocean and Caribbean Sea between the depths of 895 m to 2200 m and this study potentially expands the distribution to the Coral Sea, Australia (Figure 2.1c), a range expansion of >15,000 km. This study also potentially expands the genus (*Heteropathes*) and the family (Cladopathidae) to the Coral Sea, Australia, with range expansions of > 8,000 km and ~2,000 km, respectively.

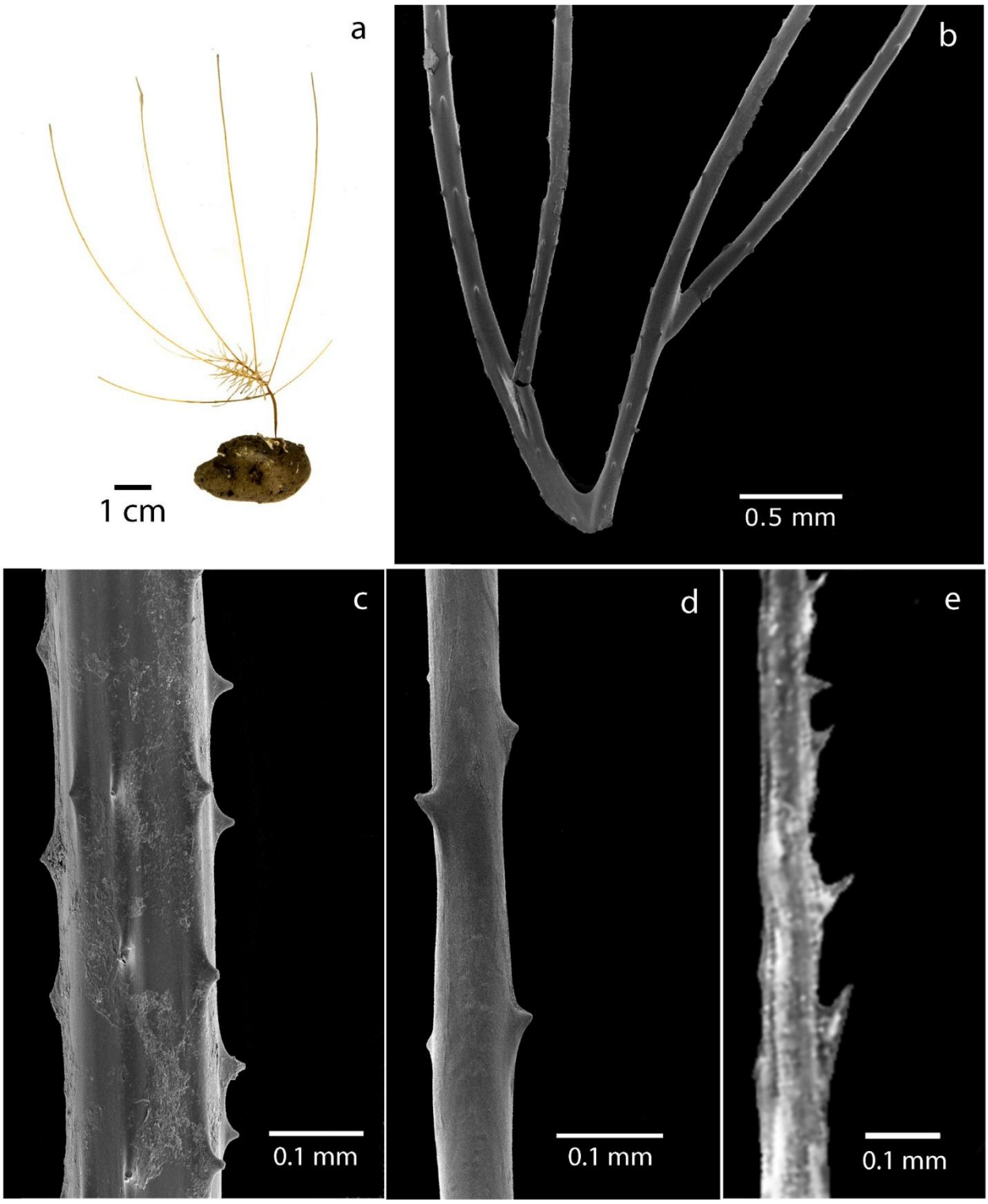

Figure 2.7 *Heteropathes cf. americana:* a, G73229, entire corallum; b, G61967, section of an anterior pinnule with subpinnules; c, G61967, section of lateral pinnule with spines; d–e, G73229, spines on sections of anterior pinnules.

2.5 Conclusion

My results demonstrate that the diversity of black corals in the deep Coral Sea is higher than the single record of *A. lyra* (Brook,1889) recorded previously. All five species identified in the collection represent new records for the region. The large range expansions (> 15,000 km) highlight the lack of sampling in the deep-sea, and I could reasonably expect species ranges to continue to expand as more deep-sea habitats are surveyed. My results show that the species reported here have much larger geographic ranges than recorded previously and provide further evidence that many black corals are cosmopolitan. However, it is important to remember that these species were identified based on traditional morphological taxonomy, and that genetic studies are needed to verify whether morphological species are comprised of multiple cryptic species complexes that are difficult to differentiate morphologically.

This study also demonstrates the utility of museum collections for documenting the occurrence of poorly known species. The specimens reported here were housed, along with other material collected on the CIDARIS expeditions, in the MTQ collection for almost 30 years. The CIDARIS expeditions remain the most complete sampling of biodiversity in the deep western Coral Sea, but much of the material still awaits examination from researchers with sufficient taxonomic expertise. Despite receiving little attention for 30 years, these specimens have now provided new insights into the diversity and taxonomy of black corals in the deep sea. Therefore, my results demonstrate the value of museum collections for documenting biodiversity, particularly in poorly known habitats such as the deep sea. Such knowledge can provide important insights into the consequences of rapid environmental change on species distributions and provide valuable information to inform conservation and management.

Chapter 3 Morphological and molecular description of a new genus and species of black coral (Cnidaria: Anthozoa: Hexacorallia: Antipatharia: Antipathidae: *Blastopathes*) from Papua New Guinea

The proposed revisions of the taxonomy of black corals presented in this chapter are **informal** to avoid *nomen nudum* designations, as per the International Code of Zoological Nomenclature, Article 13 (Ferraris & Eschmeyer 2000). Consequently, this chapter is not a verbatim reproduction of the published version of this paper.

This chapter is published as:
Horowitz, J., Brugler, M. R., Bridge, T. C. L., & Cowman, P. F. (2020). Morphological and molecular description of a new genus and species of black coral (Cnidaria: Anthozoa: Hexacorallia: Antipatharia: Antipathidae: *Blastopathes*) from Papua New Guinea. Zootaxa, 4821(3). https://doi.org/10.11646/zootaxa.4821.3.7

This chapter follows a standard taxonomic format. The results section 3.4 is formatted as follows:

1) Name: Family, genus, and species (when applicable)
2) Diagnosis: succinct description of the family, genus, or species.
3) Discussion: morphological comparison with related families, genera, or species.
4) Molecular results: molecular summary statistics and molecular comparison with related families, genera, or species.
5) Etymology: description of the origin of the name
6) Type material: collection metadata pertaining to the specimens (e.g., date, accession number, latitude, longitude, depth, name of collector, etc.)
7) Type locality: site where specimen(s) were collected
8) Description: detailed description of the species
9) Discussion: evidence incorporated to make taxonomic decision

3.1 Abstract

Blastopathes medusa is described from Kimbe Bay, Papua New Guinea, based on morphological and molecular data. *Blastopathes*, assigned to the Antipathidae, is a large, mythology-inspiring black coral characterized by clusters of elongate stem-like branches that extend out at their base and then curve upward. Colonies are not pinnulate and contain single branches, which could represent new branch cluster formations. Morphological and molecular (mitochondrial DNA and targeted capture of nuclear loci) evidence supporting the establishment of a new genus is discussed. This is the first study to utilize the target capture of ultraconserved elements (UCEs) and exonic loci to elucidate phylogenetic relationships among black corals and to identify and place a new genus and species.

3.2 Introduction

Black corals (Anthozoa: Antipatharia) are colonial hexacorallians that live in all oceans and marine habitats, from shallow water to at least 8,600 m depth (Molodtsova 2006; Wagner *et al.* 2012). Black corals are characterized by polyps with six tentacles and microscopic spines along a skeleton composed of chitin and scleroproteins (Bo *et al.* 2012a). A majority of the 45 genera (containing around 273 species) occur beyond recreational SCUBA diving depths (> 50 m) (Cairns 2007; Opresko 2019; Wagner *et al.* 2012), which makes collecting and conducting in-situ experiments on black corals difficult. Black corals also have few and variable morphological features, leading to uncertainty regarding the number of extant species, their distributions, and phylogenetic relationships within the Order. In addition, slow evolution of mitochondrial genes in black corals (2.3 times slower than octocoral mtDNA) results in low genus and species-level resolution in corresponding phylogenetic trees, making it difficult to properly resolve the taxonomy and systematics of the Antipatharia (Bo *et al.* 2018; Brugler *et al.* 2013).

Due to the many challenges associated with sampling and identifying black corals, the group is often ignored during exploratory expeditions. For example, taxonomists have been exploring Kimbe Bay, Papua New Guinea for over 50 years which has led to the description of many new fish and hard coral species (Allen & Munday 1995; Allen & Randall 1996; Hemond & Vollmer 2010; Wallace 1999); however, not a single black coral taxonomic description has resulted from these efforts. My first visit to Kimbe Bay in 2018 led to the

discovery of a large, mythology- inspiring black coral that has to date gone unreported by researchers.

In this study, I integrate morphological and molecular data to compare *Blastopathes* with other black coral genera to formally describe the new genus based on the new species *Blastopathes medusa*. This new genus is characterized by clusters of elongate stem-like branches that extend out at their base and then curve upward. Based on mitochondrial DNA (mtDNA) *nad5*-IGR-*nad1* (NAD = Nicotinamide Adenine Dinucleotide, IGR = intergenic region) and the targeted capture of ultraconserved elements (UCEs) and exonic loci, *B. medusa* was found to group within the Antipathidae. The targeted capture of UCE/exon loci was recently used to successfully reconstruct the evolutionary origins of Anthozoa (Quattrini *et al.* 2018), with the inclusion of a number of black coral specimens. Subsequently, an enhanced bait set was designed to increase capture efficiency when targeting members of the Subclass Hexcorallia (Cowman *et al.* 2020). Using this enhanced target enrichment bait set for Hexcorallia (Cowman *et al.* 2020), I captured both UCE and exonic loci for phylogenomic analysis within the Order Antipatharia. The resulting phylogeny, in combination with morphological evidence, is used to describe and systematically place the newly discovered genus and species. My results demonstrate that much about black coral biodiversity remains to be discovered, even in a relatively shallow and well-explored habitat like Kimbe Bay, Papua New Guinea.

3.3 Materials and methods

3.3.1 Specimens

The new species was collected from Kimbe Bay, West New Britain Province, Papua New Guinea. Kimbe Bay is located on the north coast of the volcanic island of New Britain in the Bismarck Sea, Papua New Guinea. Kimbe Bay supports shallow reefs along the coast and underwater mountains (seamounts) whose bases can reach depths > 500 m. Five specimens belonging to the new species were collected in March 2019 by SCUBA diving at shallow depths (<40 m) in Kimbe Bay (Figure 3.1). Small (~5 cm) fragments were subsampled from distal sections of terminal branches of each colony for Scanning Electron Microscope (SEM) imaging of the spines. Small tissue samples were also preserved in 100% ethanol for molecular analyses. Morphological descriptions were based on colony branching, spine

morphology, and polyp characteristics. Descriptions and measurement methods of morphological features of black corals are described in Figure 1.2.

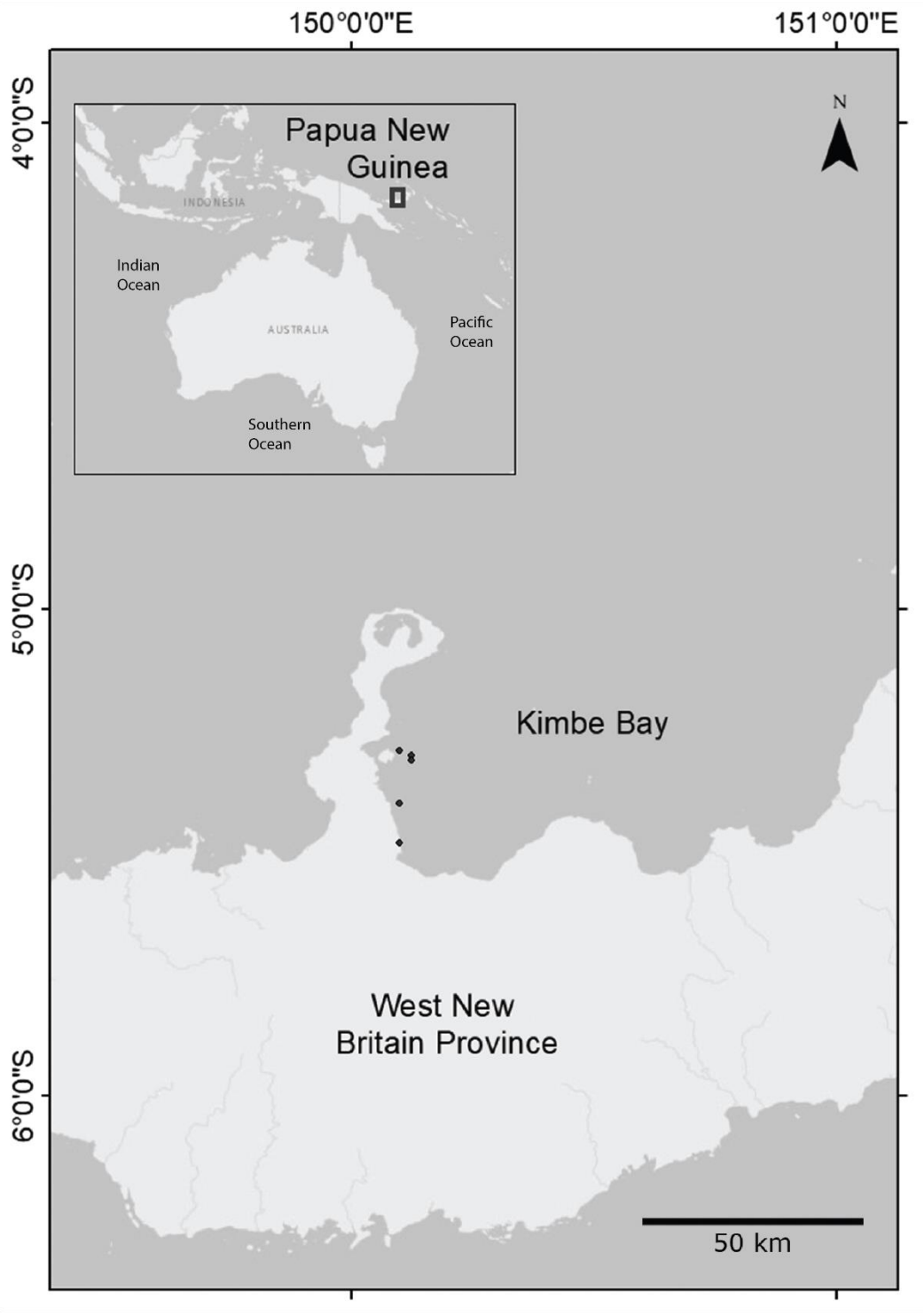

Figure 3.1 Map of collection sites for *Blastopathes medusa.*

3.3.2 Accessioning of type and molecular sequences

Type material is accessioned at the Museum of Tropical Queensland, Townsville, Australia (registration numbers: MTQ G74904–MTQ G74915) and the Papua New Guinea National Museum and Art Gallery (registration numbers: NMAG 1892, NMAG 1893, NMAG 1895–NMAG 1898. All molecular data were deposited in GenBank under accession numbers MN974021–MN974022 (mtDNA), and SRA Genbank BioProject submission PRJNA644402, BioSamples #SAMN15459017 and SAMN15461031–15461062 (UCE/exon).

3.3.3 Molecular analyses

Three specimens representing the new genus collected from Kimbe Bay in March 2019 were sampled for genomic DNA, in addition to non-*Blastopathes* museum specimens collected from all three Oceans (46 and 31 specimens for mitochondrial and UCE/exon analyses, respectively) to place the new genus in the Antipatharia. *Antipathes grandis* Verrill, 1928, (sample ANT14) was also included in the UCE/exon analysis from Quattrini *et al*. (2018) to have an additional member of *Antipathes* compared with the new genus. Occurrence data for *Blastopathes medusa* specimens and Antipatharian specimens included in UCE/exon analysis are detailed in Supplementary Table 3.1 and specimens included in nad5-IGR-nad1 (hereafter, "*igrN"*) analysis can be found in Brugler, Opresko and France (2013). The specimens underwent amplification and Sanger sequencing of the mitochondrial *nad5*-IGR*nad1* region; and high throughput next generation sequencing to target UCEs and exons. The mitochondrial region chosen was based on the results of Brugler, Opresko and France (2013) showing that *igrN* was the most variable region within the black coral mitogenome. The targeted capture approach for UCE/exon loci was chosen due to their ability to resolve phylogenetic relationships across shallow and deep time scales in corals (Cowman *et al.* 2020; Quattrini *et al.* 2018). DNA extraction for all specimens followed the protocol detailed in MacIsaac *et al*. (2013). With regard to the igrN analysis, DNA quantification, PCR primers and reagents, PCR thermocycling profiles, PCR cleanup, cycle sequencing, cycle sequencing cleanup, traditional Sanger sequencing on an ABI-3730xL, and multiple sequence alignment (exception: Gap opening penalty: 1.0; Offset value: 0.0) followed the protocol detailed in MacIsaac *et al*. (2013). While faint PCR products for *igrN* were obtained for the holotype (MTQ G74904), the resulting DNA chromatograms were unreadable; therefore, I was only able to obtain mtDNA sequence data for two specimens

(NMAG 1893 and NMAG 1895). The newly obtained sequence data were added to the *igrN* multiple sequence alignment from Brugler, Opresko and France (2013) in the form of a single representative because the two sequences shared identical haplotypes across 465 comparable bases. A maximum likelihood-based phylogeny was reconstructed using IQtree v1.7 with 1,000 ultrafast bootstrap replicates (Minh *et al.* 2020b). ModelFinder (Kalyaanamoorthy *et al.* 2017) was used to determine the best model scheme for the *igrN* to infer the evolutionary relationship of *Blastopathes medusa* to known taxa within the Order Antipatharia. Species of the family Leiopathidae were used as outgroup samples to root the resulting phylogeny.

