Applying Integrative Taxonomic Approaches to Resolve Health and Resilience of the Keystone Coral Species Complex *Acropora hyacinthus* Throughout the Great Barrier Reef

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PhD by Research

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Climate Change Cluster

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“A happy taxonomist”

Orpheus Island, Great Barrier Reef 2022

Image Credit: Augustine J. Crosbie

Certificate of original authorship

I, **Sage Hannah Rassmussen** declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

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Sage Hannah Rassmussen

Date: 7th December 2023

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Last but not least, if you are reading this you also deserve a thank you for supporting my research, I hope you enjoy!

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Thesis Structure

This thesis is comprised of a general introduction (**Chapter 1**), followed by three data chapters (**Chapter 2** to **4**), and a final general discussion (**Chapter 5**). My general introduction (**Chapter 1**) is presented as a background to taxonomy and focuses on the foundational work and missing knowledge that has formed my thesis subject. I finish with a general discussion (**Chapter 5**) that summarizes the knowledge gained from each of my data chapters, provides insights from obtaining this knowledge and discusses future directions for my research topic. At the time of thesis submission, all three of the data chapters (**Chapter 2** to **4**) are in form of manuscripts for peer-review and publication to a scientific journal.

Chapter 2:

This chapter is in the form of a research article prepared for submission to *Invertebrate Systematics*.

> **Rassmussen, S. H.,** Cowman, P.F., Baird, A.H., Crosbie, A., Quattrini, A., Bonito, V., Sinniger, F., Harii, S., Fadli, N., Tan, CH., Huang, J.Y., Bridge, T.C.L. A taxonomic synthesis of the many *Acropora hyacinthus'*: resolving species boundaries of a highly diverse coral species complex.

Chapter 3:

This chapter is in the form of a research article prepared for submission to the *Journal of Biogeography*.

Rassmussen, S. H., Camp, E.F., Nitschke, M.R., Grima, A.J., Haydon, T.D., Bridge, T.C.L., Suggett, D.J. Biogeography of tabular *Acropora*-Symbiodiniaceae associations along the Great Barrier Reef.

Chapter 4:

This chapter is in the form of a research article prepared for submission to *Coral Reefs*.

Rassmussen, S. H., Suggett, D.J., Strudwick, P., Gillette, G., Roper, C., Camp, E.F. Morphology matters: congruence of morphology and molecular phylogenies for closely related taxa of tabular *Acropora.*

Thesis Abstract

Biodiversity, the variety of life on Earth, underpins much of Life Sciences, and drives conservation measures to protect the natural world. Fundamental to our knowledge of biodiversity is the species-level resolution of taxonomy, which is concerned with discovering and describing life. Tropical coral reefs are one of the most biodiverse ecosystems on Earth, covering just 1% of the world's Oceans yet supporting up to a third of marine organisms throughout their life. Success of coral reefs is built upon the scleractinian hard corals that form the complex structures of reefs, whilst also providing a myriad of other ecosystem services both to reef dwellers and humans. Recent improvements in molecular based technologies have led to the discovery of hidden diversity of corals, with mounting evidence that we lack species-level taxonomic resolution of key genera such as the abundant and ecologically significant *Acropora*. Such a lack of taxonomic resolution ultimately raises questions about scientific understanding of coral health, abundance, and threats, in turn stalling the ability to accurately research and target conservation measures of vulnerable species.

The genus *Acropora* is one of the most abundant corals on tropical reefs, with certain morphological varieties - such as the tabular growth *Acropora* - being significantly important for coral reef recovery, resilience, and ecosystem services. Corals within this tabular group are known to form species complexes, with evidence proving the existence of at least six species forming a single complex, termed the '*Acropora hyacinthus* complex'. In this thesis I have performed an integrated

taxonomic revision, combining both molecular and morphological analysis, to resolve the species boundaries of this complex. In doing so I discovered new species of coral and resurrect several nominal species to collectively increase the known diversity of this group and improve taxonomic resolution.

With improved resolution on species-level diversity of the '*Acropora hyacinthus* complex', I addressed key questions involving coral health and conservation. Firstly, I explored the biogeographic species-specific relationships between corals and their endosymbiotic algae along the latitudinal gradient of the Great Barrier Reef. This revealed both biogeographic patterns of symbiont associations, and the diversity of rare symbiont associations across species and reefs. From this work, I was able to re-define host-symbiont association specificity that has in the past remained conflated through poorly resolved host taxonomy. Finally, with my new species level resolution of taxa previously considered 'cryptic' species complex I addressed the issue of species morphology. Specifically, the ability of both scientific researchers and conservation practitioners to accurately identify diversity, especially when morphologically similar species live in sympatry. This work focussed on the Whitsundays region of the Great Barrier Reef, where little has been researched historically on tabulate *Acropora* diversity and distribution patterns. With the exploration of novel morphological features, I was able to identify several informative morphological traits that were successful in delineating between co-occuring species, thereby potentially offering a new morphological-based framework to identify amongst cryptic tabulate *Acropora* species.

Collectively, throughout this thesis I have performed a robust taxonomic revision of a keystone coral species complex, and then applied the gained knowledge to two key areas of research and conservation for coral reefs. I have demonstrated the importance of improving species-level taxonomy and provided a framework for the application of taxonomy and morphology for robust species identification to improve research and conservation efforts needed more broadly.

"…and yet the impulse which drives man to poetry will send another into the tide pools and force him to try and report on what he finds there…why do men, sitting at the microscope, examining the calcareous plates of a sea cucumber…feel and exaltation and give the new species a name…it would be good to know the impulses truly, not to be confused by the "services to science" platitudes or the other little mazes into which we entice our minds so that they will not know what we are doing."

J. Steinbeck, The Log from the Sea of Cortez

"…the last fallen mahogany would lie perceptible on the landscape, and the last black rhino would be obvious in its loneliness, but a marine species may disappear beneath the waves unobserved, and the sea would seem to roll on the same as always." Ray 1988

Chapter 1

GENERAL INTRODUCTION

1.1 Taxonomy and Nomenclature

Taxonomy and nomenclature – the science of identifying organisms and categorizing them according to a universal 'taxonomic' language – provide the foundation for the study and management of the natural world. Central to this is the ability to identify a species, one of the fundamental units of biology (de Queiroz 2007). The species unit is crucial for a variety of scientific disciplines, including ecology, genetics, physiology, microbiology, and evolutionary biology (Thiele et al. 2021; Sandall et al. 2023). In addition, the ability to identify a species underpins the interpretations of biodiversity surveys and species distributions, which in turn impact management decisions and conservation efforts (Mace 2004; Sandall et al. 2023). Linking these scientific disciplines and management of the natural world is the sharing of knowledge, which relies on agreed-upon and universal species names to transfer information relating to distinct species. Thus, the importance of taxonomy & nomenclature can be understood through the intrinsic tasks it performs: delineating and categorizing diversity, and providing species with names and tools in which they can be identified (Dayrat 2005).

1.1.1 Molecular Technologies, Cryptic Species, and the Transfer of Knowledge

Recent and rapidly advancing molecular technologies have brought the science of taxonomy into a new era, where molecular phylogenies are revolutionising our understanding of evolutionary biology and species diversity (Fišer et al. 2018). Use of genetic material to explore the relationships amongst organisms has led to the discovery of a plethora of hidden biodiversity, including the increasingly common discovery of cryptic species (Bickford et al. 2007). These cryptic species – where two or more genetically distinct species have been classified as one, commonly due to similar morphologies – have been discovered across both marine and terrestrial ecosystems (Bickford et al. 2007) and have implications for both scientific research and conservation efforts, especially when cryptic species are discovered, yet not formally (taxonomically) described and given a name (Jörger & Schrödl 2013; Pante et al. 2014).

A key challenge that arises when cryptic species are detected is the ability to taxonomically resolve species boundaries and formally describe newly discovered species (Jörger & Schrödl 2013). This process is often labour intensive and costly, involving significant and targeted sampling of voucher specimens and an integrated taxonomic analysis (Dayrat 2005), coupled with a thorough investigation of the nomenclature and type specimens to resolve boundaries and describe new species (Adams et al. 2014). Ultimately, the ability to perform this taxonomic feat often leads to a lag between initial 'discovery' of cryptic species and final diagnosis and description of taxa (Pante et al. 2014). This final process of taxonomy, the naming of new species, whilst often overlooked is one of the fundamental steps in describing biodiversity (Pante et al. 2014). Key to the

successful integration of data regarding species across scientific disciplines and policy is the binominal name in which species are identified with (Sandall et al. 2023). Importantly, if at any stage of information transfer a species is incorrectly identified, or cryptically hidden, this erroneous information can flow on to other disciplines of science and management and can have 'cascading' impacts (Bortolus 2008; Fisher et al. 2017). For example, biodiversity lists, which often form the foundation for conservation decisions and politics, generally include only taxa that have been discovered and provided with formal species names, given through the rules of nomenclature (Pante et al. 2014). Additionally, when cryptic species are undiscovered there can be gross underestimations of diversity, and overestimations of species abundance and ranges that can mask the need for conservation of threatened taxa (Bickford et al. 2007). At the end of the day, taxonomists provide species with a name so they can be included in conservation and policy, although the effective management and protection of species by conservation efforts is required to ensure they persist through time.

1.1.2 Taxonomy and Conservation

Conservation and taxonomy are intrinsically linked, as one discipline seeks to identify and describe the worlds biodiversity for it to be protected, and the other requires knowledge on species and their distributions to protect them (Mace 2004). As we are faced with the increasing destruction of the natural world, evidence is mounting that we are entering the sixth mass extinction of species, the first one caused entirely of anthropogenetic activity (Dirzo et al. 2014; Pimm et al. 2014). With this comes the sobering fact that we collectively have little knowledge about the true number of species on planet earth, and it is likely that

many taxa are highly threatened or going extinct without our knowledge of them existing (Pimm et al. 2014). Although the number of published taxonomic works has seen a rise in recent times (Costello et al. 2015), we are still faced with an overwhelming number of species on earth and in our oceans that remain undescribed, with estimations of up to a third of all life remaining undescribed (Costello et al. 2015). In part, this can be attributed to the increase in molecular based phylogenetic taxonomic works that omit any formal naming of newly discovered species (Pante et al. 2014). Without formal species descriptions we are unable to effectively study species distribution and abundance, ecology, fitness and evaluate their vulnerability to impacts (Sandall et al. 2023). This then leads to an inability to assess, conserve, and protect species from extinction and further impedes our ability to track the impact of this biodiversity loss (Pimm et al. 2014). With these impeding threats to biodiversity there is an urgent need for discovery, knowledge, and naming of undiscovered and cryptic species (Costello et al. 2015).

1.2 Coral Reefs & Biodiversity Conservation

Coral reefs are one of the most biodiverse ecosystems on earth, with up to a third of all marine life dependent on coral reefs for a portion of their lifecycle (Reaka-Kudla 1996). These “biodiversity hotspots” are immensely important for both their ecological diversity and the ecosystem services they provide. Around the world, coral reefs are essential for coastal protection, food provisions, tourism, and cultural value (Eddy et al. 2021). Ecologically, coral reefs provide shelter, food, and substrate for reef-dwelling species (Eddy et al. 2021), and the various ecosystem services returned have an estimated value in the trillions (Souter et al. 2021). Coral reef health rests on the Scleractianian (hard) corals that, along with

their endosymbiotic algae of the Family Symbiodiniaceae, provide the architectural complexity and structure that form reefs. Reef-building corals are the foundational organisms but are immensely susceptible to climate change (e.g., ocean warming, acidification and deoxygenation (Hoegh-Guldberg et al. 2017; Alderdice et al. 2022)), and local anthropogenic stressors such as pollution and over-fishing (Hoegh-Guldberg et al. 2007). Arguably the most pressing threat to coral reefs is heat-induced mass bleaching and mortality of corals (Hughes et al. 2017; Sully et al. 2019), where the coral endosymbionts in effect become toxic to their hosts (e.g., Suggett & Smith 2020). Since the 1950s, there has been an estimated 50% decline in coral cover globally and hence in a decrease in the ecosystem services and provisions they provide (Eddy et al. 2021). Occurrence of mass coral bleaching events have increased in frequency and intensity in the past 30 years from rising sea surface temperatures under climate change (SST; Hughes et al. 2018; Sully et al. 2019) raising time-critical concerns that "business as usual" conservation and management efforts will be insufficient to safeguard reefs as we know them over the coming decades (e.g., Kleypas et al. 2021).