The targeted enrichment of UCE/exon loci was carried out using the hexacoral-v2 probe design, a Hexacoral specific bait set that was designed to maximize capture of UCE and exonic loci among hexacorals (Cowman *et al.* 2020). The hexacoral-v2 bait set includes 25,514 baits targeting 2,499 loci (1,132 UCE and 1,367 exon loci) (Cowman *et al.* 2020). The initial concentration of each extracted DNA sample was measured with a Qubit 2.0 fluorometer and sent to Arbor Biosciences (Ann Arbor, MI) for library preparation and sequencing, following details in Quattrini *et al*. (2018). Post-sequencing analyses followed Cowman *et al*. (2020) using the *Phyluce* software (Faircloth 2016). Raw reads were trimmed, assembled, matched to UCE/exon probes and aligned following the steps in Cowman *et al*. (2020) and the Phyluce online documentation (https://phyluce.readthedocs.io/en/latest/tutorial-one.html). Individually aligned UCE/exon loci were filtered to include only those that were present in at least 75% of the samples, which were then concatenated into a single partitioned alignment. Phylogenetic relationships were reconstructed using maximum likelihood in IQtree v1.7 with 1,000 ultrafast bootstrap replicates (Minh *et al.* 2020b). ModelFinder (Kalyaanamoorthy *et al.* 2017) was used to determine the best model scheme for each UCE/exon partition to infer the evolutionary relationship of *Blastopathes medusa* to known taxa within the Order Antipatharia. Read and locus summary statistics from the UCE/exon analysis are detailed in Supplementary Table 3.2. Example of code used for post-sequencing analyses are detailed in section 1 of Supplementary Data 3.1.

3.4 Results

Taxonomy
Family: Antipathidae Ehrenberg, 1834
Genus*: Blastopathes* Horowitz
Species: *Blastopathes medusa* Horowitz, Brugler, Bridge & Cowman
(Figures 3.2–8; Supplementary Tables 3.1–3.2)

Diagnosis: Corallum sparsely branched to the third and sometimes fourth order, not pinnulate. Branches long (up to 1.3 m) and spaced far apart (distances between first order branches and second order branches range from 210 cm to 560 cm) and occurring singly or in verticil-like clusters of varying numbers (as many as 10). Stem and branches thick (up to 6 mm diameter) and rigid. Each branch extending out at their base perpendicular to the stem and lower order branches from which they arise, and then curving upward with distal ends being straight or curved. One branch can extend directly upwards from the center of the cluster. Spines triangular or conical, laterally compressed, smooth, up to 0.34 mm tall. Polyps, ~1.25 mm in transverse diameter, ~6 polyps per cm in one row. Sagittal tentacles (~8 mm in length, extended) are more than twice the length of lateral tentacles (~3 mm in length, extended).

Discussion: *Blastopathes* morphologically resembles *Allopathes* Opresko & Cairns, 1994, which also has stemlike branches coming from a singular location on the corallum (Opresko & Cairns 1994). However, *Blastopathes* differs from *Allopathes* by having branch clusters that do not necessarily occur near the base of the stem and in having more than one branch cluster (Figures 3.2A–B). Additionally, the abpolypar spines of *Blastopathes* are triangular, smooth, and distally slanted while all spines of *Allopathes* are conical with conical tubercles near the apex (Figures 3.2C–D). Other genera that contain sparse and elongate branches are *Pteropathes* Brook, 1889, *Hillopathes* van Pesch, 1910, and in the genus *Antipathes*, *Antipathes dichotoma* Palla, 1766; however, none contain branch clusters. Lastly, *Blastopathes* contains a stiff and non-pinnulate stem and branches that resemble unbranched genera *Pseudocirrhipathes* Bo *et al*., 2009, *Cirrhipathes* de Blainville, 1830, and *Stichopathes* Brook, 1889, all of which differ from *Blastopathes* by lacking branches.

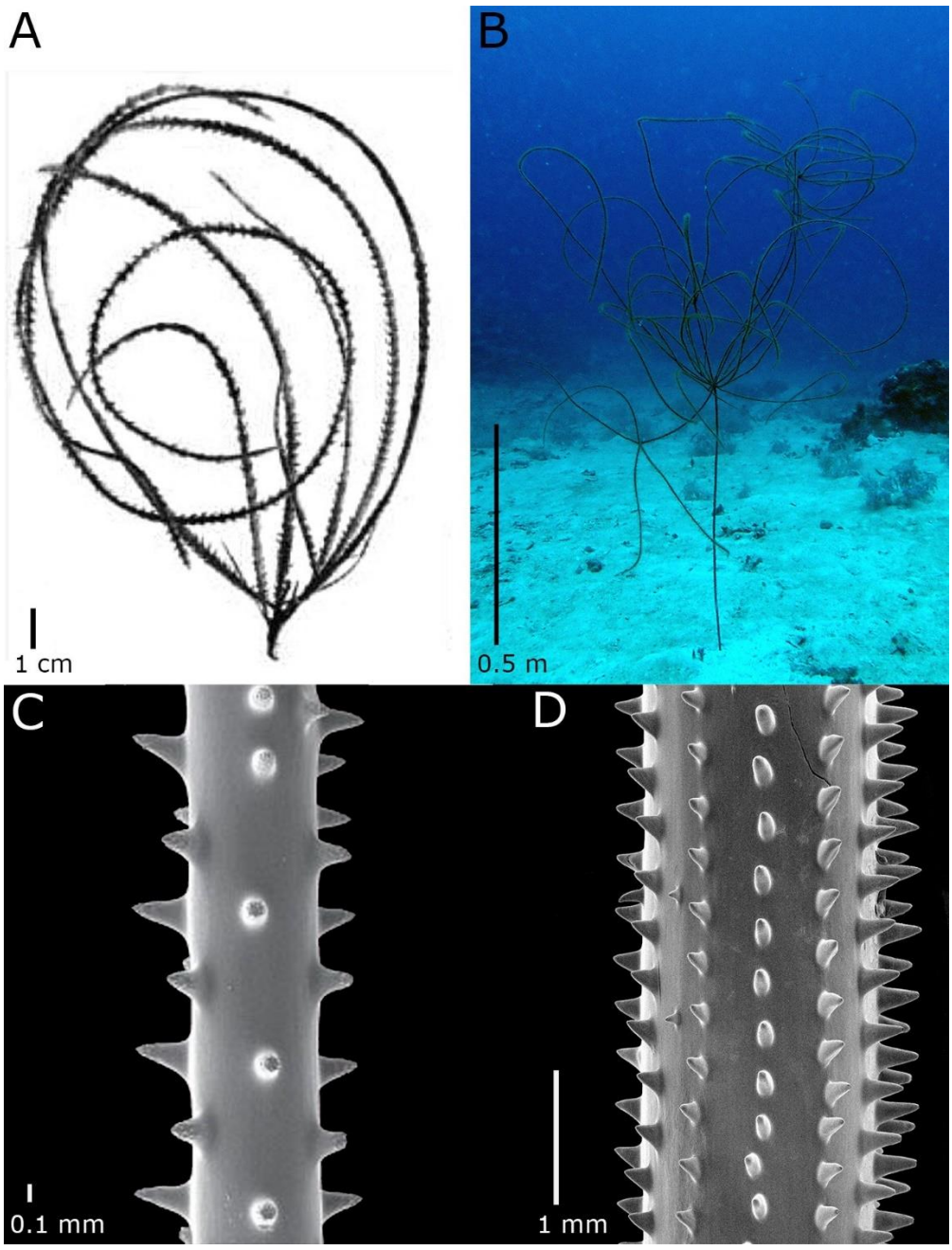

Figure 3.2 Comparison A–B, branching characteristics of *Allopathes denhartogi* and *Blastopathes medusa* (A from *A. denhartogi* holotype RMNH Coel. 31293*, B* from *B. medusa* holotype MTQ G74904); comparison C–D, spine characteristics of *A. denhartogi* and *A. medusa* (C from *A. denhartogi schizoholotype* USNM 1014577, D from B. medusa holotype MTQ G74904).

Molecular results: The mitochondrial igrN sequences for specimens NMAG 1893 and NMAG 1895 consisted of 482 base pairs. The two specimens shared identical sequences across 465 comparable bases, and therefore share one tip in the phylogenetic tree (Figure 3.3A). The complete igrN alignment consisted of 47 sequences, 682 bp, and included species from all seven black coral families. In the 682 bp alignment there were 274 parismony informative site (40%). Targeted capture data for 33 specimens that spanned six of the

seven families in the Antipatharia, resulted in a total number of raw reads ranging from 44,898 to 3,603,888. One sample (10 raw reads, C705) was removed due to sequencing failure. Quality control and adapter trimming resulting in a mean of 1,606,997 ± 1,640,018 SD trimmed reads per sample. Trimmed reads were assembled into a mean of 927 ± 154 SD contigs per sample. The total number of matched UCE/exon loci was 2,309 with an average base pair length of 752 (ranging from 83 to 18,423 base pairs). The 75% taxon occupancy matrix included 286 loci that were concatenated into an alignment with a total length of 111,929 base pairs. A total of 36,052 parismony informative (PI) sites were identified (32% of total sites), with an average of 126.06 PI sites per locus. Alignments were also constructed for the holotype specimen (MTQ G74904) and the two paratype specimens (NMAG 1893 and NMAG 1895). The total number of matched loci across the three samples was 1,290 with an average base pair length of 623 (ranging from 189 to 4,068 bp). A complete (all three samples present in each locus) concatenated matrix included 792 loci, with a total alignment length of 499,264 base pairs. There were 3,855 variable sites (~0.7% of total sites) among the three samples.

Despite the difference in species-level sampling, the maximum likelihood phylogenies displayed similar topologies for both alignment types (igrN, UCE/exon). In both cases, the new genus formed a distinct lineage within the family Antipathidae and members of the genera *Cirrhipathes* and *Antipathes* formed separate monophyletic groups (Figures 3.3A–B). Differences between the two trees include the UCE/exon tree suggesting that *Arachnopathes* Milne Edwards H., 1857, and *Stichopathes* also share a common ancestor with *Blastopathes,* while in the *igrN* tree *Stichopathes* is more closely related to another lineage containing members of the Aphanipathidae Opresko, 2004, than to *Blastopathes*.

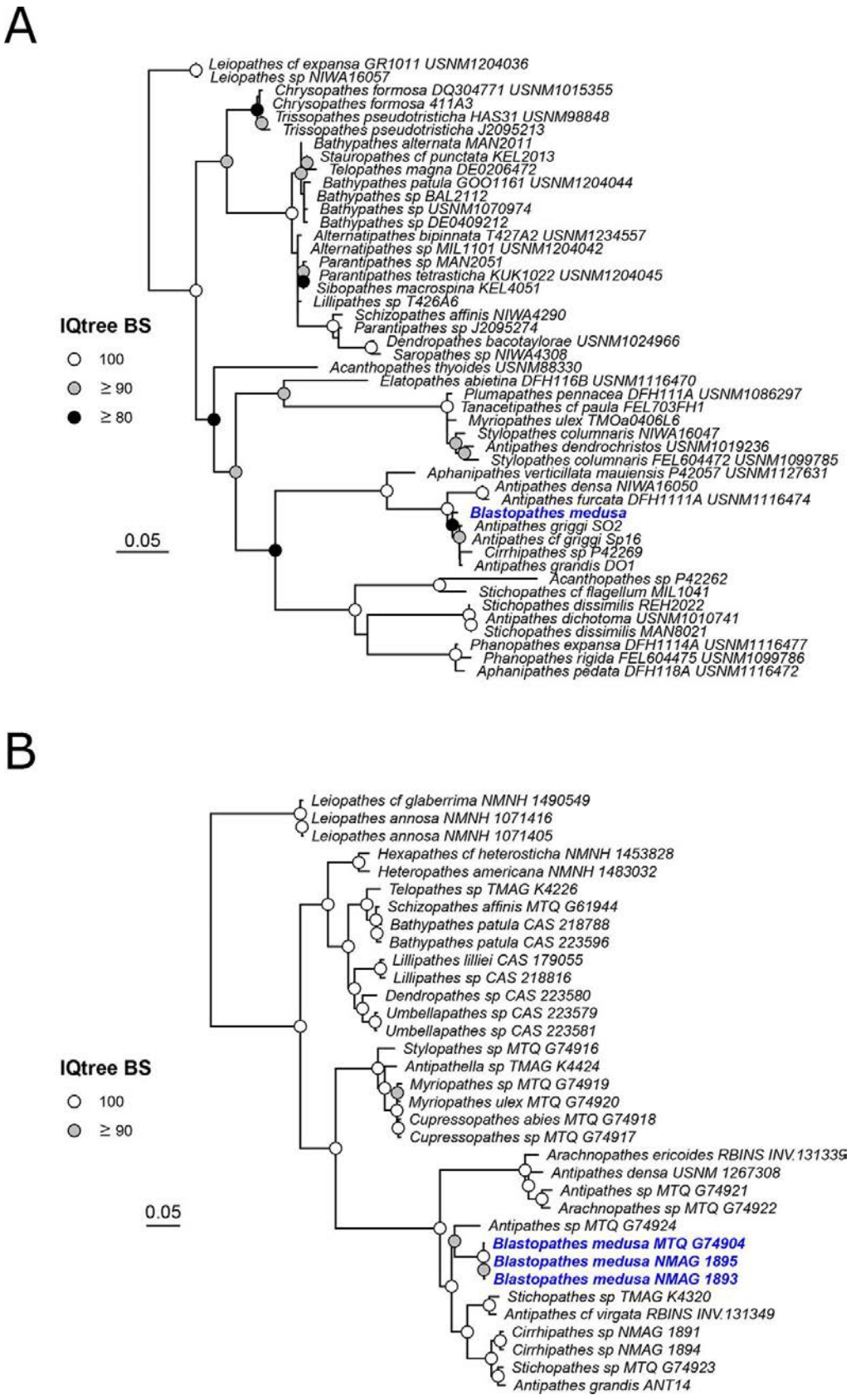

Figure 3.3 Maximum likelihood-based phylogenetic reconstructions: A, using igrN sequence data; B, using UCE and exonic sequence data. IQtree BS is the bootstrap support and relates to bootstrap support at nodes on each tree. Tip labels are as follows: genus, species, and accession identification.

Etymology: From the Greek “blastos”, germ, sprout, or shoot, in reference to the branch cluster features, and the commonly used suffix “pathes”. From the Latin “Medusa” in reference to thick and upward curving branches, like the snakes on the mythical gorgon’s head.

Type material: Holotype, MTQ G74904, Papua New Guinea, Bismarck Sea, West New Britain Province, Kimbe Bay, Vanessa’s Reef, 35m depth, 13 March 2019 (SEM stubs MTQ G74906 to MTQ G74910, schizoholotype NMAG 1892). Paratypes, NMAG 1893, Papua New Guinea, Bismarck Sea, West New Britain Province, Kimbe Bay, Christine’s Reef, 30m depth, 13 March 2019; MTQ G74911, Papua New Guinea, N Bismarck Sea, West New Britain Province, Kimbe Bay, Lady Di, 37m depth, 15 March 2019 (SEM stub MTQ G74912); MTQ G74913, Papua New Guinea, Bismarck Sea, West New Britain Province, Kimbe Bay Restrf Island, 30m depth, 16 March 2019 (SEM stub MTQ G74915); NMAG 1895, Papua New Guinea, Bismarck Sea, West New Britain Province, Kimbe Bay, Christine Reef, 30m depth, 16 March 2019.

Type locality: Kimbe Bay, Papua New Guinea. Latitude: -5.305; Longitude: 150.124

Description: The holotype is a 1.2 m tall specimen that branches to the third order (Figure 3.4). The stem is 0.6 m in length, 6.3 mm in diameter near the base, and 4 mm in diameter just below the first branch cluster. First branch cluster occurs at the apex of the stem and consists of 10 elongate branches extending in different directions (Figure 3.5A) of varying lengths (maximum length 1.3 m) and diameters (none thicker than the stem). One first order branch is 0.6 m in length, 4 mm in diameter near the base, and 2 mm in diameter just below a second order branch cluster consisting of ~10 branches (Figure 3.5B), with a maximum branch length of 50 cm. Another first order branch is 1.3 m in length, 3 mm in diameter near the base, and 0.5 mm at the branch tip and does not produce a branch cluster. Another first order branch extends from the center of the branch cluster and extends 0.9 m directly upwards with a branch thickness of 4 mm near the base and 2 mm just below a second order branch cluster consisting of four branches with a maximum branch length of 65 cm. Another first order branch extends 5 cm, is 2 mm in diameter near the base and increases to 2.5 mm just below what resembles a new branch cluster consisting of three branches of different lengths and thicknesses. The longest of the three

branches coming from the 5 cm branch is 0.6 m in length, 1.5 mm in diameter near the base, and 0.5 mm diameter near the tip. The second longest branch is 0.42 m in length, 0.2 mm in diameter near the base, and 0.1 mm near the tip. The shortest of the three branches is 5 cm in length, 0.8 mm in diameter near the base, and <0.1 mm near the tip. Branchlets are found on all branch clusters (Figure 3.5B) and locations on branches that seem to be newly forming branch clusters (Figure 3.5C) ranging from 1 cm to 8 cm in length, with ~0.5 mm diameters at their bases and <0.5 mm diameters at branch tips.

The spines (Figures 3.6A–C) on the branches and stem are smooth and laterally compressed. Polypar spines are 0.2 mm–0.34 mm tall, are conical at right angles to branch axes with rounded apexes, and spines are spaced ~0.45 mm in one row (Figure 3.6A). Abpolypar spines are 0.12 mm–0.24 mm tall and are triangular with distally slanted proximal edges and perpendicular or proximally slanted distal edges, and spines are spaced ~0.38 mm in one row (Figure 3.6B). Seven to eight, sometimes offset rows of spines can be counted in one view of a branch and stem, and approximately three spines can be counted per mm of a spine row on a branch (Figure 3.6C) and stem. The polyps (Figures 3.7A–B), olive in color when alive, are arranged in a single row on thin branches (Figure 3.7A), and in multiple rows on the stem and thick branches (Figure 3.5A). *In-situ* measurements reveal that lateral tentacles (~3 mm in length, extended) are less than half the length of sagittal tentacles (~8 mm in length, extended) (Figure 3.7A). Polyps range 0.5 mm–1.38 mm in transverse diameter and are spaced ~1 mm apart, resulting in approximately five polyps per cm in one row (Figure 3.7B). The sizes of contracted and extended polyps are quite variable.

Figure 3.4 *Blastopathes medusa* holotype (MTQ G74904): Lateral view of corallum.

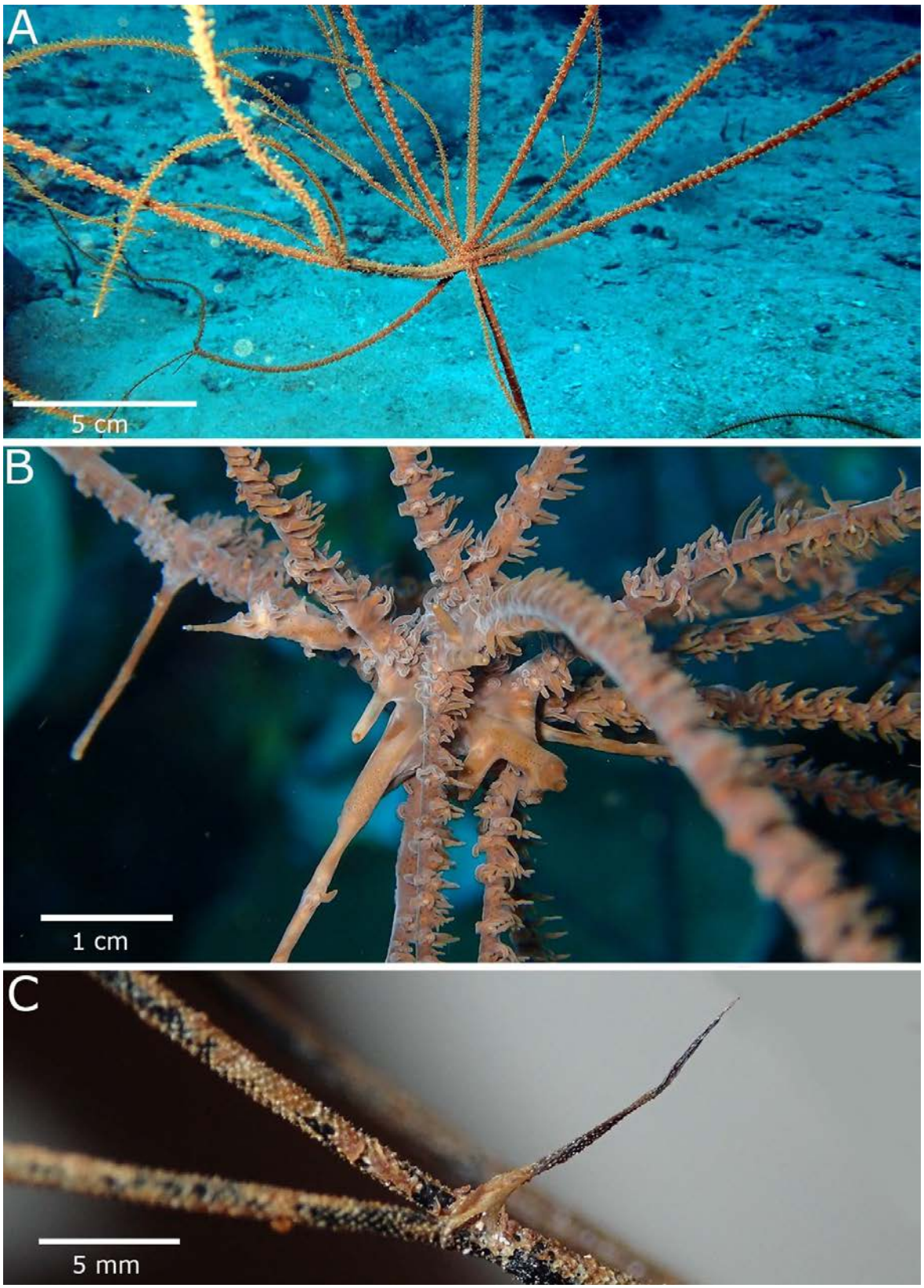

Figure 3.5 *Blastopathes medusa* holotype (MTQ G74904): A, branch cluster on stem; B, branch cluster on branch; C, branchlet on branch.

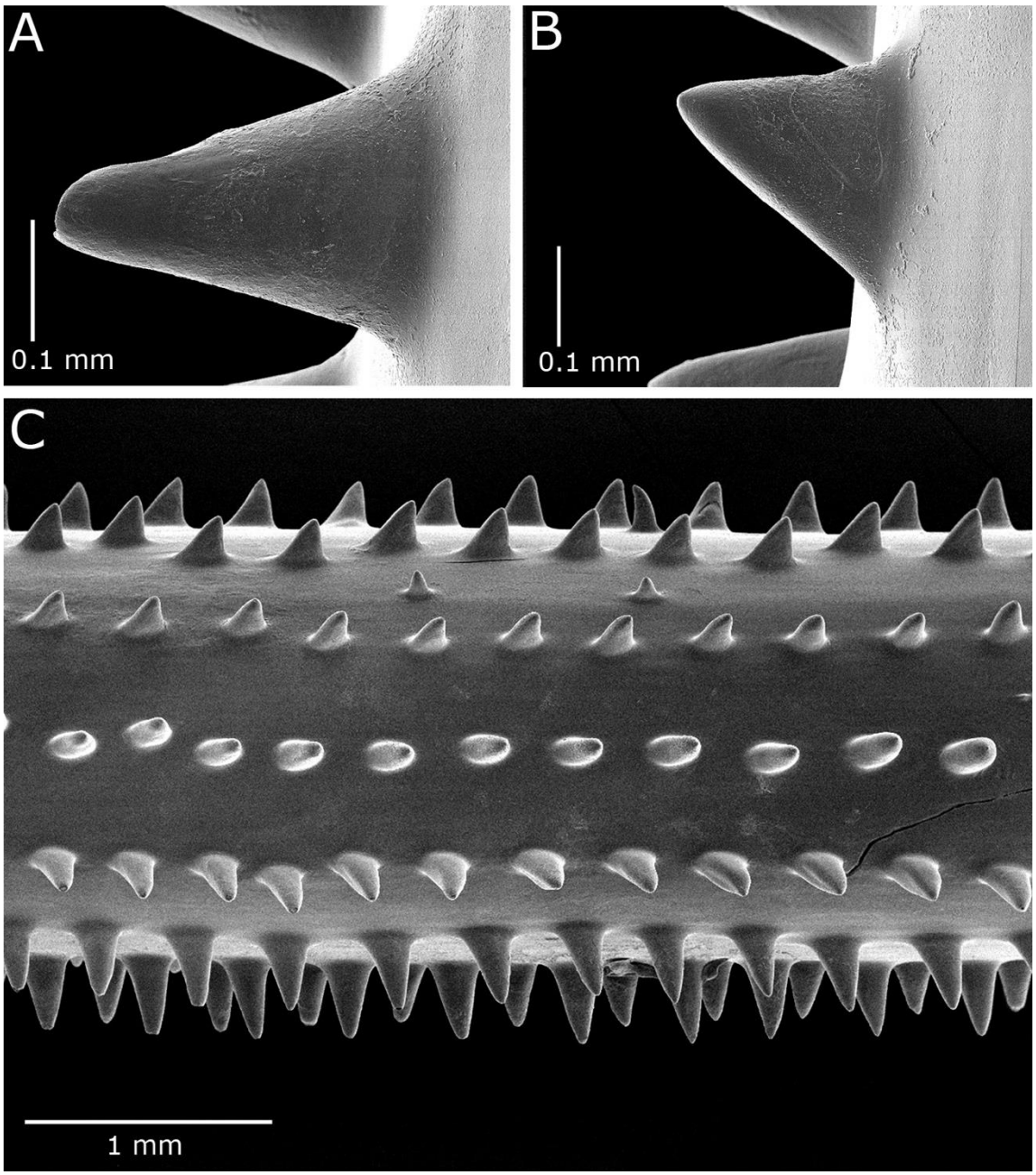

Figure 3.6 *Blastopathes medusa* holotype (MTQ G74904): A, polypar spines on terminal branch; B, abpolypar spines on terminal branch; C, section of terminal branch.

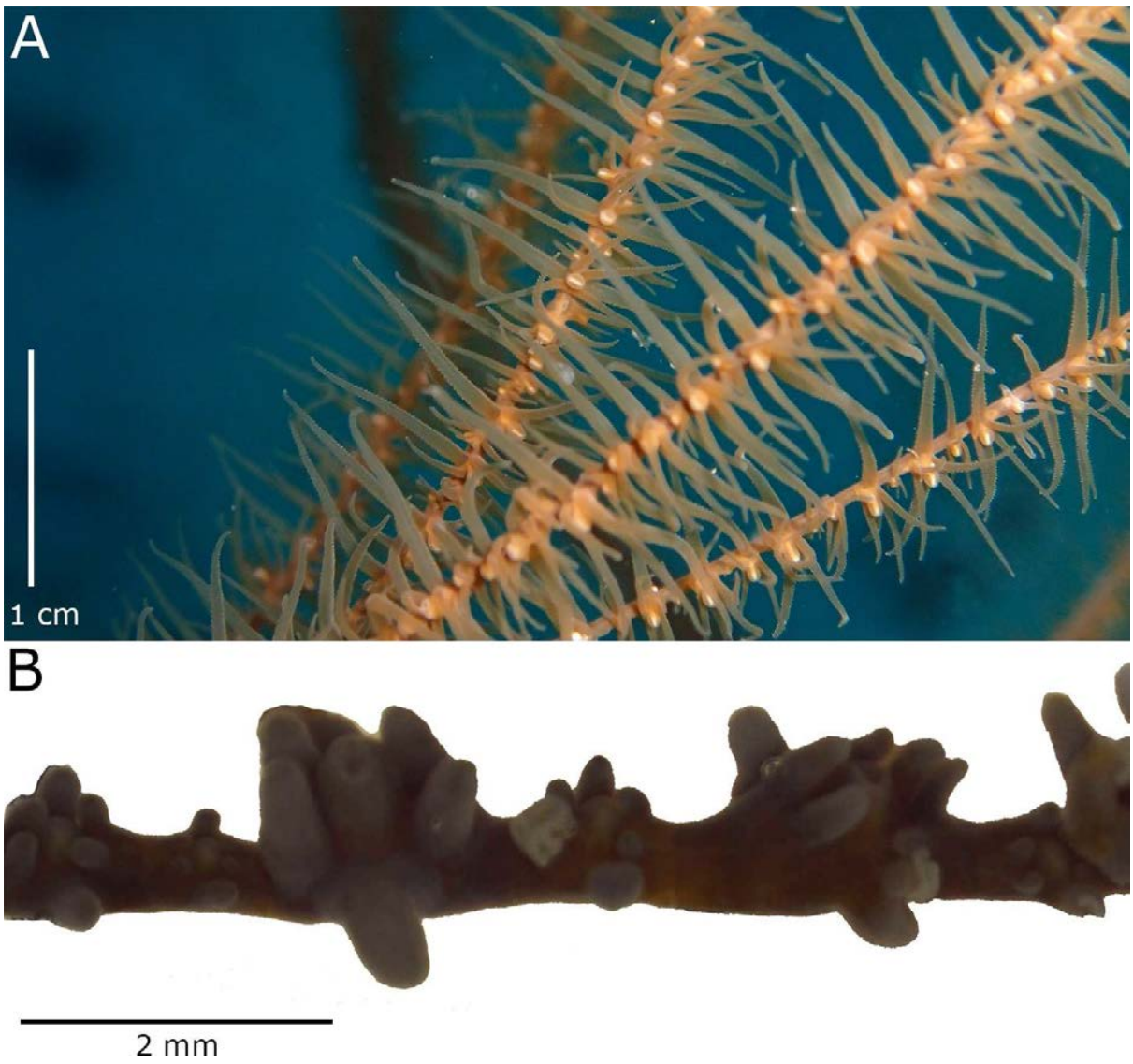

Figure 3.7 *Blastopathes medusa* holotype (MTQ G74904): A, single row of polyps on multiple terminal branches; B, interpolypar space on terminal branch.

The paratypes (Figures 3.8A–E) range from 0.4 m to 0.5 m in height. The stem lengths range from 3 to 10 cm in length from basal plate to the first branch cluster. All paratypes have branch clusters (Figures 3.8A–C), seven to eight rows of compressed spines, tall and conical polypar spines, and triangular distally slanted abpolypar spines (Figure 3.8D), with polypar spine heights ranging from 0.14 mm to 0.3 mm and abpolypar spines ranging from 0.1 mm to 0.2 mm. About five polyps per cm with lateral tentacles less than half the length of sagittal tentacles, with varying polyp sizes like the holotype (Figure 3.8E).

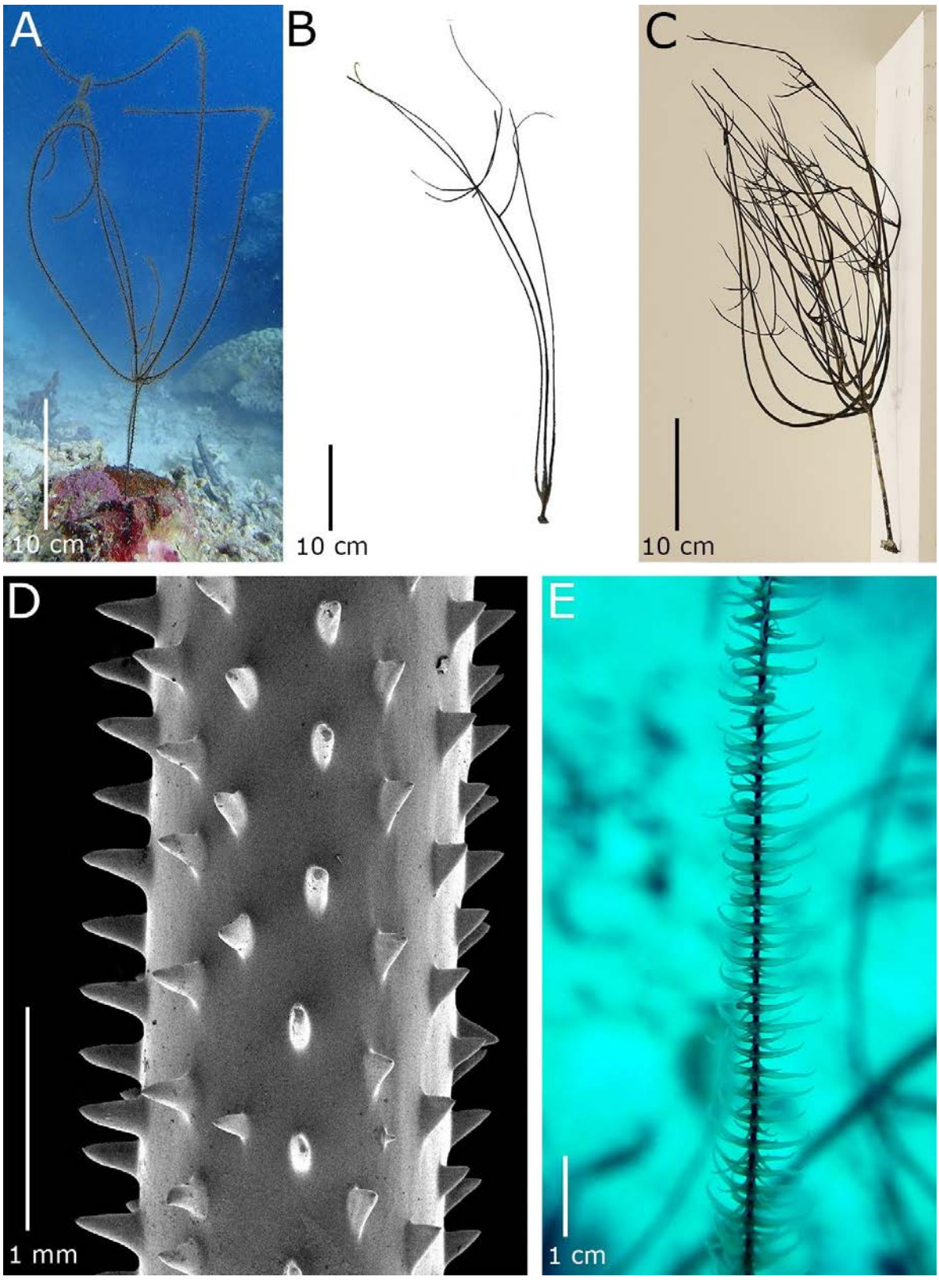

Figure 3.8 *Blastopathes medusa* paratypes: A–C, in-situ images of colonies showing branch clusters (A from paratype MTQ G74911; B from paratype NMAG 1893; C from paratype MTQ G74913); D, paratype (MTQ G74912): section of terminal branch showing eight rows of compressed spines; E, paratype (NMAG 1893): section of branch showing polyp density and tentacle lengths.

Discussion: *Blastopathes* is placed in the Antipathidae because of its morphologically distinct branch clusters that form on the stem and branches, as well as its molecular affinity to members in the Antipathidae. However, it remains unclear which morphological features, if any, represent taxonomic boundaries within the new genus. Even within the holotype specimen there is variation regarding the location of clusters along the corallum, branch thicknesses in relation to branch lengths, and polyp characteristics. It is unknown whether branch complexity relates to colony age or if it is a plastic character that differs based on predator-induced injuries (e.g., fish bites), associates (e.g., scale worms, crabs, brittle stars, etc.), epibionts (e.g., barnacles, anemones, etc.), and/or prevailing currents. The two molecular datasets used in this study (igrN, UCE/exon capture) show similar topologies, but different levels of resolution. The mitochondrial igrN sequences for the two paratypes were identical at 456 sites, while all three type specimens contained >38,000 variable sites in UCE/exon alignment. While target capture of UCE/exon loci provides higher resolution than single-locus markers and seems to resolve polytomies found in igrN and other single-locus marker analyses (Brugler *et al.* 2013), only a small percentage of the Order was included in this analysis and only three specimens representing the new genus were sequenced for this study. Therefore, it is premature to define taxonomic boundaries based on molecular distances at this stage. It is also premature to determine which genus is sister to *Blastopathes* because the closest non-*Blastopathes* specimen is a deep (440 m depth), small and short stemmed, anastomosing and complexly branched, flabellate colony with irregularly formed spines (*Antipathes* sp. MTQ G74924) that bears no resemblance to the new genus. There are likely numerous species with greater molecular affinity to the new genus that were not included in this study. Given its long and sparsely branched features, it will be interesting to determine if *Blastopathes* has non-branched ancestors to better understand the evolutionary history of branching.

3.5 Conclusion

Much remains unknown about this new genus and what variation looks like at the population level for different species with regards to UCEs and exon data. Therefore, I must compare morphological features and their associated UCE/exon data between and within all black coral species. Doing so will result in significant changes to the current understanding of black coral taxonomy and evolutionary history (Opresko 2019; Quattrini *et al.* 2018).

Chapter 4 Phylogenomic systematics of black corals (Anthozoa; Antipatharia) from the Great Barrier Reef and Coral Sea

This chapter follows a standard taxonomic format. The results section 4.4 will first have a summary of the proposed taxonomic revisions and new species descriptions with references to results that support the proposed revisions. **To avoid any potential issues with the International Code of Zoological Nomenclature surrounding the descriptions new species in a thesis, taxonomic revisions and species descriptions in this chapter are informal. Formal taxonomic revisions and species descriptions will be published in a taxonomic journal**. The manuscript version of this chapter will include the new species, genera, and family names, holotype designations, and etymologies, which are purposefully excluded from this manuscript to avoid *nomen nudum* designations, as per the International Code of Zoological Nomenclature, Article 13 (Ferraris & Eschmeyer 2000). To denote a species, genus, or family that is undescribed and informally described herein, I use sp. undesc. (i.e., undescribed species) gen. undesc. (i.e., undescribed genus), and fam. undesc. (i.e., undescribed family).