Motivation to enhance protection and conserve coral reefs has resulted in a rapid increase in 'reactive' approaches – notably coral restoration – globally (Hein et al. 2021) to augment more traditional 'proactive' management such as marine protected areas (MPA's). These reactive approaches often involve direct restoration of damaged or degraded ecosystems that support recovery and boost resilience (Boström-Einarsson et al. 2020; Hein et al. 2021). Advancements in the field of reactive management has resulted in several frameworks proposed to provide best practice measures for coral reef restoration (e.g., Vardi et al. 2021; Quigley et al. 2022; Suggett et al. 2023). A major factor to the success of reef

restoration is the resulting biodiversity it has supported and protected from loss (Quigley et al. 2022). Biodiversity in coral assemblages have been shown to have a positive effect on growth and recovery of coral reefs (Clements & Hay 2021) providing a boost to their resilience to future stress (Dury & Lirman 2017). Indeed, the importance of protecting biodiversity has resulted in a global biodiversity market worth up to US$967 billion (in 2020, Suggett et al. 2023) due to the important ecosystem services provided by the worlds biodiversity, leading to the global biodiversity targets such as the Aichi Biodiversity Targets established in 2010 by the Convention of Biological Diversity (CBD, Diaz et al. 2020). However, due to continued biodiversity loss over the last decade the CBD Parties have now adopted a new framework, the Kumming-Montreal Global Biodiversity Framework, to succeed and replace the failed Aichi Biodiversity Targets (Stephens 2023). The Kunming-Montreal declaration signed by 195 countries in 2022 now tasks ambitious targets to recover and protect biodiversity with four goals and twenty-three accompanying targets, which in turn is stimulating new biodiversity based economic markets (e.g., Biodiversity credits, Stephens 2023).

Biodiversity is a valuable resource and, with increasing importance given to the protection of the world's biodiversity, we are again faced with the recurring question: *how many species are there on earth and in our oceans* (Appletans et al. 2012). It is estimated that there are 2.2 million marine species globally (Mora et al. 2011), of which a quarter depend on coral reefs for a portion of their lifecycle, although throughout the oceans it is predicted that up to 91% of life is yet to be discovered and described (Mora et al. 2011). This is particularly true for coral reefs where much hidden diversity can be attributed to the Scleractinian hard

corals that form the foundation of these ecosystems (Cowman et al. 2020). With a lack of information about true species diversity for the corals that form reefs we are unable to truly measure the threat imposed to any one species and the success of any conservation efforts.

1.3 Coral Taxonomy & *Acropora hyacinthus* (Dana, 1846)

As with many taxa across the tree of life, the improvement in molecular phylogenies in recent years has advanced our understanding of coral evolutionary history and taxonomy (Kitahara et al. 2016; Cowman et al. 2020). However, with these advancements has come the difficult task of resolving taxonomic inconsistencies from the Family to species rank. Whilst work has begun to revise the taxonomy across many coral genera (*Craterastrea*, Benzoni et al. 2012; *Mussidae*, Budd et al. 2012; *Pocillopora*, Schmid-Roach et al. 2014; *Lobophylliidae*, Arrigoni et al. 2016; Huang et al. 2016), there are still many key groups that are lacking revisions, including within the genus *Acropora*, a prolific genus that dominates reefs of the Indo-Pacific and which contains many of the reef-forming coral species that provide architectural complexity and shelter for marine organisms (Renema et al. 2016, Mao et al. 2018). This genus is also particularly vulnerable to climate change and other stressors (Work et al. 2011; Hoogenboom et al. 2017).

Evidence has been mounting that the species diversity of *Acropora* is much larger than currently accepted, with a recent molecular phylogeny created by sequencing the ultraconserved element (UCE) region of the genome (Cowman et al. 2020) showing *Acropora* to form six clades (I – VI, Fig. 1.1) that are largely incongruent with the currently accepted taxonomic groupings based on

morphology (Wallace 1999). With this UCE phylogeny a recent integrated taxonomic revision of the *Acropora tenuis* (Dana, 1846) complex found this putatively common species thought to occur across the Indian and Pacific Oceans is made up of at least 11 genetically and morphologically distinct lineages (species) each with much smaller geographic ranges (Fig. 1.1, Bridge et al. 2023). This specific example of revision ultimately led to the resurrective of five nominal species and the description of two new coral species (Bridge et al. 2023). Indeed, across the genus *Acropora* there is further evidence of hidden 'cryptic' diversity, greater than what is reflected in the current taxonomy (*Acropora hyacinthus*, Ladner & Palumbi 2012; *Acropora samoensis*, Rosser et al. 2015; *Acropora* spp*.,* Richards et al. 2016; *Acropora pruinosa*, Pipithkul et al. 2021).

For over a decade, multiple lines of evidence have indicated that a keystone species, *Acropora hyacinthus* (Dana 1846) is made up of multiple 'cryptic species' that form a species complex (Ladner & Palumbi 2012; Cros et al. 2016; Suzuki et al. 2016; Rose et al. 2018; Sheets et al. 2018; Ramírez-Portilla et al. 2021). The species *A. hyacinthus,* and others identified in this 'species complex' form a tabular colony gross morphology (Fig. 1.2), and in the six clade UCE phylogeny for *Acropora* (Cowman et al. 2020), tabular *Acropora* were generally found in Clade VI which was one of the largest clades (Fig. 1.1), indicating the possibility of increased species abundance not reflected in the current taxonomy. Indeed, in this *Acropora* phylogeny over 50% of specimens could not be confidently assigned to any of the 408 nominal species of the genus (Cowman et al. 2020), further highlighting the likelihood that biodiversity is underestimated in the currently accepted taxonomy of *Acropora* (Wallace et al. 2012).

An abundance of research has been performed on tabular *Acropora* and *A. hyacinthus* in particular, with a Google Scholar search revealing 80 scientific reports containing "*Acropora hyacinthus*" in their title, 712 scientific reports mentioning "tabular *Acropora*", and 3,090 scientific reports mentioning "*Acropora hyacinthus*" within the main text – 2,600 of which were published in the last 20 years. Despite this plethora of study indicating *A. hyacinthus* to be one of the most studied species of *Acropora* globally, and the evidence that species diversity of tabular *Acropora* – particularly *A. hyacinthus* – is greater than currently accepted, to date no taxonomic revisions have been performed to resolve the species boundaries and describe the 'cryptically' hidden species of this complex.

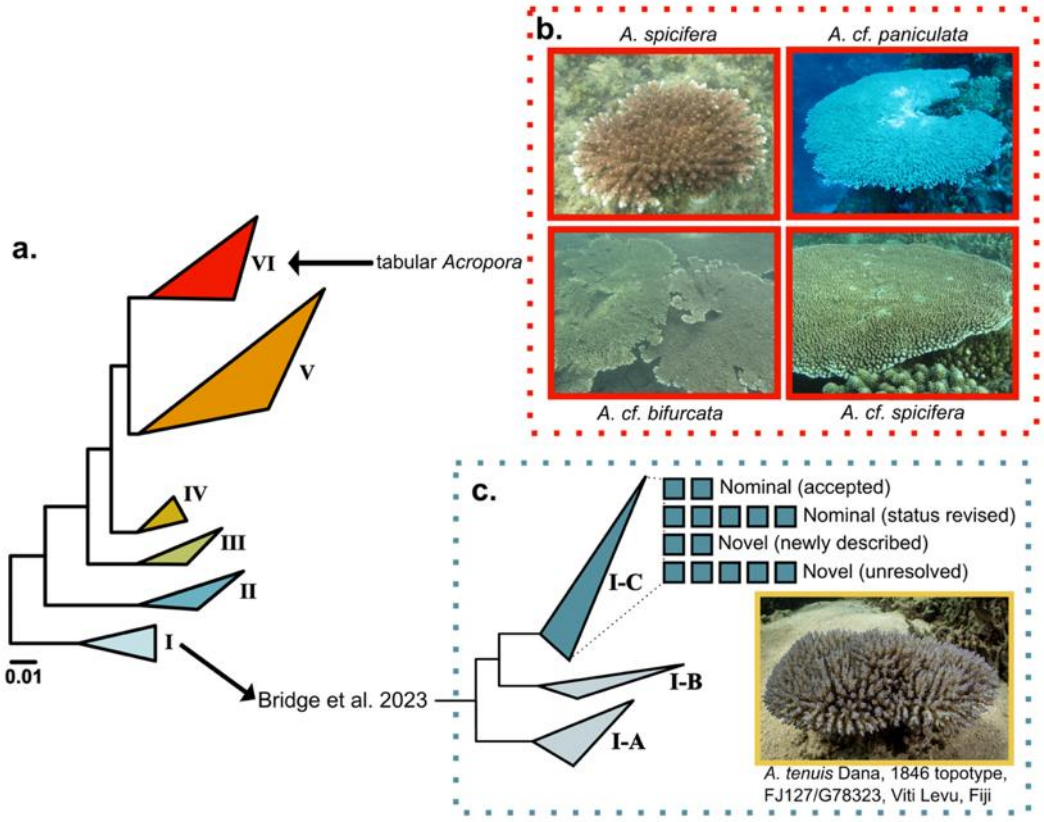

Figure 1.1 Schematic showing the **a)** six clade structure of the *Acropora* phylogeny (Cowman et al. 2020) with **b)** images of four tabular *Acropora* colonies and species assignments according to Cowman et al (2020) that were all resolved in Clade VI of this phylogeny. The use of *cf.* in species names indicate resemblance to the species with some level of uncertainty. Also, **c)** improved resolution of Clade I resolved by Bridge et al. (2023) that focused on one (I-C) of three subclades. Here, each box next to Clade I-C represents a single lineage (species) and the status of each species resulting from this taxonomic revision is shown.

Acropora hyacinthus is considered to be part of the '*Acropora hyacinthus*' group of tabular corals according of Wallace (1999), alongside *Acropora tanegashimensis* Veron, 1990, *Acropora anthocercis* (Brook, 1893), *Acropora cytherea* (Dana, 1846), *Acropora microclados* (Ehrenberg, 1834), *Acropora paniculata* Verrill, 1902 & *Acropora Indonesia* Wallace, 1997. These corals are grouped according to a shared common tabular colony growth with short vertical branchlets, similar coenosteum and radial corallite shapes that attribute to this morphological grouping (Fig. 1.2, Wallace 1999). Amongst these seven corals of the "*Acropora hyacinthus*" group there are also 17 nominal species that have been synonymized, equating to a total of 24 nominal species of tabular *Acropora* that are considered to have similarities to *A. hyacinthus*, although with only seven of these being currently accepted. With evidence of hidden diversity within both *A. hyacinthus* and *A. cytherea* (Ladner & Palumbi 2012), and with recent taxonomic revisions of other *Acropora* (*A. tenuis*, Bridge et al. 2023) showing increased diversity and errors in historical taxonomic revisions, it is now plausible that some of these nominal taxa have been incorrectly synonymized and are in fact valid

species, and that there may be undescribed species of tabular *Acropora* in our oceans.