After the summary of results, the rest of the section will follow the following format:

1) Name: order, family, genus, and species (when applicable).
2) Synonymies: previous taxonomic placement of family genus, or species (if applicable).
3) Type species: species that represents the family or genus (if applicable).
4) Species assigned to family or genus: list of species proposed to be included in new family or genus (if applicable).
5) Material examined: information about where and when the specimen(s) was collected (if applicable).
6) Diagnosis: succinct description of the family, genus, or species.
7) Discussion: morphological and/or molecular comparison with related families, genera, or species.
8) Type locality: site where specimen(s) were collected.
9) Description: detailed description of the species.
10) Discussion: evidence incorporated to make taxonomic decision.

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Chapter 5 Bathymetric evolution of black corals through deep time

This chapter is under review by the Proceedings of the Royal Society B: Biological Sciences

5.1 Abstract

Deep-sea lineages of many benthic invertebrates are thought to have evolved from shallow-water ancestors, but this hypothesis has yet to be tested for many taxonomic groups. Corals are significant contributors to the faunal diversity of the deep sea, but how and when lineages invaded this biome remain unclear. Here, I show that black corals (Anthozoa: Antipatharia) originated in slope depths (~250–1,999 m depth) in the early Ordovician and diversified in both offshore and onshore directions. Offshore-onshore diversification occurred once in 400 My, suggesting a lack of evolutionary refugia for shelf taxa and a substantial risk of losing these lineages during large-scale shallow-water disturbances. All subsequent transitions are offshore, one shelf lineage expanding back to the slope, and four independent slope lineages in the last 30 My have invaded the abyss (2,000 to >8,000 m). I also show that pinnules, specialized branch features that maximize surface area, first appeared following the Triassic-Jurassic extinction, a time in which marine productivity collapsed. This morphological adaptation, which was never lost, enhanced the ability to filter feed when nutrition levels were low, and potentially aiding in some black coral lineages invade deeper habitats with lower nutrition availability.

5.2 Introduction

How taxa invade novel habitats is fundamental to understanding the evolutionary processes underpinning global patterns of biodiversity. Colonization of novel habitats aided by evolutionary innovations across deep time has led to the radiation of groups across wide bathymetric ranges, high species diversity, and evolutionary success of lineages across the tree of life (Campoy *et al.* 2020; Rabosky 2017). However, there is a lack of knowledge surrounding the mechanisms that facilitate lineage expansion into new habitats, such as the environmental or ecological conditions that precipitate invasion of novel habitats, how frequently these events occur, which morphological features enable successful expansion into new habitats, or the ancestral origins of these lineages (Campoy *et al.* 2020; Ord & Cooke 2016; Ord & Hundt 2020). These knowledge gaps are especially pronounced for

groups with limited fossil records and lineages that occur across a wide range of biomes, such as the surface waters to the deep sea (Costello & Chaudhary 2017; Gaither *et al.* 2016).

One prevailing hypothesis to explain the origin of deep-sea fauna is an onshore-offshore pattern of faunal change where species originate in shallow shelf habitats (0–249 m depth) and subsequently invade slope (~250–1,999 m depth) and abyssal habitats (>2,000 m depth) (Berry 1972; Eldredge 1974; Jablonski *et al.* 1983). This onshore to offshore transition was first proposed by Jablonski et. al. (1983) who found that marine benthic fossil shelf communities of the Cambrian-Ordovician (~500 Ma) were more similar taxonomically with slope fauna of the Cretaceous (~100 Ma) than to slope fauna of the Cambrian-Ordovician. This hypothesis is supported by some marine groups (Anon 1880; Chan *et al.* 2021; Jacobs & Lindberg 1998; Smith & Stockley 2005), but other groups demonstrate onshore patterns of diversification (Lindner *et al.* 2008; Pante *et al.* 2012; Stolarski *et al.* 2011; Thuy 2013). This suggests that an onshore-offshore pattern might not be a general rule, and there has been little research to specifically investigate which morphological innovations and/or adaptive features are required to invade and persist within new habitats (Ord & Hundt 2020).

Anthozoans (sea anemones and corals) provide a model taxon to understand invasion and persistence in novel habitats because the group has a long evolutionary history spanning the entire Phanerozoic and has colonized every marine habitat (Gadelha *et al.* 2012; Quattrini *et al.* 2020). Black corals (Hexacorallia: Antipatharia) are an anthozoan lineage whose origins can be traced back over 300 Ma (Quattrini *et al.* 2020) and occur across a wide range of habitats from the tropics to the poles and from surface waters to depths over 8,000 m (Molodtsova *et al.* 2008; Pasternak 1977). Black corals are ecologically important because they provide habitat to many other invertebrates, with a recorded 2,554 invertebrates found living on one black coral colony (Love *et al.* 2007). They are also economically important because they are thought to ward off evil spirits and disease (Anti= against, pathos= disease) and are therefore harvested and sold all over the world, often as jewellery (Wagner *et al.* 2012). Black corals also have distinct morphological differences between shallow and deep taxa, most notably the dominance of pinnules among branching species in the lower slope and abyss. Pinnules are specialized branches of consistent length,

spacing between branches, and angles (Opresko 2002; Opresko *et al.* 2016) (Figures 5.1A-C; comparison of non-pinnulate and pinnulate morphologies). Pinnules increase the ability of a coral to filter-feed, and potentially has facilitated invasion into, and persistence within new habitats.

Here, I test the onshore-offshore pattern of evolution through deep time and assess whether the acquisition of novel morphological adaptations can explain these patterns. To test this hypothesis, I reconstruct a time-calibrated phylogeny based on target-capture enrichment of 2,479 conserved loci (ultraconserved elements and exonic loci) (Cowman *et al.* 2020) from 50 taxa (Supplementary Table 5.1). I then used a Dispersal-Extinction-Cladogenesis (DEC) model to estimate ancestral depth ranges. I also traced the evolution of pinnules to determine if they were inherited from ancestral antipatharians or derived as an innovation that enabled invasion into novel depths.

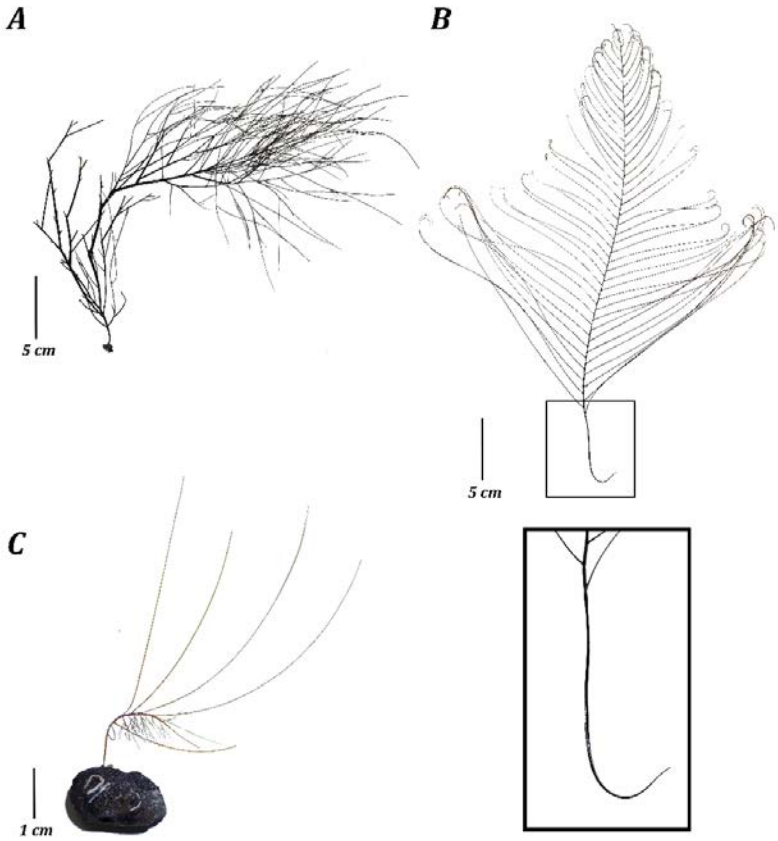


Figure 5.1 Morphological features of the Antipatharia. a *Antipathes sp.* Pallas, 1776 colony that is branched and non-pinnulate (A), a *Schizopathes affinis* Brook, 1889 with pinnulate branches and a basal hook, enlarged in inset (B), and a Heteropathes americana with a curved stem and pinnulate branches (C).

5.3 Methods

5.3.1 Sample collection

Fifty taxa were chosen for this study to incorporate pinnulate and non-pinnulate black coral species that occur at shelf, slope, and abyssal depths; with additional non-antipatharians to serve as outgroup taxa. Nine black coral taxa were included from Quattrini et al. (2020), 24 black coral taxa were included from Horowitz et al. (2020 [Chapter 3 in this thesis]), and building upon these studies I include targeted capture data for nine new black coral specimens (Supplementary Table 5.1). Specimens were collected by hand, trawl, or via remotely operated vehicle and deposited in museums. Among the nine new black coral taxa, seven taxa had tissue subsampled and donated from various museums to the Museum of Tropical Queensland, and two were hand-collected by JH in 2019 at 14 m depth from Orpheus Island, Great Barrier Reef under permit G19/39364.1, and deposited in the Museum of Tropical Queensland. Eight samples with UCE/exon sequence data representing the Actiniaria and the Zoantharia from Quattrini et al. (2020) were included as outgroups for time-calibration purposes (specimen information detailed in Supplementary Table 5.1).

5.3.2 DNA Extraction, library preparation and targeted enrichment

Details about DNA extraction, library preparation and targeted enrichment can be found in Section 3.3.3. In this chapter, individually aligned UCE/exon loci were filtered to include only those that were present in at least 50% of the samples, resulting in 1,063 loci that were then concatenated into a single partitioned alignment. Example code of the post-sequencing analyses are detailed in Sections 1 of Supplementary Data 3.1.

5.3.3 Phylogenomic reconstruction and time calibration

IQtree v1.7 (Minh *et al.* 2020a) was used to reconstruct a maximum likelihood tree and estimate ultrafast bootstrap support, gene concordance factor, and site concordance factor at each branch node (see explanation of metrics in Section 4.3.2). IQtree v1.7 was also used to create 1,063 individual bootstrap trees, one for each locus post-filtering 50% taxon occupancy. Newick utilities v1.6 (Junier & Zdobnov 2010) was used to remove low support branches (< 30% bootstrap support) following the Astral III (Zhang *et al.* 2018a) online tutorial (https://github.com/smirarab/ASTRAL/blob/master/astral-tutorial-template.md). TreeShrink was used to remove outlier long branches from individual gene trees and corresponding gene alignments, following the online documentation

(https://github.com/uym2/TreeShrink) (Mai & Mirarab 2018). IQtree was again used to reconstruct individual bootstrap trees from the cleaned alignments, and then ASTRAL-III, a multi-species coalescent method, was used to estimate the resulting species tree (Zhang *et al.* 2018a) from the individual gene trees. Example of the code used to create individual bootstrap trees and the ASTRAL tree is detailed Section 2 of Supplementary Data 3.1.

SortaDate (Smith *et al.* 2018) was used to identify the 25 most 'clock-like' loci (i.e., loci with properties of moderate length trees) from the set of 1,063 loci, which were used for this analysis, as per Oliveros (2019). The maximum likelihood phylogeny was used as a starting tree for time-calibration using BEAST v2.6.3 with four secondary calibration points selected from Quattrini et al. (2020); Zoantharia crown node (436 Ma, 95% HPD 336-531), Actiniaria crown node (513 Ma, 95% HPD 424-608), the black coral crown node excluding the Leiopathidae (321 Ma, 95% HPD 249-407), and the Zoantharia + Actiniaria crown node (642 Ma, 95% HPD 542-746) with normal distribution priors matching these HPDs. A relaxed clock model was used with a lognormal distribution on the ucld mean and uniform distribution on the ucld. stdev (initial 0.1, 0-1 bounds), as per Quattrini et al. (2020). The topology was fixed with the maximum likelihood tree created in IQtree tree, to ensure deep nodes were congruent with the corresponding node calibration from Quattrini et al. (2020). Three individual BEAST runs (see BEAST xml file in Dataset S2) of 250 million generations were completed, with resulting log and tree files combined in LogCombiner (Bouckaert *et al.* 2019) after the removal of 10% of generations as a burnin period. Tracer v1.7.1 (Rambaut *et al.* 2018) was used to assess convergence of parameter values and age estimates, and TreeAnnotator (Bouckaert *et al.* 2019) was used to produce a maximum clade credibility tree using the ML phylogeny as the target tree and mean node heights. Example of the code used to produce the BEAST phylogeny are detailed in Section 3 Supplementary Data 3.1.

5.3.4 Ancestral state reconstruction

Stochastic character mapping (Huelsenbeck *et al.* 2003) was used to estimate ancestral states of pinnulation. Each taxon was assigned a state for pinnulation (See Supplementary Table 5.2; pinnulate or non-pinnulate). Posterior probabilities using a non-symmetric model were generated from 100 stochastic character maps using the make.simmap function in the R package Phytools (Revell 2012) where pie charts at nodes

represent state likelihoods. A Dispersal-Extinction-Cladogenesis (DEC) model was implemented in RevBayes (Höhna *et al.* 2016) to estimate ancestral states of depth ranges, following the DEC analysis online tutorial (https://revbayes.github.io/tutorials/biogeo/biogeo_simple.html). Expert opinions (D Opresko, T Molodtsova and M Bo) were used to assign each taxon a depth range (shelf 0-249 m, slope 250-1,999 m, abyss >1,999 m), or a combination of depth ranges for wide ranging taxa (See Supplementary Table 5.2). An MCMC analysis was run to produce a maximum clade credibility tree using plot_anc_states in R package RevGadgets. The ggtree package (Yu 2020) was used to combine pinnulation and depth ancestral states on one time-calibrated tree, following code provided in McFadden *et al.* (2021). Example of the code used for the ancestor state reconstructions are detailed in Section 4 Supplementary Data 3.1.

5.4 Results

5.4.1 Black coral evolution and systematics

This study provides a phylogenomic perspective of the Antipatharia resolving the relationships of 26 out of 45 valid genera in the Order, representing diverse morphologies from upper shelf (<5 m) to abyssal (>8,000 m) depths. From a dataset of 1,063 conserved loci (50% taxon occupancy), both maximum likelihood (ML) and multi-species coalescent (MSC) analyses recovered congruent topologies with strong node support (Figures Supplementary Figures 5.1 and 5.2). A time-calibrated phylogeny estimated from the 25 most 'clock-like' loci dates the oldest black coral node at 443 Ma (95% HPD 347–544) which represents the crown divergence of the Antipatharia. This robust, time-calibrated phylogeny allowed us to estimate the depth ranges of ancestral antipatharians, thereby testing the onshore-offshore hypothesis.

5.4.2 Radiation from slope to shelf and abyss

Black corals first diversified 443 Ma (95% HPD 347–544) at slope depths, between 250 and 1,999 m (Figures 5.2 and Supplementary Figure 5.3). Taxa representing the two oldest extant lineages likewise occurred at slope depths (*Leiopathes glaberrima* (Esper, 1792) and *Leiopathes annosa* Wagner & Opresko 2015) [443 Ma], and *Acanthopathes thyoides* (Pourtalès, 1880) [336 Ma]). A deep divergence for the group occurred 295 Ma (95% HPD 235-354 Ma) during the Carboniferous-Permian. One lineage has a crown node

date of 245 Ma (95% HPD 191–300) and consists of mostly shelf taxa between 0 and 249 m depth (hereafter ‘Clades A1 and A2’) and the other with a crown node date of 183 Ma (95% HPD 121–250) and consists of slope (250 to 1,999 m) and abyssal (> 1,999 m) taxa (hereafter ‘Clade B1’) that collectively contain 263 out of 279 extant species. The estimated ancestral depth state of Clade A is in the shelf (Figures 5.2 and 5.3), suggesting that around 250 Ma, black corals invaded shelf habitats from the slope. The estimated ancestral depth state of Clade B is in the slope (Figures 5.2 and 5.3) where associated lineages remain until ~30 Ma when four different lineages that contain five extant taxa transitioned offshore to invade the abyss. Although both onshore-offshore and offshore-onshore patterns occurred, offshore-onshore movement occurred only once in over 400 My.

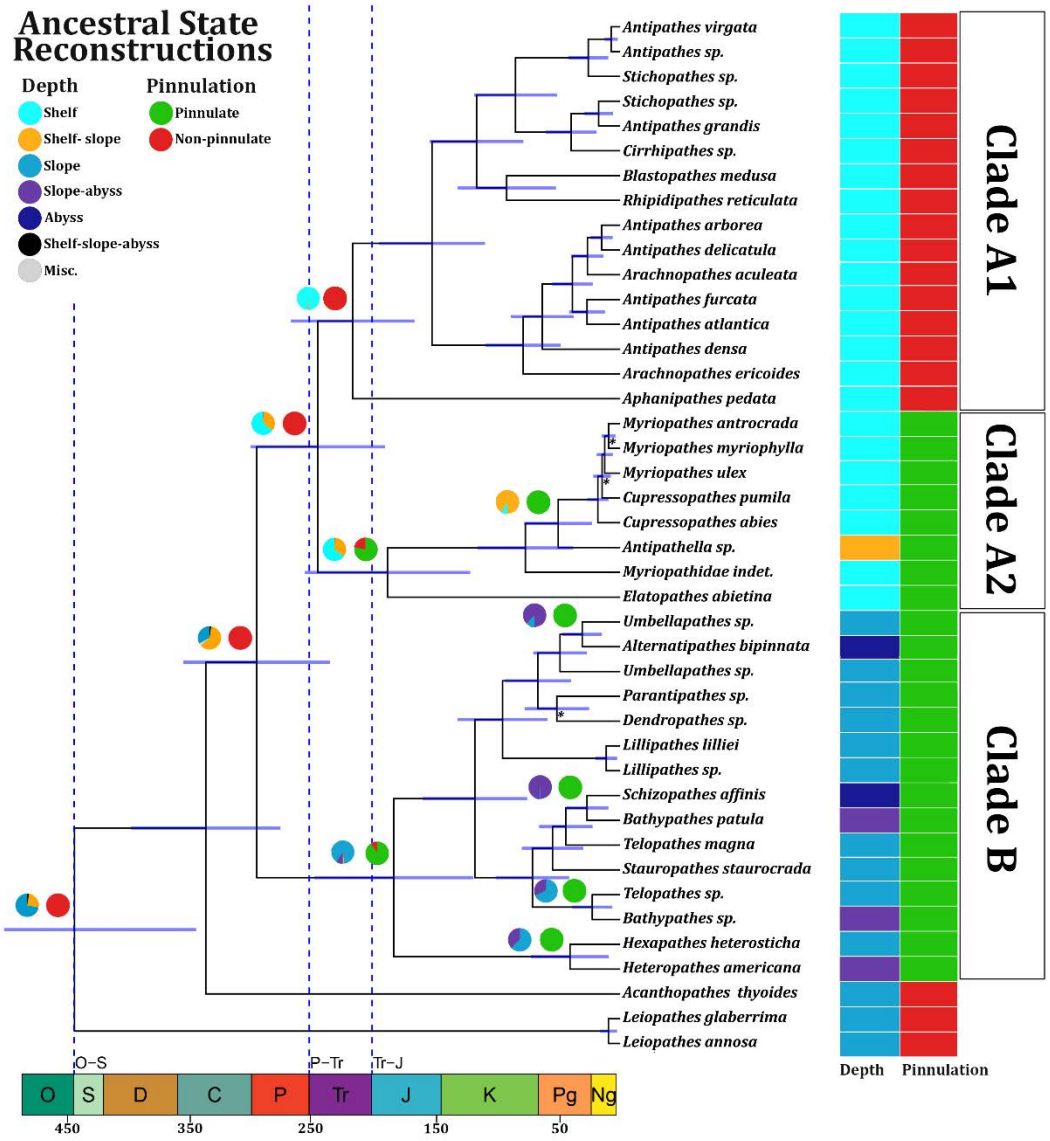


Figure 5.2 Time-calibrated phylogeny of the Antipatharia with ancestral character states of depth and pinnulation. BEAST2 dated phylogeny constructed from 25 clock-like loci with 95% highest posterior densities (horizontal blue bars). Depth ranges for each depth zone are as follows: shelf (0–249 m), slope (250–1,999 m), and abyss (>2,000 m). Posterior probability values at each node are >0.99 unless indicated by '*'. Tree is scaled to time in millions of years. Mass extinction events O-S (Ordovician–Silurian), P-Tr (Permian-Triassic), and Tr-J (Triassic-Jurassic) are shown (dashed vertical lines). Ancestral state reconstructions for depth and pinnulation traits are illustrated with pie diagrams at nodes.

5.4.3 Evolution of pinnulation

The most recent common ancestor of extant antipatharians was likely non-pinnulate (Figures 5.2 and Supplementary Figure 5.4). Based on this reconstruction, pinnules first appeared in two lineages at approximately the same time (188 Ma, 95% HPD 119–253 and 183 Ma, 95% HPD 121–250 in Clade A1 and Clade B, respectively). These two lineages occupied different habitats, separated by 295 My of divergence following the Tr-J extinction event (Figures 5.2 and Supplementary Figure 5.4). The temporal concordance in the appearance of this trait in shelf and slope taxa suggests that the evolved pinnules increased the fitness of black corals in both locations through an enhanced ability to obtain nutrition.

The ancestral reconstruction of Clade B identifies the most recent common ancestor of that lineage as having pinnules and originating in slope habitats (Figure 5.2). Most extant taxa in this clade occur at slope depths; however, in the last 30 My, at least four independent lineages transitioned offshore to the abyss.

5.5 Discussion

5.5.1 Reconstruction and comparison to limited fossil data

This phylogenomic reconstruction traces black corals to the early Ordovician (443 Ma 95% HPD 347–544), suggesting a much older date compared to a recent assessment of Anthozoa (Quattrini *et al.* 2020) (321 Ma 95% HPD 249–407) (Figure 5.2). This is due to the inclusion of the monogeneric family Leiopathidae Haeckel, 1896 in this study, which is the first lineage to branch off from the Antipatharia and is the sister lineage to all other black corals (Figure 5.2). This date (443 Ma) coincides with the Great Ordovician Biodiversification Event (485 to 443 Ma), which gave rise to suspension feeding metazoans with the ability to consume highly diverse zooplankton in the water column (Ausich & Kammer 2001; Servais *et al.* 2010). Based on fossil records, these taxa came to dominate marine ecosystems for the remainder of the Paleozoic Era (Grimmer & Holland 1979; Servais *et al.* 2010). This reconstruction also indicates that ancestral antipatharians were originally non-pinnulate and slope-dwelling. Black corals invaded novel depths bidirectionally (onshore-offshore and offshore-onshore), first invading shelf habitats 250 Ma, and only 30 Ma invading abyssal depths. Reconstructions of ancestral antipatharians can only be inferred from lineages of extant species and from the very limited fossil record of the group (Balinski *et al.* 2012; Baliński & Sun 2017). Black coral fossils have only been described from one location in the

world, the Lower Ordovician (~470 Ma) Fenxiang Formation of Hubei Province in southern China, where two genera and three species of shallow water, branched, and non-pinnulate black corals were described (Balinski *et al.* 2012; Baliński & Sun 2017).

These fossil records support the estimation of the black coral lineage being older than 321 Ma (as estimated in Quattrini et al., (2020) and the ancestral reconstruction of black corals being branching and non-pinnulate at this time. However, these fossils are thought to come from shelf depths. It is difficult to confirm the number of extinct lineages or at what depths their taxa occupied. If ancestral shelf and abyssal lineages were present, an explanation for shelf and abyssal extinctions and why the two oldest extant lineages occur on the slope could be that at ~250 Ma the Permian-Triassic (P-Tr) extinction event eradicated dominant shelf fauna (driven by extremely high temperatures) and abyssal fauna (driven by anoxia), thus restricting habitable areas of the Antipatharia to intermediate slope depths (Song *et al.* 2015). Subsequently, radiation into the shelf and the abyss occurred, likely due to the P-Tr extinction event creating vacant niches (Quattrini *et al.* 2020), but invasion into the shelf happened much earlier than into the abyss.

5.5.2 Evolution and implications of pinnules and other morphological adaptations

Gross morphological traits of the oldest extant slope taxa are described as complexly branched and non-pinnulate and are more like branching shelf taxa (consisting of pinnulate and non-pinnulate taxa) than abyssal taxa, which are generally simple colonies that are all pinnulate). This suggests that limited morphological adaptations were required to invade and survive in the shelf. However, nutrition availability is lower in deeper habitats, meaning black corals needed to improve their ability to filter feed if they wanted to expand their ranges offshore. Pinnules likely evolved as a direct response to the Triassic-Jurassic (Tr-J) extinction event that drove ~30% of marine genera to extinction (Ryder *et al.* 1996). During this time, oxygen depletion and hydrogen sulfide poisoning limited the availability of nutrients (Schobben *et al.* 2015) and a pronounced productivity collapse occurred (Ward *et al.* 2001), which would have resulted in non-pinnulate black corals having difficulty obtaining nutrients. These pinnule features enabled lineages to persist when nutrition levels were low, and invade deeper habitats with lower nutrition availability, which is supported by all lower slope and abyssal branching species having pinnules. Additionally, many abyssal species have further modifications to survive in this nutrient-poor habitat including bent

stems and pinnules, resembling wind-tunnels that funnel nutrients through the colony (Figure 5.1c), and basal hooks (derived from ancestral basal plates) to settle in soft mud and muck (Molodtsova & Opresko 2017), thereby adapting to the abyss which has little hard substrate (Riehl *et al.* 2020) (Figure 5.1b). Few other anthozoan lineages (i.e., sea pens) can survive in this environment due to selective pressures combined with a lack of reproductive opportunities for sessile species (DiMichelle *et al.* 1992; Jennings *et al.* 2013).

Pinnulation is not restricted to slope and abyssal taxa. Rather, pinnules also arose in a clade of shelf taxa, Clade A2 (Figures 5.2 and Supplementary Figure 5.4). The persistence and diversification of pinnulate species in shelf habitats might, at first may suggest that pinnules are not a prerequisite for survival where food is scarce. However, I hypothesize that in Clade A, the presence/absence of pinnules as the ancestral character state could correspond with the absence/presence of photosymbionts, which could have provided significant nutrition in non-pinnulate species and thus negate the need for pinnules. Photosymbioses were present in hexacorals long before the Tr-J extinction (>300 Mya) (McFadden *et al.* 2021) and their presence/absence may be linked to the degree of pinnulation in black corals on the shelf. However, photosymbioses in black corals are poorly known (Gress *et al.* 2021; Wagner *et al.* 2011, 2012) and therefore further research is required to understand the nature and extent of photosymbiosis in black corals. Alternatively, the persistence of non-pinnulate shelf taxa (Clade A1) throughout the Tr-J extinction event could be due to other selective pressures in shelf waters. Pinnules could create greater drag and resistance in shallow waters where wave action and currents can be strong enough to rip colonies from their basal plates (Cromroy *et al.* 1976).

5.5.3 Predominantly offshore invasions

This reconstruction demonstrates that onshore invasions are rare, only happening once in the last 400 My while five offshore invasions from independent lineages have occurred in the past 50 My, once from the shelf to the shelf-slope (Figure 5.2; *Antipathella sp*. Brook, 1889), and four lineages from the slope to the slope-abyss or directly to the abyss (Figure 5.2; Clade B). This suggests that offshore transitions are most common, but onshore transitions are possible under the right conditions (e.g., substantially low competition following extinction events). This reconstruction shows that extant species originate from slope habitats. However, the reconstruction lacks information about extinct lineages like

shelf black coral fossils (Balinski *et al.* 2012; Baliński & Sun 2017). Given how quickly onshore invasion to the shelf occurred after the P-Tr extinction, and how similar shelf and slope morphologies are compared to abyssal species, it is likely that black corals originated in the shallows (shelf or slope) and then adapted morphologically to invade and survive in low nutrient environments like the abyss. While this finding supports Jablonski et. al. (1983), the bidirectionality of transitions demonstrate that origin and invasion dynamics of the Anthozoa can be complex and hard to decipher due to lack of knowledge about extinct lineages that lack a fossil trace.

5.6 Limitations and conclusion

Only five abyssal specimens representing four abyssal genera were included in this study because trawling and dredging collection methods, which are common in the abyss, often result in specimens without tissue viable for DNA extraction and sequencing. Based on Molodtsova and Opresko (2017), This analysis includes three of the four deepest black coral genera, lacking only *Abyssopathes* Opresko, 2002. *Abyssopathes* is a genus within the Schizopathidae, and if included in this study, it would likely represent a lineage that invaded the abyss with another species in the Schizopathidae or it would be a separate offshore invasion event. In addition, there are unbranched black coral species ('whip' like) also occur in the abyss, notably in a newly established genus *Aphanostichopathes* Bo and Opresko, 2021 (Opresko *et al.* 2021); however, tissue from this genus was not available for this study. If these taxa were included, it is likely that these deep lineages would have represented additional invasion events into the abyss, further supporting Jablonski's hypothesis that deep sea lineages have shallow water ancestors. Lastly, present day black coral assemblages are a product of ancestral bathymetric and geographic transitions. Geographic transitions were not included in this study because the current geographic ranges of most species are unknown. A three-dimensional (bathymetric and geographic) approach to this study should be conducted when geographic ranges of species are more certain.

Our findings advance our understanding of the evolutionary history of this widespread anthozoan lineage. Black corals originated at slope depths, and subsequent invasions into novel depths occurred bidirectionally (onshore-offshore and offshore-onshore). Pinnules are an innovation that could have enabled offshore invasion and

persistence in environments with limited nutrition. Additional morphological innovations include basal hooks and curvature of stem and pinnules to foster success in the abyss. This new information about the evolutionary history of black corals fills knowledge gaps pertaining to how anthozoans have evolved and moved bathymetrically across deep time, which has conservation implications. Predominantly offshore transitions suggest that shelf taxa are particularly vulnerable due to their lack of evolutionary refugia and risk of losing these lineages during shallow-water disturbances (e.g., sea level, temperature, nutrient level fluctuations, oxygen content, and ultraviolet radiation). Slope taxa perhaps contain the greatest evolutionary distinctiveness because the Leiopathidae has inhabited the slope environment for 400 My, and this family consists of extremely long-lived species; individual colonies within the family have been aged at 4,265 years old (Roark *et al.* 2009). The ability for black corals to adapt and subsequently invade novel environments at depths down to 8,000 m has driven their persistence through deep time. Innovative morphologies have provided recent access to the abyss and suggest that black corals could be in the early stages of diversification in this biome.

Chapter 6 : Concluding Discussion

The overarching aim of my thesis was to overcome three biodiversity shortfalls (Linnean, Wallacean, and Darwinian) for black corals by revising the taxonomy, expanding species' ranges, and unravelling the group's evolutionary history. Addressing these shortfalls in conjunction with an integrated (morphological, molecular, and biogeographic) approach led to the following results: In **chapter 2**, I used museum samples to increase the number of known black coral species in the deep Coral Sea and synonymized a junior synonym species with its senior. In **chapter 3**, I tested a new molecular approach to identify a more useful way to reconstruct molecular relationships between species, and I described a new species and created a new genus. In **chapter 4**, I collected black corals from the Great Barrier Reef and Coral Sea to increase the number of known species in the region and provided evidence to describe new species and to create multiple new genera and families, thereby resolving longstanding taxonomic quandaries. In **Chapter 5**, using a time-calibrated phylogeny I pushed back the diversification date of black corals by 110 My and identified the predominantly offshore-directed invasions of lineages over the last ~400 million years. This thesis demonstrates the importance of using multiple lines of evidence to improve the taxonomy, and our understanding about the biodiversity and evolutionary history of black corals, which is also applicable for any branch on the tree of life. Below, I discuss concluding remarks related to my thesis aims.

6.1. Museum collections and specimens collected from under surveyed locations

Museums contain a treasure trove of biodiversity information that can provide insight into knowledge about species' occurrences and range extents (Drinkrow & Cherry 1995; Skelton *et al.* 1995; Väisänen *et al.* 1994). Museum collections often represent a checklist of species present in a region (Tracey & Hjorvarsdottir 2019); however, due to various taxonomic impediments, there is often a lag of time of years to decades between when a specimen is deposited in a museum and when it is identified, allowing biodiversity information to be extracted from the specimen (Kemp 2015). This is demonstrated in **chapter 2**, where I used black corals that were collected over 30 years ago but not identified beyond the Order Antipatharia. I identified the specimens in this collection to species level, which allowed the data to be used to better understand taxonomic diversity in the region and to increase the ranges of these species. For **chapter 4**, I spent hundreds of hours SCUBA

diving and directing remotely operated vehicles onboard research vessels to collect new specimens, which I subsequently examined alongside the specimens examined in **chapter 2**. These specimens were accessioned into the collections of the Queensland Museum, and as a result the Queensland Museum now houses the largest collection of black corals in the Southern Hemisphere. The collection also represents a checklist of black coral diversity in the Great Barrier Reef and Coral Sea region. Despite being a hotspot for research (Emslie *et al.* 2020), prior to this thesis, very few black corals were recorded from the region. This highlights the need to focus on minor-taxa (i.e., understudied taxa) because they can have important ecological, economic, and cultural importance. There is a large quantity of material across many taxa that requires examination (Graça *et al.* 2017; Jiménez-Valverde & Lobo 2006) that can assist with resolving long-standing taxonomic and evolutionary knowledge gaps that this thesis has filled for black corals. With new technologies, tools, and techniques, overcoming these gaps is becoming a reality. It is important that taxonomic effort is spread across diverse groups and regions that are often overlooked, like developing and/or remote regions that have poor infrastructure conditions, and consequently lower research efforts (Souza *et al.* 2012).

6.2. An integrated approach to resolving longstanding issues

Morphology and molecular data are two lines of evidence that can be used to identify systematic relationships among taxonomic groups and subsequently update taxonomies. The use of morphological data alone can result in analogous features being mistaken for evidence of close systematic relationships, particularly in taxa lacking a fossil record such as black corals. Conversely, the use of molecular data alone cannot allow identification of taxonomically informative morphological features that can be used to identify species in the field. Both are important for developing a robust taxonomy that accurately reflects a group's evolutionary history. In rare cases, taxonomic revisions *can* be possible if there is enough morphological data. For example, in **chapter 2** an abundance of specimens representing two closely related species led to an examination of the singular feature thought to separate the two species and results demonstrated that the "informative" feature could be explained by colony height, leading to synonymization. However, the addition of molecular data would increase certainty that the suite of

specimens considered in the chapter are indeed closely related, and the informative feature in question collapses when compared molecularly.

This thesis demonstrates the importance of an integrated approach to describing new species and notably, creating new genera and families to resolve longstanding taxonomic issues and non-monophyletic taxonomic groups. One such issue relates to varying informative features at different taxonomic levels. In **chapter 4**, I showed that identifying species in the family Myriopathidae requires consideration of branching characteristics while spine characteristics are deemed uninformative because there is simultaneously too much variation among spine characteristics within a species and too little variation between species and even genera (Opresko 2001). Comparatively, in the family Antipathidae, I primarily used spine characteristics to separate closely related species, and to provide evidence to describe new species, and even divide the family; creating an entire new family based mainly on differences in spine characteristics and branching characteristics being secondary considerations.

Another longstanding issue resolved via this integrated approach is confirmation and resolution of the mismatch between morphological and molecular relationships. Notably, the unbranched genera within Antipatharia (*Stichopathes*, *Cirrhipathes*, and *Pseudostichopathes*) are polypheletic, suggesting that unbranched morphologies have evolved multiple times over the group's evolutionary history. Although this issue has been known for many years (Bo *et al.* 2012a; Tazioli *et al.* 2007), the informativeness of molecular data and number of specimens sequenced that were required to resolve this issue were lacking prior to this thesis. In **Chapter 3**, I tested the targeted capture and sequencing of ultraconserved elements and exonic loci as an alternative to mitochondrial markers. This chapter led to the realization that targeted capture led to relatively higher phylogenetic resolution at all taxonomic levels (i.e., resolved earlier polytomies), and supported the description of a new species and a new genus. In **chapter 4**, I sequenced many branched and unbranched taxa, and with detailed morphological descriptions of the specimens and a robust phylogenetic tree of the order. I was able to confirm that genera *Stichopathes* and *Cirrhipathes* are problematic and require a focused examination of holotypes to resolve. I was also able to confirm that *Pseudocirrhipathes* is highly divergent from the other two

unbranched genera and the family Antipathidae, and is more closely related to at least one species in the family Aphanipathidae (*Aphanipathes verticillata*). Morphologically, the genus and *Ap. verticillata* also share a unique morphological trait (spines arranged in longitude rows and verticils), which allowed me to suggest creating a new family for this clade.