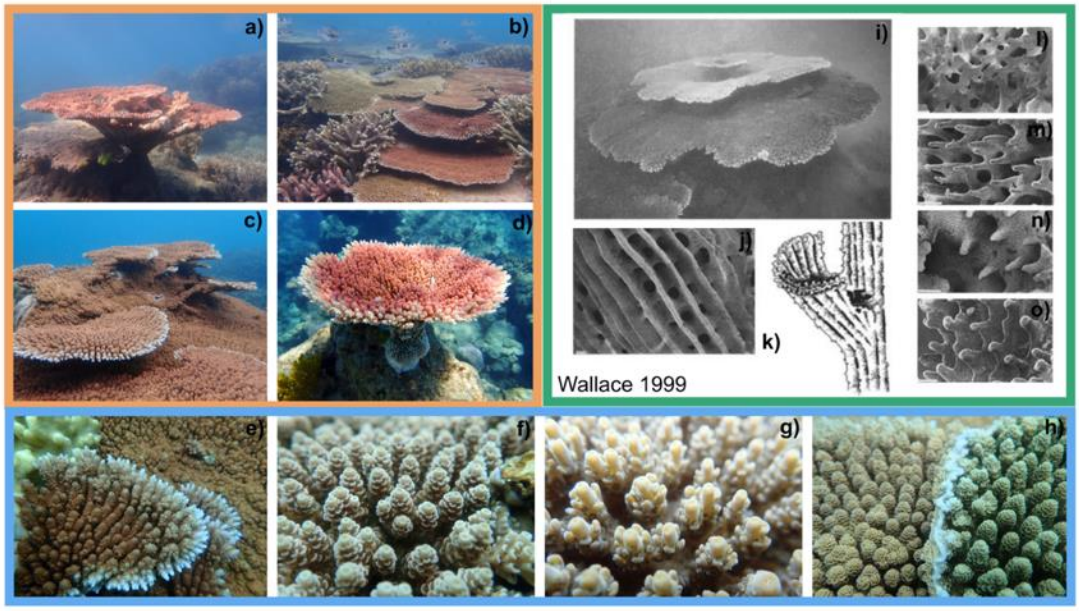

Figure 1.2 Images of tabular growth *Acropora* from the Great Barrier Reef, showing **a-d)** macro colony growth displaying tabular gross colony morphology in the field, **e)** close-up image of branchlet structure, **f-g)** close-up of branchlets showing radial corallite formation, **h)** two colonies growing next to each other, showing colour and growth variation. The images in the top right are from Wallace (1999) and show the morphological features that distinguish the '*hyacinthus* group' of species, which are **i)** tabular colony morphology, **j)** costae coenosteum on radials, **k)** labellate radials with a rectangular lip and **l-o)** reticulate coenosteum with simple spinules between corallites.

Implications of an unresolved taxonomy for this group of tabular *Acropora* are immense – whilst errors in taxonomy lead to theoretical problems, the applications of an incorrect taxonomy in scientific study and conservation have very real and practical problems that carry negative consequences on the natural world (Bortolus 2008; Costello et al. 2015). To begin with, biodiversity metrics that are unable to account for cryptic diversity can vastly underestimate the true

species diversity and richness across space and time. Species lists and extinction status, such as those provided by the International Union for the Conservation of Nature Red List (IUCN Red List) provide measures of extinction threat based on ecological assessments of species, although erroneous taxonomic assessments can heavily impact the stability of these assessments (Mace 2004). For *A. hyacinthus*, the current IUCN Red List status marks this species as 'near threatened' with a global distribution (Fig. 1.3), However, taxonomic uncertainties render this assessment unstable, as it is unknown what the true diversity, distribution and consequently threat this species faces when it is known that there is a minimum of six 'cryptic species' residing within this complex (Ladner & Palumbi 2012). Such problems become more concerning where it has been shown that tabular *Acropora* are keystone species for reef architecture, recovery, and for supporting biodiversity (Kerry & Bellwood 2015; Ortiz et al. 2021), but also highly threatened by bleaching (Brodnicke et al. 2019; Sakai et al. 2019), disease (Brodnicke et al. 2019) & storm damage (Madin et al. 2012). If individual species of tabular *Acropora* are found to be less common, with the potential of being regionally endemic as has been found species within the *A. tenuis* complex (Bridge et al. 2023), then these threats faced by tabular *Acropora* may lead to local extinctions of key taxa. In turn, without such knowledge, we are further hindered in our ability to assess the impact of these biodiversity losses.

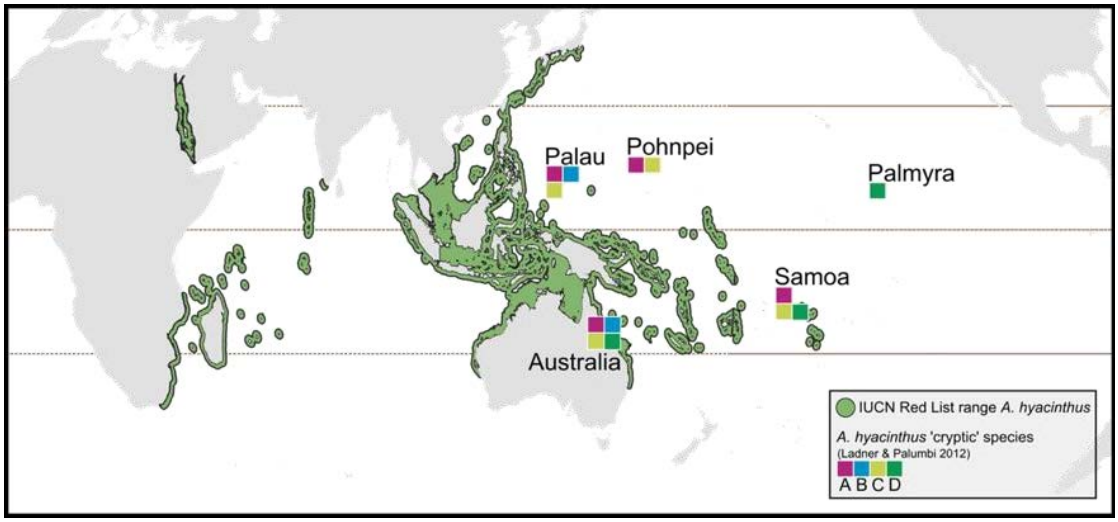

Figure 1.3 Distribution map showing the International Union for the Conservation of Nature (IUCN) Red List accepted range for *A. hyacinthus* (green shading) based on assumed biogeography for this species. Overlaid is the distribution for each of the four (A, B, C & D) cryptic *A. hyacinthus* species discovered by Ladner & Palumbi (2012).

1.4 Coral-Symbiodiniaceae Associations

As corals face impending bleaching episodes – including another global event underway at the time of writing this thesis in 2023 – there is a need to understand the mechanisms that drive differences in bleaching tolerances and survival across species to better understand resilience over time (Suggett & Smith 2020). One such measure of health is by quantifying the species of endosymbiotic algae that live within the coral tissue. Whilst some corals harbour heat tolerant species that can protect them when faces with increased ocean temperatures, others may instead favour species that provide increased energy for growth whilst performing poorly during increased temperature conditions (Leinbach et al. 2023). Understanding the symbiotic associations that corals form, both inter- and intraspecific across different environments and regions can provide tools in which we can further quantify threat levels of certain species, and to explore ways in

which we can boost favourable associations according to the conditions (Suggett et al. 2017). Both situations, being conservation and scientific study require an understanding of species boundaries to interpretate results and pass on knowledge relating to a distinct species. For example, when species are used as bioindicators for health and resilience of a system we rely on a resolved taxonomy to ensure that measurements made from such species are not confounded by phenotypic differences in closely related and morphological similar taxa (Bortolus 2008).

For the species complex discussed above, *A. hyacinthus*, a lack of knowledge for species boundaries and diversity hinders our ability to measure intra-specific symbiotic associations, thereby leading to an inability to measure inter-specific differences in bleaching tolerances that could explain patchiness in bleaching observed for tabular *Acropora* in the past (Hoogenboom et al. 2017). Past reports from the GBR, South China Sea and French Polynesia have indicated that *A. hyacinthus* colonies switch their dominant symbiont types when faced with increase thermal stress (Quigley et al. 2022; Zhu et al. 2022, Leinbach et al. 2023), aligning with the "adaptive bleaching hypothesis" (Baker et al. 2004), with environment also indicated as a key factor in shaping symbiont associations. However, reports from the GBR have also shown this species to host a stable community over time, with seasonality not affecting dominant symbiont communities (Epstein et al. 2019). With taxonomic uncertainties, it is unclear if differences in dominant communities are driven by different cryptic species, or if changes across environments are driven by host environmental niches of cryptic coral taxa. Indeed, in America Samoa investigations into the symbiont communities amongst different species within the *A. hyacinthus* complex indeed

observed inter-specific differences in bleaching tolerances correlating with symbiont types hosted by host species (Rose et al. 2021).

Knowledge of the factors that influence coral-algal symbiosis could prove useful for reef restoration, where thermally tolerant species or associations may be targeted to boost reef health when faced with future stressors, although higher thermal tolerance may come as a trade-off for fast colony growth (Cornwell et al. 2021). Such trade-offs may be problematic, as fast growth is a trait often attributed as a key factor to the success of tabular *Acropora* corals in boosting recovery and biodiversity of a reef through the architectural complexity and cover they provide (Ortiz et al. 2021). Ultimately, the inability to assess diversity impacts our understanding of the algal associations and the spatial distribution and relative abundance of each of these cryptic species through time, ultimately leading to a lack of information about which taxa may be at higher risk of bleaching and therefore in greatest need of protection, but also which of the associations may be explored further as tools to mitigate future bleaching episode and to boost resilience when restoring damaged reefs (Suggett et al. 2017).

1.5 Morphology & Species Identification

An important task for taxonomists is providing reliable and easy to use tools for identifying species, once they have been taxonomically delineated (Dayrat 2005; Costello et al. 2015). Non-taxonomists, including biologists, ecologists, conservation practitioners and citizen scientists require the tools in which they can identify species without expert taxonomic knowledge. Morphologically similar species living in sympatry may have different ecological and behavioural patterns, disease resistances, competitive interactions and more

(Bortolus 2008). An inability to correctly identify diversity can thus lead to poor interpretation of experimental results and success and has potential to affect the relative abundance and consequently the biodiversity of an ecosystem, leading to a decline in redundancy and resilience of such systems (Bortolus 2008). This is particularly true for reef restoration, where the acceleration of interest is often via local stakeholders with limited means to robustly taxonomically resolve cryptic species, such as those of tabulate Acropora (see for example Suggett et al. 2022) Indeed, on the GBR there are several active reef restoration programs (McLeod et al. 2022) and recent evidence of the importance of tabular *Acropora* for reef recovery and resilience (Ortiz et al. 2021) has driven restoration interest in *Acropora* corals with these morphologies. However, with evidence indicating that at least four morphologically similar species of the *A. hyacinthus* complex occur on the GBR (Fig. 1.3, Ladner & Palumbi 2012) there is reason to believe that targeted diversity may be lacking in restoration efforts. With an inability to identify species morphologically, and lacking information on extinction threat, range, and stress response of any one species of this complex these restoration efforts are lacking vital information and tools to target distinct species. Whilst there is the promise of tools such as DNA barcoding to identify species (Blaxter 2004), this still carries limitations such as time and resources (e.g. funding) constraints and impedes immediate identification in the field. Further, these technologies are only useful once reliable 'barcode' sequences are identified which can successfully delineate between species (Weins et al. 2004; Will et al. 2005). Traditionally, morphology has been the common tool for species identification across the tree of life, however, using morphology for species identification of corals has been questioned in recent times, as molecular

phylogenies and environmental plasticity has uncovered errors in past taxonomic assignments made using morphology alone (Kitahara et al. 2016; Cowman et al. 2020; Bridge et al. 2023; Keating et al. 2023). However, the importance of correctly identifying species and diversity drives the need for morphologically reliable identification tools.

Whilst micromorphological and microstructural features of the coral skeleton have been informative in delineating between some Scleractinia (Kitahara et al. 2016), it has been found to be uninformative for others (Flot et al. 2011). Further, these features are often only observable with specialised equipment, such as scanning electron microscopy which is both costly and time consuming, hindering field based and non-specialist species identification. Success has been made in identifying a small number of reliable diagnostic traits for species delimitations for three morphologically similar tabular *Acropora* corals in Japan (Ramírez-Portilla et al. 2021). These traits, which included colour and corallite morphology, were easily identifiable, providing evidence that novel morphological features may indeed be a useful tool for species delimitations amongst morphologically similar or putatively 'cryptic' species. Whether this applies to likely similarly cryptic tabular *Acropora* species elsewhere remains unknown.

1.6 Thesis Roadmap & Aims

Conservation effectiveness is – in part – strongly governed by an accurate understanding of biodiversity and hence a sound taxonomic foundation where individual taxa can be readily identified, and agreed upon species names allow study and integration of data across platforms through space and time (Fig. 1.4).

Such a concept has recently been further fuelled by "biodiversity accounting" where fully resolving species diversity underpins ecological value to new economic markets. Coral reefs have always been renowned for their exceptional taxonomic diversity, but taxonomic uncertainty of corals continues to impact research and conservation based around species-specific responses of corals to stress events, specificity of coral-algal symbiosis, biogeography and abundance of distinct taxa and ultimately threatened species status, which collectively affect management decisions. Difficulties in resolving taxonomic boundaries for corals is impacted by the morphological plasticity of many taxa which lends to the presence of cryptic species complexes – with little taxonomic resolution – that hinder our ability to successfully study and conserve key taxa. Therefore, the overarching goal of this thesis is to first address knowledge gaps in the taxonomic resolution and consequent diversity of a keystone group of tabular *Acropora* – commonly referred to as the '*Acropora hyacinthus* complex' (**Chapter 1**) and resolve how this hidden diversity affects biogeography, symbiont associations (**Chapter 2**), and conservation efforts (**Chapter 3**) of this group (Fig. 1.4). Taxon of this group are considered key in providing architectural complexity and microhabitats for coral reefs and their inhabitants, although are often severely impacted by heat, motion (i.e. coral bleaching and cyclones) and disease induced stressors (Ortiz et al. 2021). Thus, resolving species and consequently investigating the impact of a resolved taxonomy on key coral processes such as symbiont associations and conservation practices is time critical as reefs face an increase in predicted future stress.

The specific questions posed – that in turn form the aims – of this thesis are:

i) What is the true diversity of tabular *Acropora* corals erroneously conflated taxonomically as *A. hyacinthus* (**Chapter 2**)?

ii) How does improved taxonomic resolution affect our understanding of the stability of coral-Symbiodiniaceae associations and thus health of individual taxa (**Chapter 3**)?

iii) Can we develop widely applicable tools to ensure species and consequently genetic diversity of morphologically similar species are correctly identified (**Chapter 4**)?

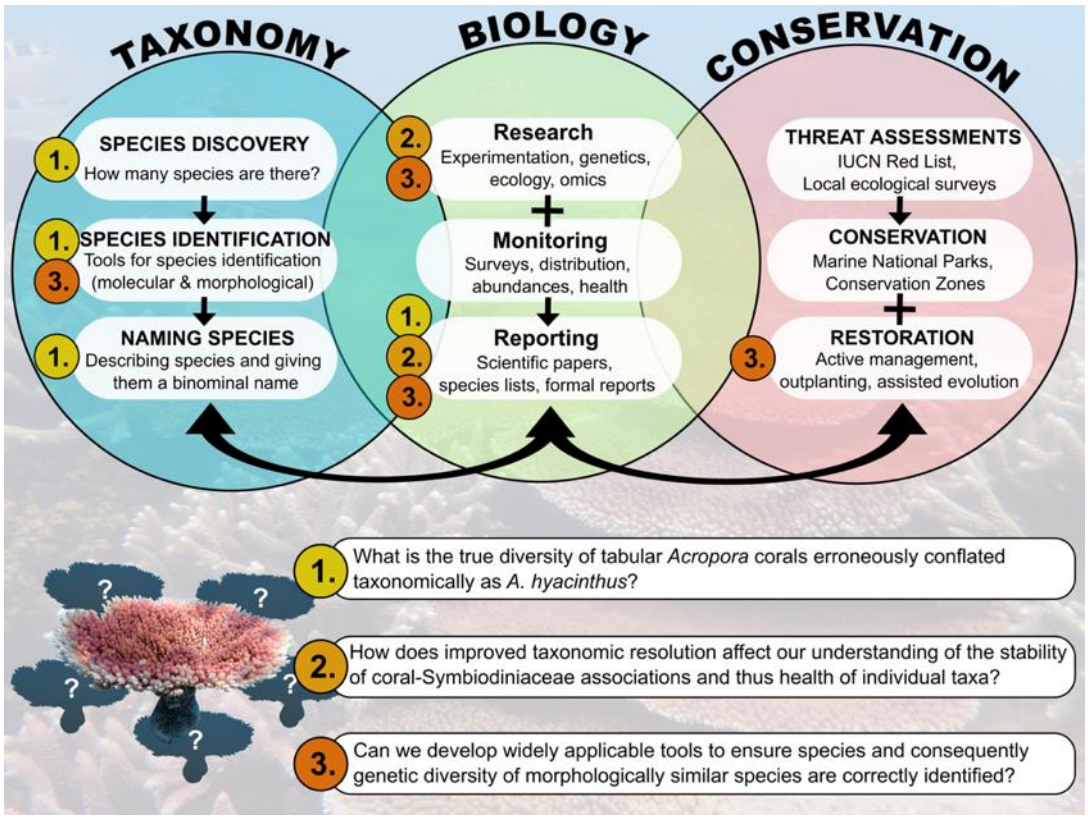

Figure 1.4 Biological research and conservation are governed by the taxonomic resolution of the targeted taxon. An inability to correctly identify distinct taxonomic units hinders the success of conservation efforts and prevents study and integration of data across space and time. The schematic here shows the dependency of these disciplines on each other with integration of knowledge flowing in both directions. Within disciplines, arrows depict the direction of the

relationships of each activity and plus signs indicating independent activities. The three knowledge gaps identified (1 = **Chapter 2**, 2 = **Chapter 3**, 3 = **Chapter 4**) are addressed in this thesis by the activities indicated in each discipline shown.

These aims are directly addressed in the three data chapters below:

Aim 1 (Chapter 2). Resolve taxonomic boundaries between closely related species of the cryptic '*Acropora hyacinthus* complex'. Advancements in molecular technologies has rapidly uncovered the presence of species complexes throughout the tree of life, including for Scleractinia where complex and plastic morphologies, sympatric speciation and poor phylogenetic resolution has lent to confusions between species boundaries. In this first chapter I performed an integrated taxonomic revision of the highly diverse and keystone '*Acropora hyacinthus*' complex throughout the Indo-Pacific. By using a state-of-the-art approach combining a molecular phylogeny generated by sequencing the Ultraconserved Element (UCE) region of the genome, along with Single Nucleotide Polymorphism (SNP) population genetics as well as morphological trait analysis, I delineate species within this complex, resurrecting previously synonymised taxa and describing several new species. This led to the next question as to how this new taxonomic framework affects our understanding of coral-symbiont association stability.

This chapter has been prepared for submission to *Invertebrate Systematics:*

Rassmussen, S. H., Cowman, P.F., Baird, A.H., Crosbie, A., Quattrini, A., Bonito, V., Sinniger, F., Harii, S., Fadli, N., Tan, CH., Huang, J.Y.,

Bridge, T.C.L. A taxonomic synthesis of the many *Acropora hyacinthus'*: resolving species boundaries of a highly diverse coral species complex.

Aim 2 (Chapter 3). Explore coral-algal symbiotic associations along the latitudinal gradient of the GBR for several closely related coral taxa. In this second chapter, I explored coral-Symbiodiniaceae associations along the latitudinal gradient of the Great Barrier Reef (GBR) in the framework of the new taxonomic resolution resolved in my first chapter. Here, I sequenced the ITS2 region of *Acropora* algal symbionts and explored both the intra- and inter-specific symbiotic associations amongst a group of six closely related tabular *Acropora* coral species. By sampling the latitudinal gradient of the GBR, which is characterized by strong environmental gradients, I further explored how changes in coral-Symbiodiniaceae association correspond with those of key factors know to influence coral fitness (e.g. sea surface temperature, irradiance, and chlorophyll-*a* concentration). I show a latitudinal gradient of symbiont associations that correlates with cooler temperature maximum reefs and high interspecific associations, emphasising the need to resolve the identity of the host taxa in interpreting biogeography and eco-evolutionary change.

This chapter has been prepared for submission to the *Journal of Biogeography*:

Rassmussen, S. H., Camp, E.F., Nitschke, M.R., Grima, A.J., Haydon, T.D., Bridge, T.C.L., Suggett, D.J. Biogeography of tabular *Acropora*-Symbiodiniaceae associations along the Great Barrier Reef.

Aim 3 (Chapter 4). Discover which morphological features are phylogenetically informative in delineating four morphologically similar and closely related taxa of tabular *Acropora* living in sympatry on the GBR. In this final chapter, I performed a targeted morphological and molecular analysis on four sympatric and closely related species of tabular *Acropora* within an applied research framework (accurately selecting coral species for propagation-based reef restoration), at five selected reef sites in the Whitsundays region of the GBR. The aim was to resolve key – but easily diagnosed – morphological markers that were informative for species identification, and to determine if morphological variability (both intra- and interspecific) corresponded with genetically-resolved populations. As such, whether morphology could provide tool in the field for both scientists and restoration practitioners to immediately identify closely related and morphological similar species and genetic populations without the need for costly and time-consuming DNA sequencing. I show for the first time the usefulness of several novel morphological traits in delineating species in congruence with molecular population structure.

> This chapter has been prepared for submission to *Coral Reefs*:
> **Rassmussen, S. H.,** Suggett, D.J., Strudwick, P., Gillette, G., Roper, C., Camp, E.F. Morphology matters: congruence of morphology and molecular phylogenies for closely related taxa of tabular *Acropora.*

The knowledge obtained through addressing these aims is synthesized in **Chapter 5** where I discuss the value of this integrated taxonomic approach in resolving further groups of coral, specifically of the genus *Acropora*. I explore the steps

required to improve the collective taxonomic resolution of corals and the ways which this can support ongoing research and conservation efforts. Additionally, the expert taxonomic knowledge gained through delivering these thesis aims contributed to additional collaborative research presented via further publications and invitations to taxonomic workshops attended throughout my PhD candidature.

Publications:

Smallhorn-West, P. F., Garvin, J. B., Slayback, D. A., DeCarlo, T. M., Gordon, S. E., **Fitzgerald, S. H.**, Halafihi, T., Jones, G. P., & Bridge, T. C. L. (2020). Coral reef annihilation, persistence and recovery at Earth's youngest volcanic island. *Coral Reefs*, *39*(3), 529-536. https://doi.org/10.1007/s00338-019-01868-8

Suggett, D. J., Nitschke, M. R., Hughes, D. J., Bartels, N., Camp, E. F., Dilernia, N., Edmondson, J., **Fitzgerald, S.**, Grima, A., Sage, A., & Warner, M. E. (2022). Toward bio-optical phenotyping of reef-forming corals using Light-Induced Fluorescence Transient-Fast Repetition Rate fluorometry. *Limnology and Oceanography: Methods*, *20*(3), 172-191. https://doi.org/https://doi.org/10.1002/lom3.10479

Howlett, L., Camp, E.F., Locatelli, N., Baums, I., Strudwick, P., **Rassmussen, S.**, Suggett, D.J. (2023) Population and clonal structure of *Acropora hyacinthus* to inform coral restoration practices on the Great Barrier Reef. *Coral Reefs* (in review)

*During my PhD studies I took on a new last name (Rassmussen), and in publications prior to 2023 I am identified by my maiden name (Fitzgerald).