6.3. The evolutionary history of black corals

There is a growing body of literature that has investigated the bathymetric evolution of invertebrates; however, the methods used to study this knowledge gap have evolved with the advent of multi-loci and high-throughput sequencing techniques. Jablonski (1983), used fossils to demonstrate that lineages are evolving as they move offshore to the deep sea. As molecular methods have developed, mitochondrial markers were used to depict evolutionary histories; however, a lack of resolution at the species level limits our ability to detect transitions at small scales (Brugler *et al.* 2013). In **chapter 5**, targeted capture data were used to confirm that black corals move predominantly offshore in evolutionary time, supporting Jablonski's hypothesis. However, I also detected some onshore transitions, which suggest that bathymetric evolutions through deep-time are not unidirectional. These findings for black corals, which are hosts to many invertebrate species in the shallow and the deep (Wagner *et al.* 2012) have important conservation implications. Notably, understanding how lineages respond to extinction-level events and adapt to invasions in new environments provides insight into how future lineage-threatening events will affect black corals (Quattrini *et al.* 2020). Predominantly offshore movements means that the shallow water populations, which are threatened by harvesting for the purposes of making fine jewelry (Wagner *et al.* 2012), might not easily be replenished by deeper populations. Deep populations are also threatened by dredging and trawling for metals that are required to convert renewable energy into stored energy, which are found in their highest concentrations within ferromanganese crusts and nodules (Hein & Koschinsky 2014). As discussed in **chapter 5**, black coral lineages took a very long time to invade in the abyss (~200 million years) because of the notable abyssal adaptations that were required to persist in nutrient poor and soft sediment habitats. A loss of these abyssal populations could result in an absence of black corals in the abyss for a very long time, affecting the abyssal associated species that rely upon black corals for habitat (Molodtsova & Budaeva 2007; Wagner *et al.* 2012).

6.4. Implications of findings

This thesis addressed biodiversity shortfalls, which can be used to inform conservation decisions and conserve biodiversity. Specifically, this thesis provides new insight about how many black coral species occur in Australian waters (Linnean shortfall) how far these species ranges extend (Wallacean shortfall), and the bathymetric evolutionary history of the order (Darwinian shortfall). However, there is still uncertainty whether the Australian black corals identified in this thesis represent range expansions of species, or undescribed species that look like accepted species. Comparison of molecular data between Australian specimens and holotype specimens will determine which is the case, each of which will have different conservation implications (Mace 2004; Thomson *et al.* 2018). If Australian black coral species represent mainly new-to-science species, the implications are that more black coral species exist than are currently thought, and that species can have regional ranges compared to cosmopolitan ranges. This will require different and more geographically focused conservation action to address local threats to the potentially endemic Australian species. Alternatively, if Australian specimens collected in this thesis mainly represent range expansions of valid species, many species can have very large-to-cosmopolitan range sizes, which increases resilience towards extinction due biodiversity threats (e.g., warming waters in specific regions of the Great Barrier Reef or deep-sea mining).

6.5. Future research

This thesis provides new molecular data for many species in the order; however, over half of nominal species have yet to be sequenced. A complete phylogeny is required to complete the revision of the order because species gaps in the tree make it difficult to determine boundaries between species, notably among species with slight morphological differences. For example, **chapter 4** shows that the family Myriopathidae contains two very closely related genera with very similar spine characteristics: *Myriopathes* and *Cupressopathes*. Sequence data of all species in the family (~34 species) could reveal that certain morphological “differences” are environmentally driven and that many “closely related species” could represent singular species. It might be the case that each of these genera represent just one species, given how close they are compared to other sister genera in neighboring families. Another taxonomic question to explore is the relationship between

Rhipidipathes and *Blastopathes*. Based on sequence data to date, these two genera are closely related; however, they are morphologically very different, as discussed in **chapter 4**. This could infer ghost lineages (e.g., extinct, unknown, or unsampled species) that would otherwise further separate the two morphologically distinct genera.

In addition to producing a complete phylogeny, it is important that the phylogeny contains sequence data representing holotype or topotype specimens, especially for specimens representing species assigned as types for new families and genera. For example, in **chapter 4**, *A. curvata* is assigned as the type for Fam. undesc. 1 and *Gen. undesc. 1* but the specimen included in the chapter is not from the type locality. Therefore, it must be confirmed that the Australian *A. curvata* is the same species as *A. curvata* from the type locality of Papua New Guinea. Morphologically, the Australian specimen fits the type of *A. curvata*; however, to avoid complicated taxonomic revisions rectifying a potential error such as misidentifying a specimen representing a type of the new family, I will sequence a topotype before establishing the new family.

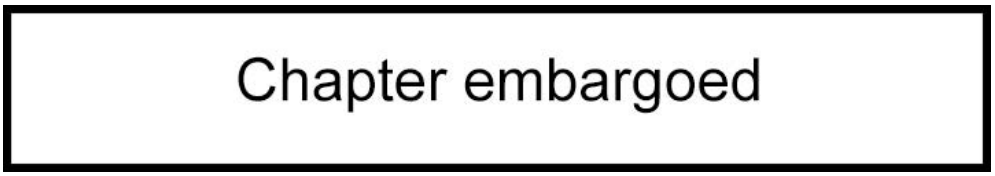
Greater efforts should also be made to survey regions that remain poorly sampled. For example, in **chapter 3**, the first survey of black corals in Papua New Guinea led to the discovery and description of a new species and creation of a new genus. Many countries, notably countries with poor infrastructure, are understudied and likely contain undescribed species and specimens representing range expansions for many species (Souza *et al.* 2012). Sequencing all species, including species from under surveyed countries will help to overcome the three biodiversity shortfalls, and allow for conservation interventions to achieve their intended impact (Musvuugwa *et al.* 2021).

This thesis provides the first view into the evolutionary history of black corals; however, **chapter 5** considers only bathymetric transitions through deep time, and does not consider geographic evolution. This is because such an analysis would require a near complete phylogeny with sequence data of representative species across the world. This should be the next step after producing a complete phylogeny that has representative species in all major oceans.

In summary, this thesis provides new biodiversity data to overcome the Linnean, Wallacean, and Darwinian shortfalls, which has led to a greater understanding of how

species are related, distributed, and how they have evolved over time. This thesis can be used as a guide for other understudied taxa to revise taxonomy, update knowledge on occurrences and ranges, and to understand how lineages have evolved, with the overarching aim of conserving global biodiversity.

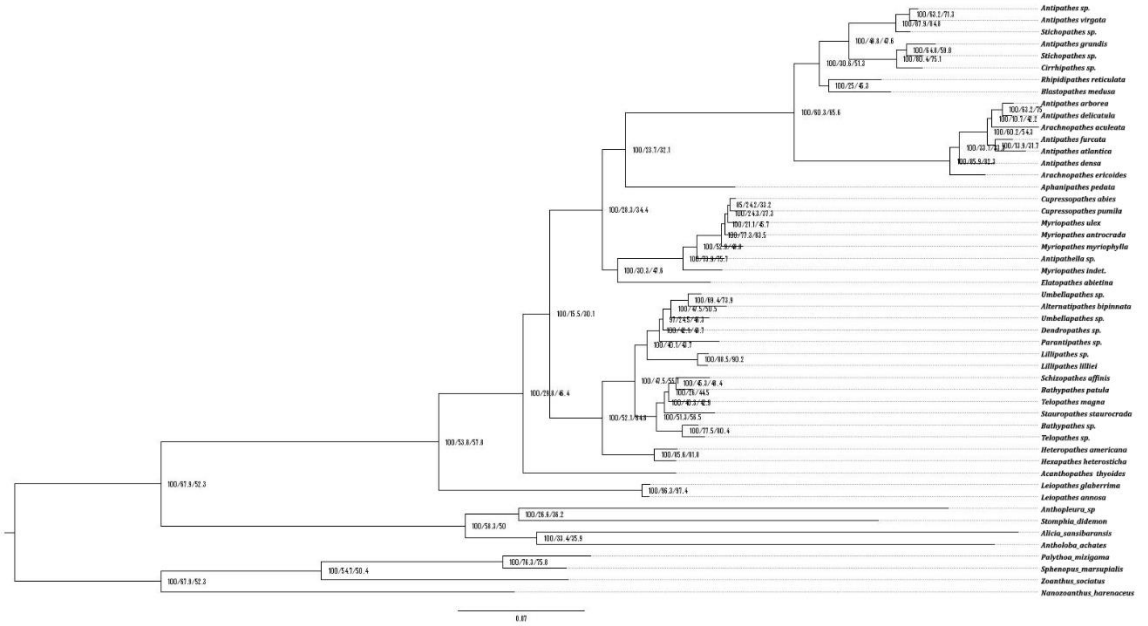
Supplementary Figures



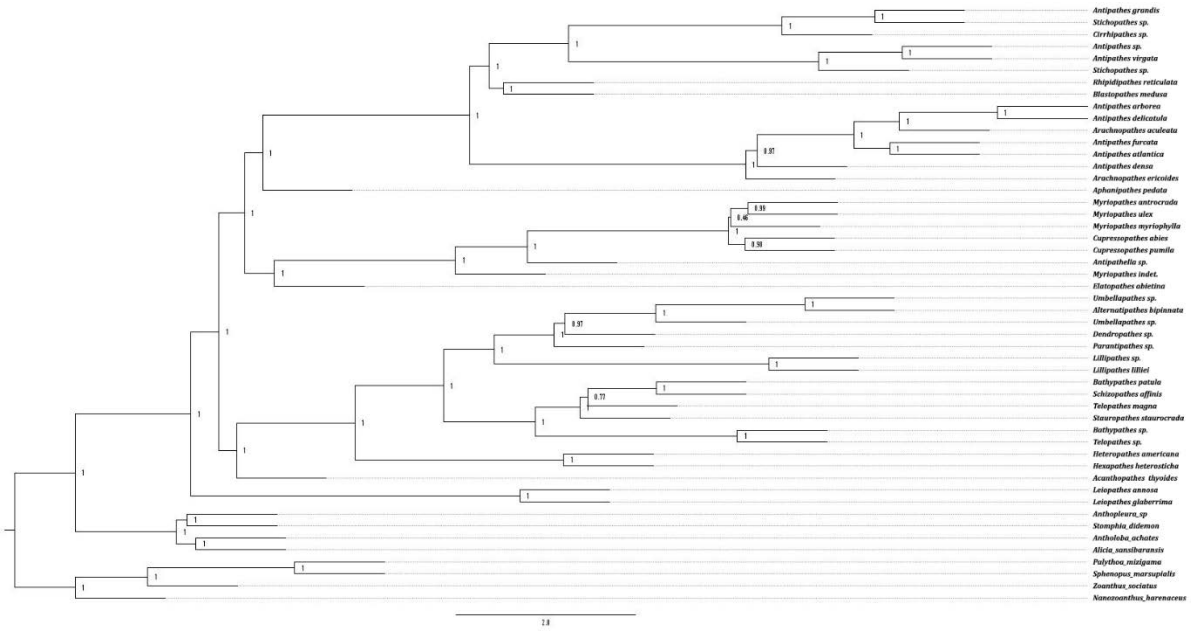
Supplementary Figure 4.1.

Chapter embargoed

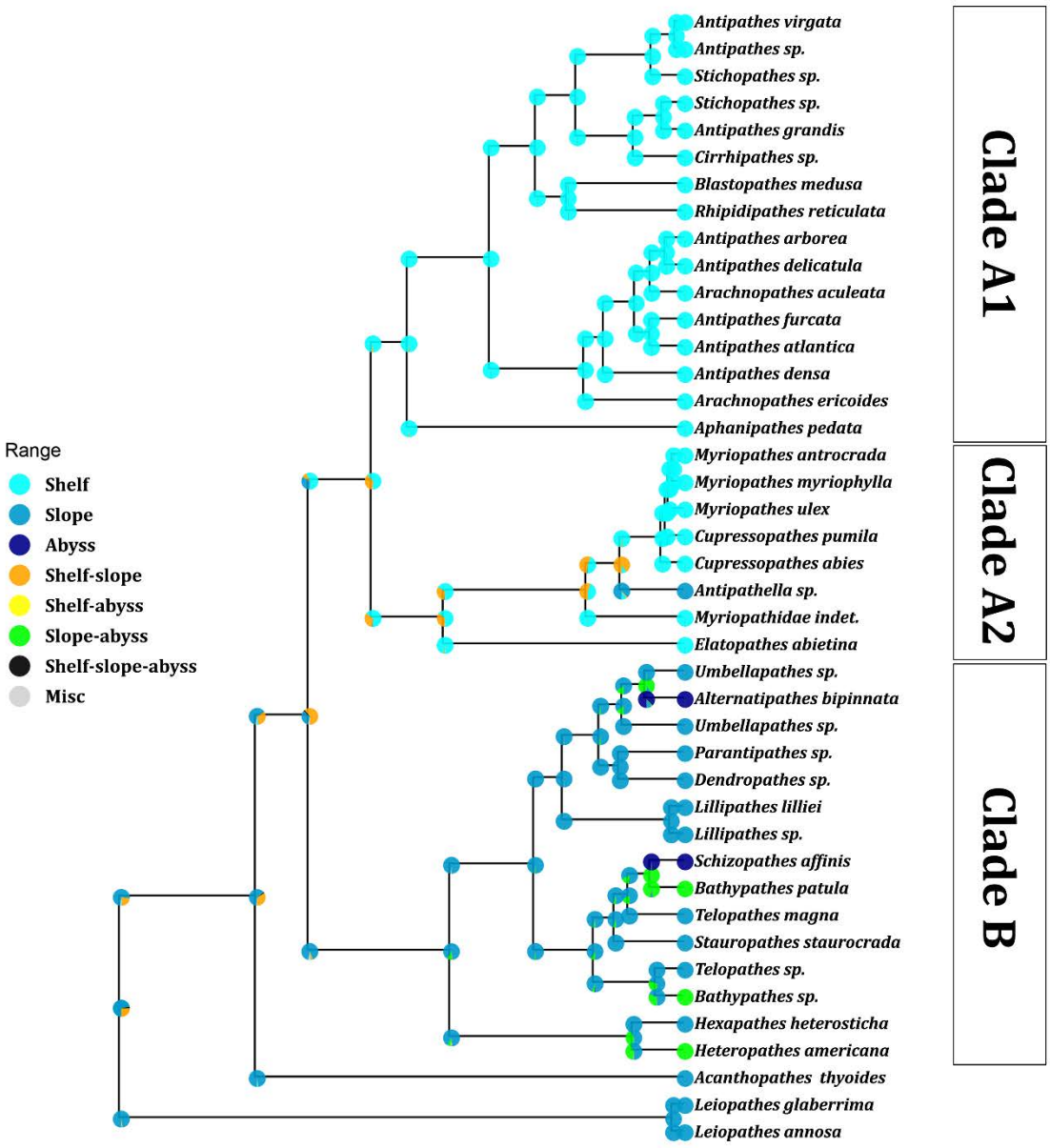
Supplementary Figure 4.2.



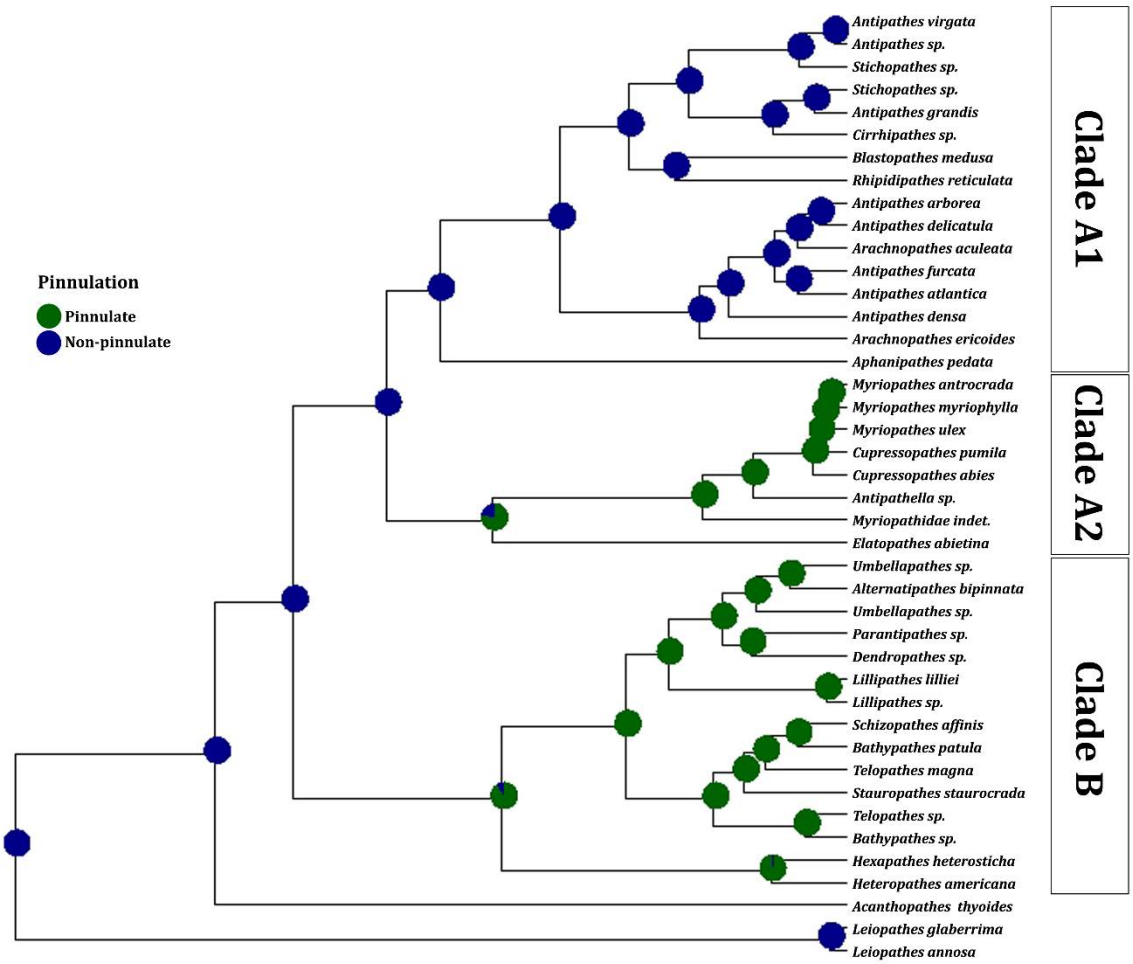
Supplementary Figure 5.1. Maximum-likelihood phylogeny of the Antipatharia produced from IQtree v1.7. Phylogenetic tree based on a 50% complete matrix containing 1,063 Ultraconserved element and exonic loci. Values at nodes separated by "/" represent ultrafast bootstrap branch supports, gene concordance factors, and site concordance factors. The tree was rooted with zoantharian taxa.



Supplementary Figure 5.2. Species tree with posterior probabilities calculated in ASTRAL. Species tree based on individual gene trees from a 50% complete matrix that were created with Astral III after long branches and low support were removed using TreeShrink and Newick utilities respectively.