Workshops:

2022: Project Phoenix Taxonomy Workshop held at Orpheus Island on the Great Barrier Reef and hosted by the Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, organised by Prof. Andrew Baird. In November 2022 I was invited to join this taxonomy workshop alongside an international team of researchers, each with an interest in coral systematics and taxonomy and each a member of Project Phoenix (coralprojectphoenix.org), of which I am also a member. At this week-long workshop I was able to present my own research, discuss taxonomy with peers and collaboratively we worked on a manuscript covering the status of coral taxonomy and the way forward.

2023: Red Sea *Acropora* Workshop hosted by the Red Sea Research Centre, King Abdulla University of Science and Technology, Saudi Arabia, organised by Ass. Prof. Francesca Benzoni. In March 2023 I join (by invitation) this weeklong workshop aimed at the re-assessment of the *Acropora* diversity and taxonomy from the Red Sea and surrounding regions. This workshop included an international team or researchers, each with an interest in coral taxonomy and systematics. I was able to present my research to an audience from the Red Sea Research Centre and learn about Red Sea *Acropora* whilst collaborating with

peers on future projects to aid in resolving taxonomic diversity of this genus from the Red Sea.

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Chapter 2

A taxonomic synthesis of the many *Acropora hyacinthus'*: resolving species boundaries of a highly diverse coral species complex.

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Taxonomic note:

The proposed taxonomic revision in the following chapter (**Chapter 2**) is considered **informal**, as species names and taxonomic designations used throughout this thesis are currently unpublished. To avoid *nomen nudum* designations (per the International Code of Zoological Nomenclature, Article 13 (Ferraris & Eschmeyer 2000)) we formally recognise this thesis, and specifically **Chapter 2**, as informal works at this stage. To avoid potential issues, new species are herein referred to as sp. undesc. (undescribed species), new species names have been removed (here referred to as *Acropora* coralA – coralD) throughout, and holotype specimen voucher numbers have been omitted (also referred to throughout as sp. undes. In replacement of specimen voucher number). Outside of this thesis, species names and taxonomic revisions are not valid until formal publication of each Chapter in peer reviewed scientific Journals.

2.1 Abstract

Molecular phylogenomics have revealed that traditional coral taxonomy based on skeletal morphology does not accurately reflect the true diversity of the order Scleractinia. Here, I apply an integrated taxonomic approach combining quantitative morphological and molecular analysis to delineate species boundaries and evolutionary history in a clade of tabulate corals of the genus *Acropora* that contains the species *Acropora hyacinthus* (Dana 1846) and related taxa (termed the '*hyacinthus* species complex'). Recent research based largely on molecular data has challenged the traditional taxonomic view that *A. hyacinthus* is geographically widespread across the Indo-Pacific; however, no attempt has been made to resolve the taxonomy and provide a nomenclature of this species complex. Using a unique collection of tabulate *Acropora* specimens collected from 22 regions spanning the Indian and Pacific Oceans I formulated a morphometric trait analysis which was analysed alongside a phylogenomic reconstruction using genome capture data (ultraconserved element; UCEs) of this clade. By calling Single Nucleotide Polymorphism (SNP) sites from the UCE dataset, I used species delimitation approaches to further delineate species boundaries for this group. In contrast to the most recent taxonomic revision of the genus based on morphology which recognized only four species within this complex, I recovered sixteen lineages sufficiently delineated by multiple lines of evidence to be designated as distinct species. Based on comparison of our specimens with relevant type material, I resurrect five species previously considered junior synonyms in recent taxonomic revisions based on morphology: *A. turbinata* (Dana, 1846), *A. sinensis* (Milne Edwards & Haime, 1860), *A. conferta* (Quelch, 1886), *A pectinata* (Brook, 1892) and *A. bifurcata* Nemenzo,

1971 and describe four new species: *A. coralA* **sp. undes.** from subtropical south-eastern Australia, *A. coralB* **sp. undes.** from the Western Pacific, *A. coralC* **sp. undes.** from the eastern Indian Ocean and Coral Triangle and *A. coralD* **sp. undes.** from the north-west Pacific. With an integrated taxonomic approach, our data reveals that few species within this clade are geographically widespread and rather most species are restricted to distinct geographic regions. Furthermore, the species richness within this clade of *Acropora* is far greater than currently thought. Given the key role tabular *Acropora* play on Indo-Pacific reefs, our findings have significant implications for reef conservation and management. In particular, a number of the species in the *A. hyacinthus* complex are likely to have a high extinction risk due to small populations and narrow biogeographys.

2.2 Introduction

Species are the fundamental units of biological organization, therefore the capacity to correctly identify species is important for research and management of the natural world. However, a significant portion of species on Earth remain undiscovered and not yet formally described (Bickford et al. 2007; Mora et al. 2011; Appeltans et al. 2012). Confounding this lack of species identification is the fact that molecular phylogenomics is revealing that many 'species' defined based on morphological characters are actually species complexes or even distantly related lineages that have evolved similar morphological characters independently (Bickford et al. 2007; Jörger & Schrödl 2013). Consequently, many of the morphological characters traditionally used to delineate species and higher taxonomic groups (e.g. genera, families) are revealed to be homoplasious, meaning it is not possible to accurately identify independently evolving species

based solely on these characters in many taxa (Appeltans et al. 2012; Adams et al. 2014).

In biodiversity hotspots such as coral reefs, as little as 9% of all species have likely been described (Fisher et al. 2015). This can be attributed to both a lack of taxonomic research in hyper-diverse invertebrate groups (Cardoso et al. 2011) and a high occurrence of putatively 'cryptic' speciation in marine ecosystems (Pante et al. 2015b; Pearman et al. 2016; Bongaerts et al. 2021). The inability to correctly identify taxa can have cascading effects through study and management of reef organisms (Bortolus 2008). For example, incorrectly identifying several species as one can lead to underrepresented diversity and an overrepresentations of abundance, and incorrect assumptions about spatial distribution leading to misleading conclusions regarding the ecology and biology of reef organisms. This consequently misguides management and restoration practices that rely on species lists and threatened status to make conservation decisions (Bickford et al. 2007; Pante et al. 2015a; Cros et al. 2016; Sheets et al. 2018; Gomez-Corrales & Prata 2020). Ultimately, this can lead to potentially rare or endemic species being missed in conservation and increases the possibilities of threatened species going extinct without our knowledge (Pimm et al. 2014). This then limits our ability to assess the impacts of biodiversity losses on the wider ecosystem.

Reef-building corals of the genus *Acropora* (Order Scleractinia) are the most abundant and taxonomically diverse corals on Indo-Pacific reefs (Wallace, 1999). Species of *Acropora* exhibit a diverse range of morphologies that contribute significantly to reef productivity and architectural complexity that supports biodiversity on most Indo-Pacific coral reefs (Hongo & Kayanne 2010;

Graham & Nash 2013). Over 400 nominal species of *Acropora* have been described, making this the most species rich extant genus of reef corals. However, taxonomic revisions of the genus in the late 20th century based solely on morphology (Veron & Wallace, 1984; Wallace, 1999; Veron 2000) recognized only one-third to one-quarter of these species as valid. The large number of synonymies was largely attributed to the fact that unlike other coral reef taxa (e.g. fishes), most species were thought to be geographically widespread across the Indo-Pacific (Veron, 1995; Hughes et al. 2002), and the considerable morphological variation among species was due to habitat-mediated plasticity rather than interspecific variation (Veron & Pichon 1976; Veron & Wallace 1984; Veron, 1995; Wallace, 1999; Todd et al. 2008). However, molecular phylogenomic data are increasingly revealing that these morphological taxonomic works underestimate the true diversity of *Acropora*, and that many, if not most, of these synonymies are likely to be incorrect (Ramirez-Portilla et al. 2021; Bridge et al. 2023).

The genus *Acropora* contains a diverse range of growth forms (Wallace, 1999). Tabular growth forms– that is, species that display a tabular or plating gross colony morphology – are disproportionately abundant and ecologically significant components of reefs across the Indo-Pacific (Hongo & Kayanne 2010; Nakabayashi et al. 2019; Ortiz et al., 2021). The fast growth rates of tabular *Acropora* species also enables them to rapidly recover after disturbances, and has seen them become increasingly dominant components of Indo-Pacific coral communities as disturbance frequency increases (Johns et al. 2014; Morais et al. 2023).Tabular *Acropora* also provide key ecosystem services including providing canopy and shading microhabitats which act as protection for fish and other

organisms at different lifecycle stages (Pratchett et al. 2008; Kerry & Bellwood 2015). Consequently, tabular *Acropora* are of increasing interest to reef managers (Ortiz et al. 2021) and are often the focus of reef restoration activities (Boström-Einarsson et al. 2020; Ortiz et al. 2021).

Of the 140 currently accepted *Acropora* species, 20 exhibit a predominantly tabular growth form. Among the most putatively common and widespread of these species is *Acropora hyacinthus* (Dana, 1846) a species originally described from Fiji but currently considered to occur from Hanga Roa (Easter Island) in the Eastern Pacific, across the Pacific Ocean as far north as Tokyo Bay (35° N) and across the Indian Ocean as far north as the northern Red Sea and as far south as Geographe Bay in Western Australia and East London, South Africa (34° S). However, molecular evidence has revealed at least six distinct evolutionary lineages within specimens identified as *A. hyacinthus* in the Pacific Ocean alone (Ladner & Paulmbi 2012; Suzuki et al. 2016; Sheets et al. 2018; Nakabayashi et al. 2019). However, all of these studies refer to the molecular diversity as 'cryptic speciation' but do not report on examination of morphological characters that may delineated these lineages or attempt to resolve the taxonomy of the group. More recently, Ramirez-Portilla et al. (2021) conducted an integrated taxonomic examination of three sympatric tabular *Acropora* species in Japan and were able to delineate three species on the basis of both morphological and molecular markers (Ramírez-Portilla et al. 2021). Interestingly, breeding trials also showed that these species did not hybridise, calling into question the widespread but largely unproven assumption that hybridization is common in *Acropora (*Willis et al. 2006*)*. Additional reports from America Samoa have identified lineages of the 'hyacinthus complex' that occur in

different microhabitats and display a range of bleaching tolerances and host different strains of algal symbionts (Rose et al. 2018, 2021).

The overwhelming evidence that the diversity of *A. hyacinthus* is greater than currently appreciated clearly warrants a formal taxonomic investigation of the group, and whether the lineages identified in molecular studies are indeed cryptic, or whether there are morphological differences indicative of distinct species that have been lumped together under the assumption of extensive morphological plasticity within *A. hyacinthus*. Indeed, the recent taxonomic revision of *Acropora tenuis* (Dana, 1846) another putatively widespread *Acropora* species with extensive 'cryptic' diversity (Rosser et al. 2016; Zayasu et al. 2021; Cooke et al. 2020; Matias et al. 2023) revealed that the species comprised at least 11 distinct species across the Indo-Pacific (Bridge et al. 2023).

Approximately 44 *Acropora* species with a tabular growth form have been described to date (Supplementary Information. Table S2.1), mostly from the 19th century (35 species) and all based solely on morphological features. The vast majority of nominal species (n= 31) were synonymized in taxonomic revisions the late 20th century (Veron & Wallace, 1984; Veron & Hodgson, 1989; Wallace 1999, Supplementary Material, Table S2.1), which, owing to a lack of phylogenetically informative molecular markers in *Acropora* (see Cowman et al. 2020), still underpin most contemporary research on reef corals. For example, Wallace (1999) lists eight nominal species of *Acropora* from across both the Indian and Pacific Oceans as junior synonyms of *A. hyacinthus* based on similarity of morphological features: *Madrepora surculosa* Dana, 1846 from Fiji; *M. turbinata* Dana, 1846 from Tahiti; *M. patella* Studer from Papua New Guinea, 1879; *M. conferta* Quelch, 1886 from Fiji; *M. recumbens* Brook, 1892 from north-

east Australia; *M. pectinata* Brook, 1892 from north-east Australia; *M. sinensis* Brook, 1893 from Taiwan & *Acropora bifurcata* Nemenzo, 1971 from the Philippines (Fig. 2.1). Wallace (1999) also considered another species, *M. flabelliformis* Milne Edwards, 1860 from the Indian Ocean to also be *A. hyacinthus* although a formal evaluation of the type specimen was still required to confirm this synonymy, which has not yet been completed. The taxonomic revision of Wallace (1999) also used morphological characters to examine the evolutionary history of the genus, and characterized accepted species into 'species groups', which were originally based solely on morphological similarity but later assumed to reflect the evolutionary history of the genus. In Wallace (1999), most of the species examined here, including *A. hyacinthus*, were included in the '*hyacinthus* group' along with *A. microclados* (Ehrenberg, 1834); *A. cytherea* (Dana, 1846); *A. anthocercis* (Brook, 1893); *A. paniculata* (Verrill, 1902); *A. tanegashimensis* Veron, 1990 and *A. indonesia* Wallace, 1997. There has since been a lack of molecular investigations to confirm the relatedness of these taxa, however, a recent phylogenomic analysis of the genus *Acropora* (Cowman et al. 2020) suggests the '*hyacinthus* group' may not form a natural (monophyletic) group.

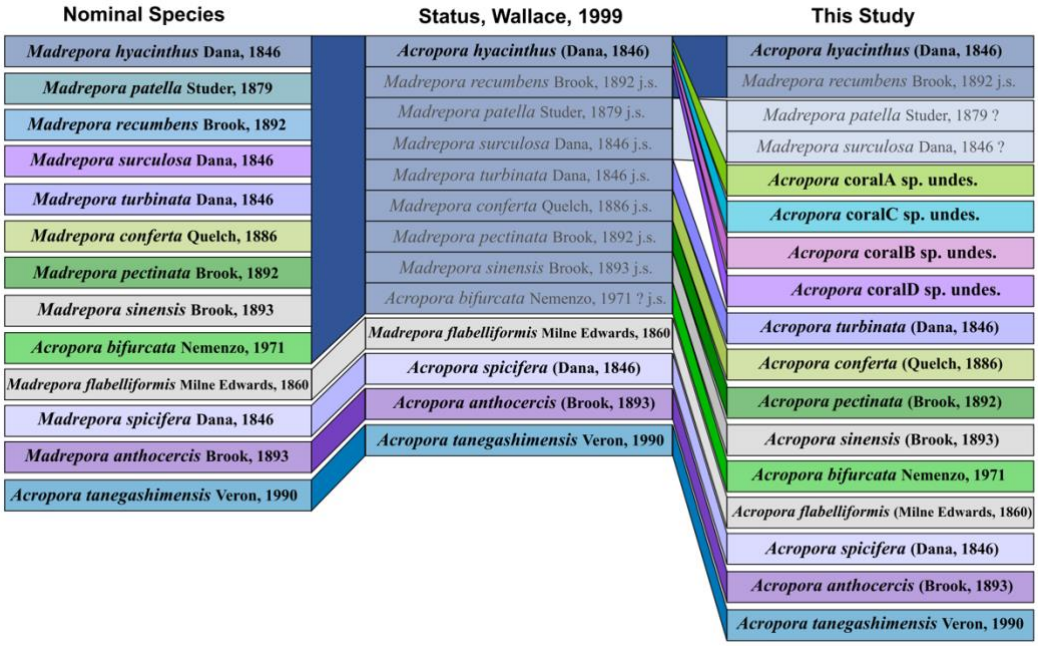

Figure 2.1 Tabulate *Acropora* nomenclature. First panel shows nominal species and authority, middle panel shows each nominal species status according to the Wallace (1999) revision where many nominal taxa were synonymized with *Acropora hyacinthus*, with synonymised or unresolved taxa are shaded. The last panel shows the status of each nominal species resulting from this revision, including novel species (sp. undes). Throughout, j.s. indicates species that are considered a junior synonym of *A. hyacinthus*, and question marks indicate that the species could not be adequately tested with the material available. Such species should nonetheless be considered valid until proven otherwise.

Molecular evidence indicates that specimens identified as *A. hyacinthus* based on morphology comprised of up to six 'cryptic' – or pseudocryptic - species that each have smaller geographic ranges (Ladner & Palumbi 2012; Suzuki et al. 2016). However, in the absence of any taxonomic assessment it remains unknown if these molecular lineages represent population structure within species, nominal species that have been synonymized incorrectly or undescribed species. Recent taxonomic research utilizing phylogenomic data have begun to resolve systematic relationships amongst *Acropora* (Cowman et al. 2020; Bridge et al. 2023), and

suggest several tabulate *Acropora* species, including species from the '*hyacinthus* group' (Wallace 1999), occur within the most recently derived of the six Acropora clades delineated by Cowman et al. (2020). However, to date there has been no taxonomic research focusing on resolving species-level relationships within this clade.

Resolving these inconsistencies between morphological and genetic identification has become time-critical for such species. Acroporids have been recently catastrophically impacted by climate change, and most recently recurrent heat waves that have resulted in mass coral bleaching (Hughes et al. 2017, 2018). Throughout the Indo-Pacific, bleaching of any one "species" (e.g. *A. hyacinthus*; Hoogenboom et al. 2017; Hughes et al. 2017, 2018) of tabulate Acropora from the 2016/17 events was highly patchy within and between reefs. Whilst such patchiness may reflect differences in environmental stress throughout reefs (Hoogenboom et al. 2017, Gardner et al. 2019), it is also highly plausible that this is also confounded by the poor capacity to resolve species diversity amongst this tabulate complex (Gold & Palumbi 2018; Rose et al. 2021). Efforts to fast-track reef recovery currently focus on attempting to propagate "species" of *Acropora hyacinthus* (e.g. Morikawa & Palumbi 2019; Suggett et al. 2019; Howlett et al. in review), with the success dependent on confidently resolving how functional diversity is driven by species and within-species genotypic variation (Morikawa & Palumbi 2019, Baums et al. 2019). The ecological importance and increasingly high study rate of species within the '*Acropora hyacinthus* complex' necessitates a taxonomic revision of the group.

Identifying and describing species comes with its own challenges and delineating species boundaries has proven especially difficult for Scleractinian

corals. While traditional coral taxonomy has relied on the comparison of morphological characters of the coral aragonite skeleton (Kitahara et al 2016), this approach is notoriously problematic because of morphological plasticity within species due to both environmental and genetic factors (Todd 2008) and convergence (Arrigoni et al. 2016; Ladner & Palumbi 2012; Quattrini et al. 2019). Increasingly sophisticated molecular techniques have widely aided in the phylogenetic classification of species across the tree of life whilst disrupting traditional taxonomic classifications and necessitating innumerable taxonomic revisions; however, slow rates of mitochondrial DNA evolution (van Oppen et al. 1999; Shearer et al. 2002; Schmidt-Roach et al. 2013), recent divergence, incomplete lineage sorting (Johnston et al. 2017) and past hybridisation events (Richards & Hobbs 2015; Quattrini et al. 2019) in Scleractinia have made molecular systematics within this Order difficult. Recent success has been made in delineating molecular species boundaries for Anthozoa - and more recently for Acropora - by using target enrichment methods, capturing ultra-conserved elements (UCE) loci and exon regions of the DNA (Quattrini et al. 2018; Cowman et al. 2020). The UCE region is highly conserved across the tree of life, with informative flanking regions which are ideal for exploring phylogenies across shallow and deep timescales across a range of taxa (Faircloth et al. 2012).

Molecular phylogenies, however, are not enough alone to form taxonomic decisions on species delineations. Increasingly, multi-factored approaches are being used to delimit species and provide taxonomic resolution, commonly referred to as integrated taxonomy (Dayrat 2005; Will et al. 2005). This approach - using multiple lines of evidence to form robust “species hypotheses” - has been used for several taxonomic revisions within Scleractinia for the genera

Psammocora (Benzoni et al. 2010), *Craterastrea* (Benzoni et al. 2012), *Pocillopora* (Schmidt-Roach et al. 2014), *Micromussa* & *Homophyllia* (Arrigoni et al. 2016), *Acropora* (Bridge et al. 2023) and the family Lobophylliidae (Huang et al. 2016). Such studies have in fact combined traditional methods of assessing morphological traits with modern molecular phylogenies, resulting in identification of key informative physical traits that can be used for species or genus identification, and in showing morpho-molecular clusters likely indicative of species or genera.

Here, I conduct a formal taxonomic revision of a clade within the genus *Acropora* containing the abundant and putatively widespread species *A. hyacinthus* and close relatives using an integrated approach. To achieve this aim, colleagues and I collected 139 tabulate Acropora coral specimens from across the Indo-Pacific region and employed a target sequence capture of UCE/exon loci (Cowman et al. 2020), to reconstruct a phylogeny for the group to provide a framework to identify primary species hypothesis (PSH) (Puillandre et al. 2012). I then extracted Single Nucleotide Polymorphism (or SNP) loci from the target capture data and performed several species delimitation techniques to examine species boundaries. I then performed a morphological trait assessment as an additional line of evidence in support of PSH, including analyses of type material for all nominal tabulate *Acropora* species. This was combined with a thorough taxonomic investigation to form final species hypothesis for this group.

2.3 Methods

2.3.1 Sampling

Sampling was conducted in a way to obtain morphological and geographic representation for tabular *Acropora* from across the Indian and Pacific Oceans. *Acropora* colonies (n = 139) were sampled via SCUBA or snorkel from 13 regions across the Indo-Pacific (Fig. 2.2). Sampling was across a broad geographic range and aimed to collect the full range of morphological variability among tabular *Acropora* from the Indian Ocean through to the central South Pacific to ensure sufficient genetic and morphological variation was captured for all known and unknown species to allow for a comprehensive taxonomic revision of this group. In the Indian Ocean, sampling at Christmas Island, Cocos-Keeling Islands and the Chagos Archipelago was performed however no specimens from the hyacinthus complex were recovered.

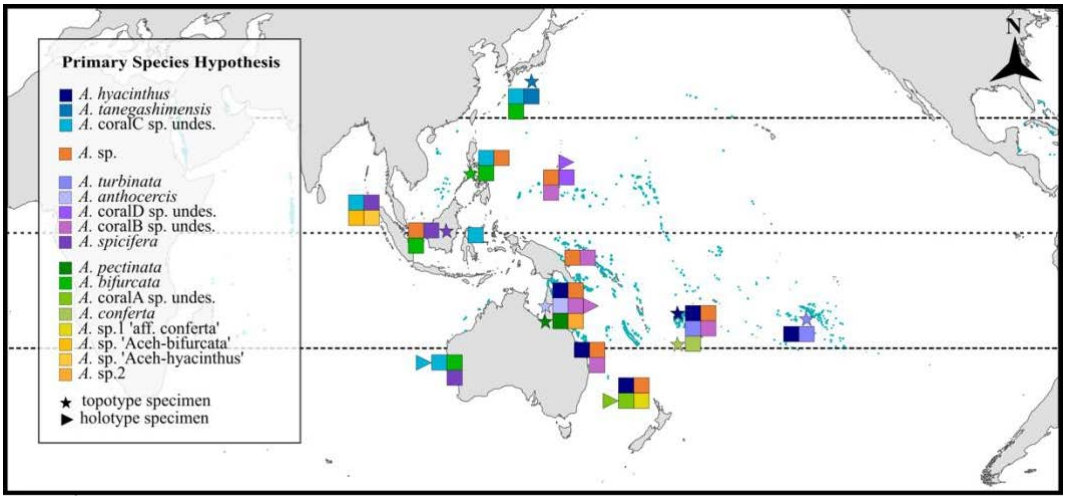

Figure 2.2 Map indicating geographic regions where samples included in the current study were collected. Species listed are according to the Maximum Likelihood phylogeny Primary Species Hypothesis and coloured squares indicate species listed in the key. Stars indicate location of topotype material for nominal taxa, and triangles indicate collection location of holotype specimens for novel species described in the current study.