Supplementary Figure 5.3. Phylogeny of the Antipatharia with ancestral depth states at all nodes. Ancestral depth states estimated using a Dispersal-Extinction-Cladogegnesis model with RevBayes. Ancestral state reconstructions are illustrated with pie diagrams.



Supplementary Figure 5.4. Phylogeny of the Antipatharia with ancestral pinnulation states at all nodes. Ancestral pinnulation states estimated using the make.simmap function in the R package phytools. Ancestral state reconstructions are illustrated with pie diagrams.

Supplementary Tables

Supplementary Table 2.1. Species identifications and station data for CIDARIS specimens

Species ID	Museum Number	Station Number	Latitude	Longitude	Depth (m)	Date
Abyssopathes lyriformis	MTQ G62078	15.1	-13.484	147.211	2542	05-09-88
Bathypathes patula	MTQ G35428	9-4	-18.157	148.368	1122	05-07-86
Bathypathes patula	MTQ G35430	11-4	-18.167	148.54	1121	05-08-86
Bathypathes patula	MTQ G61917	16.3	-17.785	148.224	1141	09-05-86
Bathypathes patula	MTQ G61948	3.3	-11.39	144.603	1999	09-02-92
Bathypathes patula	MTQ G61966	6.1	-10.013	145.004	1777	10-02-92
Bathypathes patula	MTQ G61967	3.2	-11.364	144.583	2016	09-02-92
Bathypathes patula	MTQ G61977	7.2	-9.791	145.263	1764	11-02-92
Bathypathes patula	MTQ G61978	7.1	-9.784	145.269	1764	11-02-92
Bathypathes patula	MTQ G61979	11.1	-13.825	147.531	2019	03-09-88
Bathypathes patula	MTQ G61980	10.1	-14.001	147.28	1560	03-09-88
Bathypathes patula	MTQ G61981	6.3	-10.02	145.017	1779	11-02-92
Bathypathes patula	MTQ G62049	20.3	-17.775	147.813	1224	10-05-86
Bathypathes patula	MTQ G73228	10.1	-10.525	146.115	1576	14-02-92
Heteropathes cf. *americana*	MTQ G73229	3.2	-11.364	144.583	2016	09-02-92
Parantipathes cf. *hirondelle*	MTQ G35429	49-2	-17.851	147.164	916	17-05-86
Parantipathes cf. *hirondelle*	MTQ G62019	49.3	-17.861	147.163	920	17-05-86
Schizopathes affinis	MTQ G61837	3.2	-11.364	144.583	2016	09-02-92
Schizopathes affinis	MTQ G61944	10.1	-10.525	146.115	1576	13-02-92
Schizopathes affinis	MTQ G61951	14.2	-11.776	146.354	2474	15-02-92
Schizopathes affinis	MTQ G73230	11.1	-13.825	147.531	2019	04-09-88

Supplementary Table 2.2. Corallum and pinnule descriptions for Bathypathes seculata and Bathypathes patula specimens, including data used to construct the growth profile (arranged by increasing colony size). Specimens collected from the CIDARIS expeditions are highlighted in **bold**.

Species	Specimen/ station	Total colony size (cm)	Length: longest pinnule (cm)	Length: pinnulate portion of the stem (cm)	Ratio: length of longest pinnule to length of pinnulated stem	Position of longest pinnule pair from the bottom	Pinnule density per side
B. patula	G35430	3.30	1.6	1.10	1.45	1st out of 3	2/ 1cm
B. *seculata*	G61917	5.80	6.0	3.10	1.94	4th out of 5	4/ 2cm
B. patula	74 A	6.00	4.0	2.50	1.60	1st out of 4	DNA
B. seculata	G61977	6.10	5.0	3.00	1.67	3rd out of 4	3/ 2cm
B. seculata	G61966	7.00	7.8	4.50	1.73	4th out of 6	4/ 3cm
B. seculata	G62049	7.50	7.0	4.40	1.59	4th out of 7	5/ 3cm
B. seculata	G61979	7.75	6.5	4.35	1.49	4th out of 6	5/ 3cm
B. seculata	G73228	8.00	6.4	4.00	1.60	2nd out of 4	4/ 3cm
B. seculata	G35428	8.50	8.2	5.00	1.64	5th out of 7	5/ 3cm
B. seculata	G61978	8.60	9.5	5.00	1.90	5th out of 7	4/ 3cm
B. seculata *	USNM 53430	9.00	7.5	5.00	1.50	6th out of 6	3/ 3cm
B. seculata	G61981	9.10	10.5	5.80	1.81	6th out of 7	3/ 3cm
B. patula	214 B	10.25	5.5	4.50	1.22	4th out of 7	DNA
B. patula	St. 453 III	10.60	4.2	3.00	1.40	no data	DNA
B. patula	214 A	10.90	5.8	3.30	1.76	5th out of 7	DNA
B. seculata	G61948	11.00	10.5	6.40	1.64	6th out of 9	5/ 3cm
B. patula	St. 453 II	11.50	4.1	2.60	1.58	no data	DNA
B. seculata	G61967	11.60	8.6	7.60	1.13	6th out of 9	5/ 3cm
B. patula	St. 190	13.60	9.2	8.80	1.05	no data	DNA
B. patula	74 B	15.00	6.4	4.50	1.42	4th out of 8	DNA
B. patula *	195	19.00	7.5	9.50	0.79	5th out of 9	4/ 3cm
B. patula	G61980	24.00	7.8	16.10	0.48	9th out of 16	6/ 3cm
B. patula	Plate 41	25.00	10.0	15.00	0.67	4th out of 9	DNA

*—Holotype specimen
DNA—Data Not Available

Supplementary Table 3.1. Occurrence data for *Blastopathes medusa* and antipatharian specimens included in UCE/exon analysis.

Sample ID	Family	Genus	Species	Museum	Museum Registration ID	Latitude (Decimal degree.)	Longitude (Decimal degree)	Depth (Meters)
C727	Antipathidae	*Blastopathes*	medusa	MTQ	G74904	-5.300	150.124	35
C728	Antipathidae	*Blastopathes*	medusa	NMAG	1893	-5.309	150.126	30
C729	Antipathidae	*Blastopathes*	medusa	NMAG	1895	-5.309	150.126	30
Unseq1	Antipathidae	*Blastopathes*	medusa	MTQ	G74911	-5.443	150.097	37
Unseq2	Antipathidae	*Blastopathes*	medusa	MTQ	G74913	-5.295	150.104	30
C731	Myriopathidae	*Antipathella*	sp.	TMAG	K4424	-41.901	148.449	70
C1395	Antipathidae	*Antipathes*	densa	NMNH	1267308	34.555	171.228	297
C705	Antipathidae	*Antipathes*	dichotoma	NMNH	1280884	39.968	9.730	160
C724	Antipathidae	*Antipathes*	sp.	MTQ	G74924	-20.400	161.270	440
ANT14	Antipathidae	*Antipathes*	grandis	ND	ND	ND	ND	ND
C717	Antipathidae	*Antipathes*	sp.	MTQ	G74921	-31.580	159.110	65
C1414	Antipathidae	*Antipathes*	virgata sp.	RBINS	131349	-23.350	43.614	23
C1415	Antipathidae	*Arachnopathes*	ericoides	RBINS	131339	-23.350	43.615	23
C718	Antipathidae	*Arachnopathes*	sp.	MTQ	G74922	-31.580	159.110	65
C712	Schizopathidae	*Bathypathes*	patula	CAS	223596	37.725	123.030	1,258
C713	Schizopathidae	*Bathypathes*	patula	CAS	218788	37.725	123.030	1,669
C732	Schizopathidae	*Telopathes*	tasmaniensis	TMAG	K4226	-44.332	147.168	1,220
C725	Antipathidae	*Cirrhipathes*	sp.	NMAG	1891	-5.000	150.000	20
C726	Antipathidae	*Cirrhipathes*	sp.	NMAG	1894	-5.000	150.000	30
C722	Myriopathidae	*Cupressopathes*	abies	MTQ	G74918	-31.580	159.110	65
C719	Myriopathidae	*Cupressopathes*	sp.	MTQ	G74917	-31.580	159.110	65
C715	Schizopathidae	*Dendropathes*	sp.	CAS	223580	37.725	123.030	1,244
C704	Cladopathidae	*Heteropathes*	americana	NMNH	1483032	27.710	-92.220	402
C703	Cladopathidae	*Heteropathes*	heterosticha*	NMNH	1453828	5.860	-162.130	418
C706	Leiopathidae	*Leiopathes*	annosa	NMNH	1071405	27.019	-176.526	472
C708	Leiopathidae	*Leiopathes*	annosa	NMNH	1071416	21.406	-157.643	470
C707	Leiopathidae	*Leiopathes*	glaberrima*	NMNH	1490549	30.940	-77.330	1,247
C709	Schizopathidae	*Lillipathes*	lilliei	CAS	179055	57.764	-173.990	897
C714	Schizopathidae	*Lillipathes*	sp.	CAS	218816	37.725	123.030	DNA
C721	Myriopathidae	*Myriopathes*	sp.	MTQ	G74919	DNA	DNA	DNA

C720	Myriopathidae	*Myriopathes*	*ulex*	MTQ	G74920	DNA	DNA	60
C701	Schizopathidae	*Schizopathes*	*affinis*	MTQ	G61944	-10.530	146.120	1,570
C716	Antipathidae	*Stichopathes*	sp.	MTQ	G74923	-31.580	159.110	65
C730	Antipathidae	*Stichopathes*	sp.	TMAG	K4320	-31.819	159.346	88
C723	Stylopathidae	*Stylopathes*	sp.	MTQ	G74916	-20.400	161.270	170
C710	Schizopathidae	*Umbellapathes*	sp.	CAS	223581	37.725	123.030	2,126
C711	Schizopathidae	*Umbellapathes*	sp.	CAS	223579	37.725	123.030	2,639

*— cf.

MTQ—Museum of Tropical Queensland.
TMAG—Tasmanian Museum of Art and Gallery.
NMNH—Smithsonian National Museum of Natural History.
RBINS—Royal Belgian Institute of Natural Sciences.
CAS—California Academy of Sciences.
ND—Not Deposited.
DNA—Data Not Available.

Supplementary Table 3.2. Read and locus summary statistics used in the ultraconserved element and exon analysis.

Sample ID	# of raw reads	# of trimmed reads	Assemblies: Total bp	Assemblies: Mean length	Assemblies: Min length	Assemblies: Max length	**Assemblies: Total loci**
C727	486,223	922,966	645,490	616.51	189	2,050	1,047
C728	690,212	1,323,114	675,974	622.44	229	2,472	1,086
C729	609,438	1,158,646	647,701	630.67	230	4,068	1,027
C731	609,438	53,957	332,418	468.19	229	1,626	710
C1395	2,929,342	5,662,957	1,260,724	1,063.90	229	7,005	1,185
C705	10						
C724	778,785	1,524,914	994,877	879.64	232	8,970	1,131
ANT14	3,255,210	3,197,830	255,422	434.39	229	2,477	588
C717	44,898	84,485	373,753	501.01	231	1,399	746
C1414	3,300,922	6,416,063	1,421,434	1,240.34	109	5,732	1,146
C1415	3,603,888	6,995,452	1,502,106	1,207.48	194	6,399	1,244
C718	92,553	175,031	439,625	590.10	230	2,365	745
C712	576,369	1,111,464	540,474	665.61	168	4,256	812
C713	649,679	1,241,300	450,667	595.33	228	5,330	757
C732	197,543	381,590	401,523	489.07	84	4,054	821
C725	246,061	465,122	429,235	563.30	232	2,212	762
C726	475,889	931,311	603,531	647.57	229	1,799	932

C722	892,971	1,757,980	811,922	799.14	230	5,071	1,016
C719	936,858	1,837,892	953,730	951.83	170	5,365	1,002
C715	609,560	1,181,141	714,187	783.10	233	4,862	912
C704	639,504	1,232,271	863,519	880.24	213	6,149	981
C703	192,900	374,882	657,958	759.77	234	2,683	866
C706	1,162,282	2,254,705	1,054,753	973.02	209	3,688	1,084
C708	1,066,552	2,077,456	1,074,699	957.84	134	3,339	1,122
C707	485,480	943,291	894,848	877.30	143	3,323	1,020
C709	674,439	1,303,184	556,747	642.89	233	2,755	866
C714	419,483	812,809	543,060	675.45	232	4,033	804
C721	583,629	1,138,812	743,274	763.11	213	13,698	974
C720	738,221	1,450,962	999,406	994.43	181	5,389	1,005
C701	364,368	711,369	613,975	706.53	83	18,423	869
C716	468,195	905,479	483,524	580.46	167	2,807	833
C730	552,022	1,080,343	585,791	655.98	161	4,128	893
C723	969,988	1,900,012	812,630	895.95	229	4,018	907
C710	401,460	777,191	576,312	708.00	89	3,841	814
C711	647,174	1,251,253	594,088	743.54	89	3,448	799

Greyed sample was removed due to sequencing failure

Supplementary Table 4.1.

Chapter embargoed

Chapter embargoed

Supplementary Table 4.2.

Chapter embargoed

Chapter embargoed

Supplementary Table 4.3.

Chapter embargoed

Chapter embargoed

Supplementary table 4.4.

Chapter embargoed

Chapter embargoed

Supplementary table 5.1: Metadata for specimens included in study

Taxa	Order	Family	Voucher location	Accession #	Latitude	Longitude	Depth
Antholoba achates	Ac	Actinostolidae	DNA	DNA	DNA	DNA	DNA
Anthopleura sp.	Ac	Actiniidae	DNA	DNA	DNA	DNA	DNA
Stomphia didemon	Ac	Actinostolidae	DNA	DNA	DNA	DNA	DNA
Alicia sansibarensis	Ac	Aliciidae	DNA	DNA	DNA	DNA	DNA
Myriopathes ulex	An	Myriopathidae	DNA	DNA	DNA	DNA	50
Acanthopathes thyoides	An	Aphanipathidae	NMNH	1288453	27.79	-93.68	127
Aphanipathes pedata	An	Aphanipathidae	NMNH	1288458	27.81	-93.69	229
Bathypathes patula	An	Schizopathidae	NMNH	1288462	58.20	-138.98	515
Umbellapathes sp.	An	Schizopathidae	NMNH	1404092	15.47	-169.07	1529
Antipathes atlantica	An	Antipathidae	NMNH	1288454	27.84	-93.42	119
Telopathes magna	An	Schizopathidae	NMNH	1204049	37.46	-59.95	1909
Elatopathes abietina	An	Aphanipathidae	NMNH	1288451	27.79	-93.68	130
Blastopathes medusa	An	Antipathidae	MTQ	G74904	-5.00	150.00	35
Antipathella sp.	An	Myriopathidae	TMAG	4424	148.45	-41.90	70
Antipathes densa	An	Antipathidae	NMNH	1267308	34.56	171.23	297
Rhipidipathes reticulata	An	Aphanipathidae	MTQ	G74924	-20.40	161.27	440
Antipathes furcata	An	Antipathidae	MTQ	G74921	-31.58	159.11	65
Antipathes virgata	An	Antipathidae	RBINS	INV131349	-23.36	43.62	25
Arachnopathes ericoides	An	Antipathidae	RBINS	INV131339	-23.36	43.62	23
Arachnopathes aculeata	An	Antipathidae	MTQ	G74922	-31.58	159.11	65
Cirrhipathes sp.	An	Antipathidae	NMAG	NMAG1891	-5.00	150.00	29
Cupressopathes abies	An	Myriopathidae	MTQ	G74918	-31.58	159.11	65
Dendropathes sp.	An	Schizopathidae	CAS	223580	37.73	123.03	1244
Heteropathes americana	An	Cladopathidae	NMNH	1483032	27.71	-92.22	402
Hexapathes heterosticha	An	Cladopathidae	NMNH	1453828	5.86	-162.13	418
Leiopathes annosa	An	Leiopathidae	NMNH	1071405	27.02	-176.53	472
Leiopathes glaberrima	An	Leiopathidae	NMNH	1490549	30.94	-77.33	1247
Lillipathes lilliei	An	Schizopathidae	CAS	179055	57.76	-173.99	897
Lillipathes sp.	An	Schizopathidae	CAS	218816	37.73	123.03	1372
Myriopathes myriophylla	An	Myriopathidae	MTQ	G74919	-31.58	159.11	65
Myriopathes antrocrada	An	Myriopathidae	MTQ	G74920	-31.58	159.11	65
Schizopathes affinis	An	Schizopathidae	MTQ	G61944	-10.53	146.12	1576
Stichopathes sp.	An	Antipathidae	MTQ	G74923	-31.58	159.11	65
Stichopathes sp.	An	Antipathidae	TMAG	4320	-31.82	159.35	88
Myriopathidae indet.	An	Myriopathidae	MTQ	G74916	-20.40	161.27	150
Umbellapathes sp.	An	Schizopathidae	CAS	223579	37.73	123.03	2638.57
Parantipathes sp.	An	Schizopathidae	DNA	MSS29	DNA	DNA	370
Antipathes grandis	An	Antipathidae	NMNH	1096111	20.95	-156.73	81
Antipathes delicatula	An	Antipathidae	MTQ	G77187	-18.60	146.49	14

Antipathes arborea	An	Antipathidae	MTQ	G77188	-18.60	146.49	14
Antipathes sp.	An	Antipathidae	RBINS	INV131338	-23.58	43.71	15
Cupressopathes pumila	An	Myriopathidae	RBINS	INV131366	-23.36	43.62	24
Bathypathes sp.	An	Schizopathidae	NIWA	83298	-37.18	176.98	1000
Alternatipathes bipinnata	An	Schizopathidae	NMNH	1234554	35.81	-122.65	2634
Telopathes sp.	An	Schizopathidae	NIWA	86338	-34.89	179.04	1665
Stauropathes staurocrada	An	Schizopathidae	NMNH	1071042	25.70	-171.45	1489
Nanzoanthus harenaceus	Z	Nanozoanthidae	DNA	DNA	DNA	DNA	DNA
Palythoa mizigama	Z	Sphenopidae	DNA	DNA	DNA	DNA	DNA
Sphenopus marsupialis	Z	Sphenopidae	DNA	DNA	DNA	DNA	DNA
Zoanthus sociatus	Z	Zoanthidae	DNA	DNA	DNA	DNA	DNA

Order
Ac—Actiniaria
An— Antipatharia
Z— Zoantharia

Voucher Location
MTQ—Museum of Tropical Queensland.
TMAG—Tasmanian Museum of Art and Gallery.
NMNH—Smithsonian National Museum of Natural History.
RBINS—Royal Belgian Institute of Natural Sciences.
CAS—California Academy of Sciences.
ND—Not Deposited.
DNA—Data Not Available.