High-resolution photographs of each colony *in situ*, including both whole-colony and close-up images, were taken before a voucher specimen (~15-25 cm diameter) were collected using hammer and chisel. From this fragment, a 1-2 cm subsample was preserved in 100% undenatured ethanol for molecular analysis. The remaining fragment was then bleached in sodium hypochlorite for a minimum of 24 hours, rinsed in freshwater remove tissue and dried for morphological analysis. In addition to the tabular *Acropora* specimens that are the focus of this study, colleagues and I also collected and sequenced specimens of a wide range of other *Acropora* species to identify higher-level systematic relationships between the *hyacinthus* group and the rest of the genus. These include topotype specimens that closely resemble the type specimen in morphology and were collected from the type locality (see Cowman et al. 2020; Bridge et al. 2023). Given that the type material for virtually all nominal species of *Acropora* are dried calcium carbonate skeletons that lack molecular data or tissue, sequencing topotypes provides a reference point to anchor a nominal species epithet to a molecular lineage.

2.3.2 DNA Extractions and Sequencing

To obtain a phylogeny in which I could characterize species and clade level relationships I utilized target capture of the UCE/exon region of the genome, previously performed by Quattrini et al. (2018) for Anthozoa and refined by Cowman et al. (2020) for Hexacorallia and Scleractinia. To do this, I extracted DNA from tissue samples according to a modified approach of the SDS-based method (Wilson et al. 2002) following Bridge et al. (2023). Extracted DNA was quality assessed with a Qubit 2.0 fluorometer and sent to Arbor Biosciences (Ann

Arbor, MI) for library preparation and sequencing following the methods outlined in Quattrini et al. (2018) and Bridge et al. (2023). A custom bait set for capturing UCE and exon loci originally for anthozoans (anthozoa-v1, Quattrini et al. 2018) and re-designed to be hexacoral specific [hexa-v2] by Cowman et al. (2020) targeting 1,132 UCE loci and 1,365 exon loci was used to enrich libraries, which were subsequently sequenced on a single lane of Illumina HiSeq 3000. As both UCE probes designed from genomic sources, and exons probes designed from transcriptomes sources provide similar phylogenetic resolution (Quattrini 2018; Cowman 2020) and UCE dataset have been shown to map to exonic loci (Van Dam et al. 2020), I here after refer to the UCE/exon captures data exclusively as UCEs.

2.3.3 Sequence processing and alignments

Demultiplexed reads were processed according to the Phyluce pipeline (Faircloth, 2016; http://phyluce.readthedocs.io/en/latest/tutorial-one.html/), following modifications outlined in Cowman et al. (2020) for trimming and assembling reads. Initially, reads were processed alongside samples from Cowman et al. (2020) to obtain the relative phylogenetic relationship of tabulate Acropora – specifically the *Acropora hyacinthus* complex - within the six-clade structure previously outlined by Cowman et al. (2020), a procedure also adopted to examine the *Acropora tenuis* complex reconstructed to Clade I (Bridge et al 2023). Out of 143 samples in the current study, five were first published in Cowman et al. (2020) (Supplementary Material, Table S2.2). I then focused the analyses on the *A. hyacinthus* complex independently (138 samples), which resided within Clade VI (see Cowman et al. 2020, 2.3). I also included five

outgroup specimens of *Acropora* aff. *downigi* that fell in the Clade VI basal clade to root our phylogeny (Supplementary Material, Table S2.2). Briefly, reads were cleaned using illumiprocessor (Faircloth et al., 2012) for *trimmomatic* version 0.36 (Bolger et al., 2014) and assembled with the standalone version of SPAdes version 3.12 (Bankevich et al., 2012). Assembled contig sequences were then matched to the hexacoral-v2-scleractina-subset UCE bait set at 70% minimum identity and 70% minimum coverage using *phyluce_assembly_match_contigs_to_probes*. Taxon specific loci were then extracted into FASTA files using *phyluce_assembly_get_match_counts* and *phyluce_assembly_get_fastas_from_match_counts.* Loci were aligned with the standalone version of MAFFT (version 7.4.8, Katoh et al., 2002), and were both edge trimmed using *phyluce_align_get_trimmed_alignments_from_untrimmed* and internally trimmed with *phyluce_align_get_gblocks_trimmed_alignments_from_untrimmed* (Gblocks; Castresana 2000). Both a 50% and 75% matrix was generated for each alignment (edge and internally trimmed) using *phyluce_align_get_only_loci_with_min_taxa*.

2.3.4 Phylogenomic Reconstruction

The program IQ-TREE version 2.1 (Minh et al., 2020) was used to perform a ML analysis on each of the alignments (edge and internally trimmed 50% and 75%). A species tree was inferred with a partitioned analysis with ModelFinder (Kalyaanamoorthy et al. 2017) invoked in IQ-TREE to choose the best substitution model with the settings '-m TESTMERGE –merge-model GTR –merge-rate G –rcluster 10'. For each alignment I calculated ultrafast bootstrap (UFBoot) support approximation with 1,000 replicates (Minh et al. 2013; Hoang

et al. 2018), which provides a fast and effective measure of node support for large datasets.

To provide complimentary measures to UFBoot, I also calculated gene concordance factors (gCF) and site concordance factors (sCF) to further describe variations displayed in the data. Briefly, gCF is a measure of the percentage of decisive gene trees, with higher values indicating support for that clade (Minh et al., 2020), whilst sCF represents a measure of the decisive alignment sites supporting a single branch with values >34% indicating decisiveness for that node, and higher values indicating stronger support (Minh et al., 2020).

To address potential discordances, IQ-TREE 2 was run on each loci to produce individual bootstrapped gene trees. The program newick_utils (Junier and Zdobnov, 2010) was then run on each treefile to collapse any branches with lower than 30% bootstrap support and TreeShrink (Mai & Mirarab, 2018) was run to identify and remove long branches. The resulting fasta alignments were then re-processed though IQ-TREE 2 and combined before processing through Astral (Zhang et al., 2018) to calculate local posterior probability (LPP) for the final species tree. The LPP is a probability measure that each branch is true based on the given gene trees, with lower values indicating discordance of a particular branch. The resulting trees supported four concordant clades across the phylogenetic reconstructions, herein referred to as subclades Ha, Hb, Hc & Hd (2.3), although with discordant topologies within each subclade.

2.3.5 SNP calling

Single nucleotide polymorphisms (SNP) were extracted from the combined UCE/exon datasets using a modified script from Erickson et al. (2020),

which was adapted from previous taxonomic and population genetic studies (Derkarabetian et al., 2019, Zarza et al., 2018). Briefly, for each of the identified subclades the individual taxon with the highest number of recovered UCE/exon loci from *phyluce_assembly_get_match_counts* were used as a reference for SNP calling within that clade. For each reference individual, a fasta of UCE and exon contigs was created using *phyluce_assembly_get_match_counts* and *phyluce_assembly_get_fastas_from_match_counts.* The reference fasta files were then indexed using bwa v 0.7.17 (Li & Derbin, 2009). BAM files were subsequently created by mapping individual reads to the reference individual using bwa-mem (Li, 2013). Reads were sorted with SAMtools (Li et al., 2009), and duplicates removed using Picard v 2.18.29 (Picard, Broad Institute). BAM files were then realigned with GATK v 3.8 (McKenna et al., 2010) and filtered at >75% missing data using VCFtools (Danecek et al., 2011). A STRUCTURE formatted file (.str) was generated with the script adegenet_from_vcf.py (github.com/mgharvey/seqcap_pop), selecting all SNPs for downstream analysis. Due to low capture of SNP genotypes, the STRUCTURE files were filtered using poppr v2.9.4 (Kamvar et al., 2014) to remove both loci with <80% complete genotypes and individual samples with >20% missing SNP data.

2.3.6 Species delimitation (STRUCTURE, DAPC, t-SNE, SNAPP)

All specimens were assigned a Primary Species Hypothesis (PSH) according to the ML phylogeny (Supplementary Material, Fig. S2.2.1), guided by the taxonomic literature, including all nominal species descriptions and type material. Although the genus *Acropora* contains 140 currently accepted species, there are over 400 nominal species within this genus (Hoeksema & Cairns 2023).

Due to mounting evidence indicating that there are more species of coral than currently accepted I chose to accept all nominal species for PSH assessments. Taken from Cowman et al. (2020), a series of open nomenclature (ON) qualifiers were used to indicate the level of uncertainty in the given PSH. *S*pecimens designated as 'topotypes' (as previously defined) were collected for *A. anthocercis* (Great Barrier Reef), *A. bifurcata* (Philippines), *A. conferta* (Fiji), *A. hyacinthus* (Fiji), *A. pectinata* (Great Barrier Reef)*, A. spicifera* (Singapore), *A. tanegashimensis* (Japan) and *A. turbinata* (French Polynesia), (Fig. 2.2). These topotype specimens were given the nominal name with no qualifier. The qualifier cf. ('confers with') was assigned to specimens that resembled type morphology but were not sampled from the type locality. The qualifier aff. ('has affinity with') was provided to specimens that had some morphological similarity with the type and could be an undescribed species or represent a degree of morphological plasticity. Additionally, a single outlier specimen that could not be identified to have any similarity with type material or any other specimens in the phylogeny was identified with the qualifier sp. followed by the voucher number (A. sp.PN02, Supplementary Material, Fig. S2.1).

To identify optimal genetic clusters (K) within each subclade (Ha, Hb, Hc & Hd), the genetic clustering methods STRUCTURE and Discriminant Analysis of Principle Components (DAPC) were employed using the filtered SNP dataset. I chose to run the species delimitation analysis on each subclade separately as initial investigations on the whole dataset revealed only subclade-level structure was uncovered when exploring all individuals together. STRUCTURE analysis was run on each subclade using StrAuto (Chhatre & Emerson 2017) for 1M generations, 250K burn-in and five replicates for each value K which has been

shown to be ideal settings in similar datasets (Erickson et al., 2021), with the maximum K for each subclade chosen to be the number of PSH identified from the phylogenetic analysis plus one. Results were visualized via pophelper v1.0.10 (Francis 2017) and optimal $K_{STRUCTURE}$ determined based on Evanno calculations of ΔK and Mean L(K). DAPC analysis was performed in R (R Core Team 2021) using the adgenet package (Jombart 2008) with the program *find.clusters* initially run to determine the optimal K_{DAPC} required to minimize the Bayesian Information Criterion (BIC) score.

To determine if clusters identified were truly indicative of species level divergence, and not just population level structure I performed several clustering analysis methods on our data with a modified analysis from Derkarabetian et al. (2019). Again, each analysis below was performed on sublcades separately for higher resolution on finer scale species structure. Firstly, I executed t-Distributed Stochastic Neighbor Embedding (t-SNE; van der Maaten & Hinton, 2008), a nonlinear dimensionality reduction algorithm which clusters similar objects and repels dissimilar objects with high probability in a two- or three-dimensional space. From here I performed clustering analysis on the t-SNE output by running: (1) PAM clustering with the optimal K_{gap} determined by gap statistic calculated using factoextra v1.0.7 (Kassambara and Mundt, 2017); and (2) hierarchical clustering analysis (HCA) with the mclust R package (Scrucca et al., 2017) which determined optimal K_{HCA} and clustered specimens.