Supplementary table 5.2. Pinnulate or not and depth coded to specimens

Taxa	Depth code	Pinnule code
Acanthopathes thyoides	Shelf	Non-pinnulate
Alicia sansibarensis	NA	NA
Alternatipathes bipinnata	Abyss	Pinnulate
Antholoba achates	NA	NA
Anthopleura sp.	NA	NA
Antipathella sp.	Shelf-slope	Pinnulate
Antipathes arborea	Shelf	Non-pinnulate
Antipathes atlantica	Shelf	Non-pinnulate
Antipathes delicatula	Shelf	Non-pinnulate
Antipathes densa	Shelf	Non-pinnulate
Antipathes furcata	Shelf	Non-pinnulate
Antipathes grandis	Shelf	Non-pinnulate
Antipathes sp.	Shelf	Non-pinnulate
Antipathes virgata	Shelf	Non-pinnulate
Aphanipathes pedata	Shelf	Non-pinnulate
Arachnopathes aculeata	Shelf	Non-pinnulate
Arachnopathes ericoides	Shelf	Non-pinnulate
Bathypathes patula	Slope-abyss	Pinnulate
Bathypathes sp.	Slope-abyss	Pinnulate
Blastopathes medusa	Shelf	Non-pinnulate

Cirrhipathes sp.	Shelf	NA
Cupressopathes abies	Shelf	Pinnulate
Cupressopathes pumila	Shelf	Pinnulate
Dendropathes sp.	Slope	Pinnulate
Elatopathes abietina	Shelf	Pinnulate
Heteropathes americana	Slope-abyss	Pinnulate
Hexapathes heterosticha	Slope	Pinnulate
Leiopathes annosa	Slope	Non-pinnulate
Leiopathes glaberrima	Slope	Non-pinnulate
Lillipathes lilliei	Slope	Pinnulate
Lillipathes sp.	Slope	Pinnulate
Myriopathes antrocrada	Shelf	Pinnulate
Myriopathes myriophylla	Shelf	Pinnulate
Myriopathes ulex	Shelf	Pinnulate
Myriopathidae indet.	Shelf	Pinnulate
Nanzoanthus harenaceus	NA	NA
Palythoa mizigama	NA	NA
Parantipathes sp.	Slope	Pinnulate
Rhipidipathes reticulata	Shelf	Non-pinnulate
Schizopathes affinis	Abyss	Pinnulate
Sphenopus marsupialis	NA	NA
Stauropathes staurocrada	Slope	Pinnulate
Stichopathes sp.	Shelf	NA
Stichopathes sp.	Shelf	NA
Stomphia didemon	NA	NA
Telopathes magna	Slope	Pinnulate
Telopathes sp.	Slope	Pinnulate
Umbellapathes sp.	Slope	Pinnulate
Umbellapathes sp.	Slope	Pinnulate
Zoanthus sociatus	NA	NA

NA—Not Applicable (because taxa is either not a black coral or is not a branching black coral)

Supplementary Code
Supplementary Code 3.1. Scripts used for Phylogenetic analyses

##SCRIPTS USED##

###SECTION 1

###PHYLUCE WORKFLOW (following https://phyluce.readthedocs.io/en/latest/tutorial-one.html)

#Trim and clean sequences
illumiprocessor --input rawreads --output clean-fastq --config black_coral.config --cores 48
#. /rawreads/filename example:
bc1_CTCCTAGA_L001_R1_001.fastq.gz bc2_CGTACGAA_L001_R1_001.fastq.gz
bc1_CTCCTAGA_L001_R2_001.fastq.gz bc2_CGTACGAA_L001_R2_001.fastq.gz

#Blackcoral.config example
[adapters]
i7:GATCGGAAGAGCACACGTCTGAACTCCAGTCAC*ATCTCGTATGCCGTCTTCTGCTTG
i5:AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT*GTGTAGATCTCGGTGGTCGCCGTATCATT

[tag sequences]
i5-N501:AAGCCACA
i5-N502:AGAACGAG
i5-N503:CGTACGAA
i5-N504:CTCCTAGA
i5-N505:CTCGTCTT
i5-N506:GACGAATG
i5-N507:GCATGTCT
i5-N508:GGTCAGAT
i7-N703:AGGTTCGA
i7-N702:ACTCCATC
i7-N701:AAGAAGGC
i7-N708:GGTGTCTT
i7-N707:GCATGTCT
i7-N704:CGAAGAAC
i7-N709:TGTGACTG
i7-N705:CGAGACTA
i7-N706:GACATGGT

[tag map]
bc1_CTCCTAGA:i7-N706,i5-N504
bc2_CGTACGAA:i7-N701,i5-N503

[names]
bc1_CTCCTAGA:bc1a
bc2_CGTACGAA:bc2a

###Spades Assembly
spades.py -o /spades-assemblies-blackcoral/bc1 -1 /CLEANED_READ/bc1/split-adapter-quality-trimmed/bc1-READ1.fastq.gz -2 /CLEANED_READ/bc1/split-adapter-quality-trimmed/bc1-READ2.fastq.gz --careful --threads 40 --cov-cutoff 2;

#Example of matching uce probes to contigs, extracting and aligning them, and then running them through RAxML

phyluce_assembly_match_contigs_to_probes --contigs spades-assemblies/contigs --probes probes/hexa-v2-sclerac-subset-final-probes-uce.prbsrm.final.fasta --output uce-search-results --min-coverage 70 --min-identity 70

phyluce_assembly_get_match_counts --locus-db uce-search-results/probe.matches.sqlite --taxon-list-config taxon-set.conf --taxon-group 'bc_all' --incomplete-matrix --output taxon-sets/all/all-taxa-incomplete.conf

#Example of taxon-set.conf

[samples]
bc1
bc2

phyluce_assembly_get_fastas_from_match_counts --contigs ../../spades-assemblies/contigs --locus-db ../../uce-search-results/probe.matches.sqlite --match-count-output all-taxa-incomplete.conf --output all-taxa-incomplete.fasta --incomplete-matrix all-taxa-incomplete.incomplete --log-path log

phyluce_align_seqcap_align --fasta all-taxa-incomplete.fasta --output mafft-nexus-internal-trimmed --taxa 50 --aligner mafft --cores 12 --incomplete-matrix --output-format fasta --no-trim --log-path log

phyluce_align_get_gblocks_trimmed_alignments_from_untrimmed --alignments mafft-nexus-internal-trimmed --output mafft-nexus-internal-trimmed-gblocks --cores 12 --log log

phyluce_align_get_align_summary_data --alignments mafft-nexus-internal-trimmed-gblocks --cores 12 --log-path log --show-taxon-counts

phyluce_align_remove_locus_name_from_nexus_lines --alignments mafft-nexus-internal-trimmed-gblocks --output mafft-nexus-internal-trimmed-gblocks-clean --cores 12 --log-path log

phyluce_align_get_only_loci_with_min_taxa --alignments mafft-nexus-internal-trimmed-gblocks-clean --taxa 50 --percent 0.50 --output mafft-nexus-internal-trimmed-gblocks-clean-50p --cores 12 --log-path log

###IQTree
Inferring Species tree
iqtree2 -p /spades-taxon-sets-final/bc/mafft-nexus-internal-trimmed-gblocks-clean-50p --prefix concat.bc.i50 -B 1000 -nt 3 -m MFP+MERGE --merge-model GTR --merge-rate G -rcluster 10 --cptime 4000
Inferring gene trees
iqtree2 -S /spades-taxon-sets-final/bc/mafft-nexus-internal-trimmed-gblocks-clean-50p --prefix loci.bc.i50 -nt AUTO --cptime 4000
Gene concordance factor
iqtree2 -t concat.bc.i50.treefile --gcf loci.bc.i50.treefile -p /spades-taxon-sets-final/bc/mafft-nexus-internal-trimmed-gblocks-clean-50p --scf 100 --prefix concord.bc.i50 --cf-verbose

###SECTION 2
###SPECIES TREE METHODS

#Make gene trees as above from 50% data matrices
#Concatenate trees together into 1 file
cat uce* >out.tre

#Remove long branches from gene trees using TreeShrink (following https://github.com/uym2/TreeShrink)
run_treeshrink.py -t out.tre -o treeshr

#Prune low support (<30) branches using nw_ed
PROGRAMS/newick-utils-1.6/bin/nw_ed treeshr.tre 'i & b<=30' o > treeshr2

#Make individual gene trees (same as above) on each cleaned alignment

#Run astral (following https://github.com/smirarab/ASTRAL/blob/master/astral-tutorial-template.md)
java -jar /Astral/astral.5.6.3.jar -i treeshr2.v2.tre -o astral.all.50p.tre

###SECTION 3
###FIND CLOCK-LIKE GENES
#Run sortadate on rooted gene trees
python /tree_internal/root/SortaDate-master/src/get_var_length.py ./ --flend .r.tree --outf sortadate --outg Nanozoanthus_harenaceusSAMN13244957
#Run sortadate on rooted species tree
python /tree_internal/root/SortaDate-master/src/get_bp_genetrees.py ./ concat.bc.i50.treefile.r.tree --flend .r.tree --outf sortadate2
#Run sortadate to combine two runs
python /tree_internal4/root/SortaDate-master/src/combine_results.py sortadate sortadate2 --outf sortadatecomb
Run sortadate to Sort and get the list of the good genes
python /tree_internal/root/SortaDate-master/src/get_good_genes.py sortadatecomb --max 50 --order 3,1,2 --outf out.txt

```
###DATING TREE USING Penalised Likelihood Method
library(ape)
library(ggplot2)
library(ggtree)
library(phytools)
tree=read.tree("/Users/Jeremy/concord.bc.i50.cf.tree.r.tree")
print(tree)
ggtree(tree) + geom_text2(aes(subset=!isTip, label=node), hjust=-.3) + geom_tiplab()
tree4=chronopl(tree, lambda=1, age.min=c(542,336,349,249,507),
          age.max=c(746,531,512,407,699), node=c(51,52,97,57,55))
plotTree(tree2)
write.tree(tree2)

###BEAST Analyses
# xml all ran on CIPRES portal
#Tree Annotator produced "MCC.withlabs.final.nex"

###SECTION 4
####ANCESTRAL STATE CHARACTERIZATION
#Stochastic Character mapping for pinnulation
library(phytools)
tr <- read.beast("deep3state.ase.tre")
traits<- as.matrix(read.csv(file="tree_specimen_name_updates5.csv",row.names=1))

#non-pinnulate/pinnulate
var.disc<-setNames(traits[,7],rownames(traits))
var.disc<-as.factor(var.disc)
cols_p<-setNames(c("dark blue","dark green"),levels(var.disc))
smap.trees.pin<-make.simmap(tr,var.disc,model="ARD",nsim=100)
summary(smap.trees.pin)
summary_p<-summary(smap.trees.pin)
ancstats <- as.data.frame(summary_p$ace)
ancstats$node <- 1:tr$Nnode+Ntip(tr)
pinpies <- nodepie(ancstats, cols = 1:2)
pinpies <- lapply(pinpies, function(g) g+scale_fill_manual(values = cols_p))

####Revbayes for depth (following
https://revbayes.github.io/tutorials/biogeo/biogeo_simple.html)
range_fn ="input/bc.range.NEXUS"
tree_fn = "input/tree2.tre"
out_fn="output/deep4"

dat_range_01 = readDiscreteCharacterData(range_fn)
dat_range_n = formatDiscreteCharacterData(dat_range_01, "DEC")
n_areas = dat_range_01.nchar()
dat_range_01[1]
```

```
dat_range_n[1]
state_desc = dat_range_n.getStateDescriptions()
state_desc_str = "state,range\n"
for (i in 1:state_desc.size()){
  state_desc_str += (i-1) + "," + state_desc[i] + "\n"
}
write(state_desc_str, file=out_fn+".state_labels.txt")

tree <- readTrees(tree_fn)[1]

rate_bg ~ dnLoguniform(1E-4,1E2)
rate_bg.setValue(1E-2)
moves = VectorMoves()
moves.append( mvSlide(rate_bg, weight=4) )
dispersal_rate <- 1.0
for (i in 1:n_areas) {
  for (j in 1:n_areas) {
    dr[i][j] <- dispersal_rate
  }
}
log_sd <- 0.5
log_mean <- ln(1) - 0.5*log_sd^2
extirpation_rate ~ dnLognormal(mean=log_mean, sd=log_sd)
moves.append( mvScale(extirpation_rate, weight=2) )
for (i in 1:n_areas) {
  for (j in 1:n_areas) {
    er[i][j] <- 0.0
  }
  er[i][i] := extirpation_rate
}
Q_DEC := fnDECRateMatrix(dispersalRates=dr, extirpationRates=er)
clado_event_types <- [ "s", "a" ]
clado_event_probs <- simplex(1, 1)
P_DEC := fnDECCladoProbs(eventProbs=clado_event_probs,
                         eventTypes=clado_event_types,
                         numCharacters=n_areas)

m_bg ~ dnPhyloCTMCClado(tree=tree,
                        Q=Q_DEC,
                        cladoProbs=P_DEC,
                        branchRates=rate_bg,
                        nSites=1,
                        type="NaturalNumbers")
m_bg.clamp(dat_range_n)
monitors = VectorMonitors()
monitors.append( mnScreen(rate_bg, extirpation_rate, printgen=100) )
```

```
monitors.append( mnModel(file=out_fn+".params.log", printgen=10) )
monitors.append( mnFile(tree, file=out_fn+".tre", printgen=10) )
monitors.append( mnJointConditionalAncestralState(tree=tree,
                                ctmc=m_bg,
                                filename=out_fn+".states.log",
                                type="NaturalNumbers",
                                printgen=10,
                                withTips=true,
                                withStartStates=true) )
monitors.append( mnStochasticCharacterMap(ctmc=m_bg,
                            filename=out_fn+".stoch.log",
                            printgen=100) )
mymodel = model(m_bg)
mymcmc = mcmc(mymodel, moves, monitors)
mymcmc.run(5000)

#Open RB in new terminal
out_fn="deep4"
out_str="deep4"
out_state_fn = out_str + ".states.log"
out_tree_fn = out_str + ".tre"
out_mcc_fn = out_str + ".mcc.tre"
tree_trace = readTreeTrace(file=out_tree_fn, treetype="clock")
tree_trace.setBurnin(0.25)
n_burn = tree_trace.getBurnin()

mcc_tree = mccTree(tree_trace, file=out_mcc_fn)

state_trace = readAncestralStateTrace(file=out_state_fn)

tree_trace = readAncestralStateTreeTrace(file=out_tree_fn, treetype="clock")
anc_tree = ancestralStateTree(tree=mcc_tree,
                    ancestral_state_trace_vector=state_trace,
                    tree_trace=tree_trace,
                    include_start_states=true,
                    file=out_str+".ase.tre",
                    burnin=n_burn,
                    site=1)

tr <- read.beast("deep3state.ase.tre")
as_tibble(tr) %>% select(node, starts_with("end")) %>% filter(node >= 43) -> depth_anc
depth_anc %>%
  select(node, end_state_1, end_state_2, end_state_3) %>%
  pivot_longer(cols = -node, names_to = "states",values_drop_na = TRUE) -> end_states
depth_anc %>%
```

```
  select(node, end_state_1_pp, end_state_2_pp, end_state_3_pp, end_state_other_pp)
%>%
  pivot_longer(cols = -node, names_to = "states_pp", values_drop_na = TRUE) ->
end_states_pp
end_states_pp %>%
  mutate(states = gsub("_pp", "", states_pp)) %>%
  rename(pp = value) %>% full_join(end_states) %>%
  rename(depths = value) %>%
  mutate(depths = ifelse(is.na(depths), "other", depths)) %>%
  mutate(depths = ifelse(is.na(depths), "other", depths)) %>%
  select(node, depths, pp) -> depthspies_long
depthspies_long %>%
  filter(depths != "NA") %>%
  pivot_wider(names_from = depths, values_from = pp, values_fill = "0") %>%
transmute(node = as.integer(node),s = as.numeric(`1`),b = as.numeric(`2`),a =
as.numeric(`3`), sb = as.numeric(`4`), sa = as.numeric(`5`), ba = as.numeric(`6`),sba =
as.numeric(`7`),misc = as.numeric(other)) -> depth_pies

fp = "./"
plot_fn = paste(fp, "deep3.simple.range.pdf",sep="")
tree_fn = paste(fp, "deep3state.ase.tre", sep="")
label_fn = paste(fp, "deep3state.state_labels.txt", sep="")
color_fn = paste(fp, "range_colors.n4.3.txt", sep="")

#makestates
make_states = function(label_fn, color_fn, fp="./") {
  # generate colors for ranges
  range_color_list = read.csv(color_fn, header=T, sep=",", colClasses="character")

  # get area names
  area_names = unlist(sapply(range_color_list$range, function(y) { if (nchar(y)==1) { return(y)
} }))

  # get state labels
  state_descriptions = read.csv(label_fn, header=T, sep=",", colClasses="character")

  # map presence-absence ranges to area names
  range_labels = sapply(state_descriptions$range[2:nrow(state_descriptions)],
                  function(x) {
                    present = as.vector(gregexpr(pattern="1", x)[[1]])
                    paste( area_names[present], collapse="")
                  })
  # map labels to colors
  range_colors = range_color_list$color[ match(range_labels, range_color_list$range) ]

  # generate state/color labels
```

```
  idx = 1
  st_lbl = list()
  st_colors = c()
  for (j in 1:(nrow(state_descriptions)-1)) {
    st_lbl[[ as.character(j) ]] = range_labels[j]
    st_colors[j] = range_colors[j]
  }
  st_colors[ length(st_colors)+1 ] = "lightgray"
  st_lbl[["misc."]] = "misc."

  return( list(state_labels=st_lbl, state_colors=st_colors) )
}
# get state labels and state colors
states = make_states(label_fn, color_fn, fp=fp)
state_labels = states$state_labels
state_colors = states$state_colors

cols_d<-setNames(state_colors,names(depth_pies)[-1])


depthpies <- nodepie(depth_pies, cols = 2:9)
depthpies <- lapply(depthpies, function(g) g+scale_fill_manual(values = cols_p))

#to plot pinnule and depth ancestral traits
ggtree(tr)->p1
revts(p1)->p1
p1+
  coord_cartesian(xlim = c(-500,250),
                  ylim = c(-1,Ntip(tr@phylo)+1), expand = FALSE) +
  scale_x_continuous(breaks=seq(-450,0,100), labels=abs(seq(450,0,-100)))-> p2

rad <- 0.075
p2 +
  geom_inset(depthpies, width = rad, height = rad) +
  geom_inset(pinpies, width = rad, height = rad,hjust=15) ->p3
p3
p3 +
  geom_tiplab(aes(label=gsub("_"," ",label)),size=4)->p4
p4

data.frame(time= c(-443,-252),event=c("Ordovician-Silurian extinction",
                                      "Permian-Triassic extinction"))->ex
p4 +
  geom_vline(aes(xintercept=time),ex,color="blue",linetype="dashed") +
  geom_text(data=ex, mapping=aes(x=time, y=0, label=event), size=4,
            vjust=-0, hjust=-0.02)->p5
```

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