To identify the highest supported species delimitation model within subclades Ha, Hc & Hd I applied a Bayes Factor Delimitation with genomic data (BFD*; Leache et al., 2014) approach using the program SNAPP (Bryant et al., 2012) through BEAST version 2 (Bouckaert et al., 2014). For each subclade, I

performed path sampling with 48 steps (MCMC = 100,000, burnin = 10,000) across multiple species hypothesis models based on ML phylogeny topologies, biogeography, and STRUCTURE results (Supplementary Material, Table S3) following parameters in Quattrini et al. (2019) & Leaché et al. (2014). As subclade Hb resolved no alternate population models in the SNP species delimitation analysis I omitted this subclade from the BFD* analysis. Models were ranked on their marginal likelihood (MLE) and Bayes Factors (BF) were calculated [2* model 1 MLE – model 2 MLE] comparing alternate species models with the STRUCTURE models, with a positive BF value indicating support of model 1 and vice-a-versa. Topologies for the highest supported species hypothesis models were visualized in DensiTree, with branch colours represented the support of that topology with blue representing the most likely topology, red the second most likely and green the remaining topologies.

2.3.7 Morphological analysis

I examined morphological features that were phylogenetically informative of the subclades defined in the molecular analysis to explore morphological species boundaries. A trait matrix of 37 traits was developed specifically for tabulate *Acropora* using a combination of common morphological traits previously used for *Acropora* (Wallace 1999) and several newly developed traits based on morphological features of tabulate Acropora and observed morphological plasticity. The purpose of this was to see if any new (or existing) traits were useful in identifying PSH determined by molecular analysis. I chose to explore both morphometric (quantitative, n = 12) and morphological (qualitative, n = 25) traits as it was evident upon initial examinations of species that many

informative features, such as corallite structure, could not easily be measured in a quantitative way, although the use of quantitative features is still commonly used for species delineations amongst other coral taxa (e.g, *Psammocora*, Benzoni et al. 2010; *Pocillopora*, Schmidt-Roach et al. 2014). Physical voucher specimens and in situ photographs were used accordingly to measure each trait. A full list of traits and descriptions of measurements can be found in the supplementary material (Supplementary Material, Table S4).

For the analysis, traits were divided into morphological and morphometric categories and all analysis was performed in R Studio v (R Core Team 2021). As previously, both morphological and morphometric analysis were performed on subclades independently. For the morphological traits - which covers the visual look of the colony including corallite shape, colony colour and branching structure - an agglomerative hierarchical cluster analysis (HCA) was performed using the R program cluster v 2.1.4 (Maechler et al., 2022) and factoextra v (Kassambara 2020). A dissimilarity matrix was generated using the Gower clustering method due to its ability to handle categorical data (Gower 1971), outliers in the data were identified as specimens that fell outside of a 95% confidence level range (mt package R) and HCA performed with hclust function. The agglomerative coefficient was calculated to confirm the strength of the clustering, with values closer to 1 indicating a strong structure representative of the data. For the morphometric traits, which includes all physical measurements for each specimen (e.g, branch diameter, corallite diameter and septa measurements) a principal component analysis (PCA) was performed using the prcomp function. PCA is a dimensionality-reduction method that allows visualization of large data matrices while also identifying the relative contribution

of each variable to the final distribution of data. A correlation matrix was performed prior to PCA to visualise the correlation between each variable to determine if traits had positive or negative relationships and to see if any traits had strongly correlated relationships. To further reduce noise and determine which traits were most informative in explaining the data a contribution analysis was performed to identify and remove traits that contributed less than average (1 / n=traits) to the first two principle components (PC) which was calculated as [(C1 * E1) + (C2 * E2) / (E1 + E2)] where C represents the contribution of the variable, 1 & 2 represent the respective PC, & E represents the Eigenvalue. Once more, outliers in the data matrix were identified as individuals that fell outside a 95% confidence level (mt v 2.0.1.19: Lin 2022) and PCA plots were used to visualise the spread of the data, with data points coloured by PSH.

2.4 Results

2.4.1 UCE/exon capture

Through target capturing of UCE/exons based on the Hexacoral v2 bait set I enriched 144 individuals with a total of 2354 loci (2,474,864 bp). The average number of loci recovered per sample was 1153 ± 122 (range 746 – 1545, Supplementary Material, Table S2.5). Alignments spanned 1233 loci across the 50% complete alignment matrix and the percentage of parsimony informative sites was 6.71%.

2.4.2 Maximum Likelihood phylogeny

Our phylogenetic reconstructions of the ‘hyacinthus complex’ were congruent with prior analysis placing this morphological group in Clade VI of the *Acropora* phylogeny (*sensu* Cowman et al. 2020). By focusing in on this

morphological complex, I was further able to resolve concordant topologies across all reconstructions (ML in IQtree and MSC in ASTRAL) in support of four subclades (designated as Ha, Hb, Hc & Hd; 2.3) although with varying levels of support measures (UFBoot, gCF & sCF; LPP) across alignments (Supplementary Material, Fig. S2.1). Only one subclade – Ha – was not resolved amongst all reconstructions as it formed a paraphyletic group in the internally trimmed 75% complete matrix tree (Supplementary Material, Fig. S2.1). Further, I found just one individual to switch clades in the edge trimmed 75% matrix phylogeny from Hb to clade Ha (KM71, *A. sp.7,* Supplementary Material, Fig. S2.1). The inconsistencies within each clade across reconstructions likely stemmed from relatively few loci being included in the 75% complete matrices (413 loci) resulting in discordant species level topologies and low support for clade topologies in the 75% matrix phylogenies. Similarly, as our taxa were closely related, I found the edge trimmed alignments resolved higher support and concordance. Due to higher resolution (1173 loci), strong node support and alignment with primary morphological assessments the results below are shown according to the edge trimmed 50% complete matrix phylogenies.

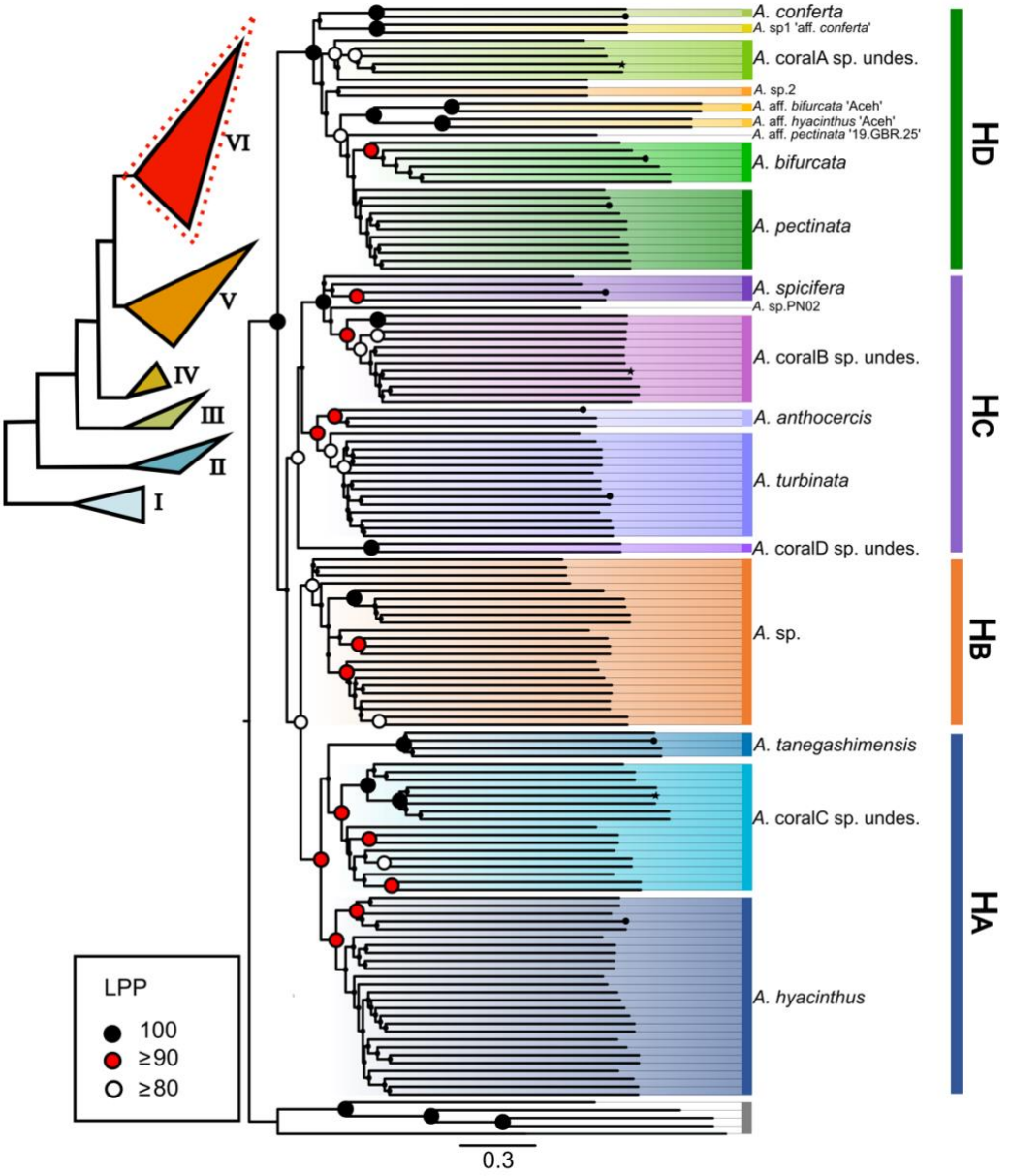

Figure 2.3 ASTRAL ML phylogeny generated with edge trimmed 50% complete matrix. The bars on the right indicate the four clades (Ha – Hd) resolved throughout the phylogenetic reconstructions (Supplementary Material, Fig. S2.1). Shaded nodes within each clade show the Primary Species Hypothesis resolved from this phylogeny. Inset tree to the left shows the six clade *Acropora* phylogeny resolved in Cowman et al. (2020) with the specimens in the current study resolved in Clade VI of this genus reconstruction. Node support shows local posterior probability (LPP) with key in the bottom left corner indicating support levels. Branches terminated by a star indicate holotypes, while those terminated by a circle indicate topotypes.

Across the ML and MSC phylogenetic reconstructions I recovered high node support for the four subclades (100% UFBoot; >37% sCF; >89% Local Posterior Probability). Across all reconstructions, gene concordance factors (gCF) were consistently low (<11%; Supplementary Material, Fig. S2.1), which was not unusual as single loci and short branch lengths in UCE datasets can be uninformative (Minh et al., 2020). In the ML phylogeny, high UFBoot support (100%) and sCF (≥37%) across subclades Ha - Hc recovered a total of 9 lineages proposed for primary species hypothesis (Fig 2). Subclade Hd resolved a further 8 lineages, although with varying levels of support (UFBoot 78 – 100%, sCF >33, 2.3, Supplementary Material, Fig. S2.1) with the *Acropora pectinata* lineage displaying unconcordant topologies and non-monophyly across phylogenies. Across the four clades, 8 lineages were anchored by topotype specimens of nominal species. Clade Ha resolved three PSHs including that of *Acropora hyacinthus* with specimens occurring in the central Pacific and eastern Australia. This subclade also comprised of *Acropora tanegashimensis* (Japanese archipelago), and one novel PSHs with a range from Western Australia to Japan. Clade Hb contained just one novel PSH (*Acropora sp7*) with a biogeograpic range as far west as Singapore, north to Palau, south to Lord Howe lsand off eastern Australia and west to Fiji. Clade Hc comprised of five PSHs each confined to distinct geographic regions being the Great Barrier Reef in Australia (*Acropora anthocercis*); the central Pacific (*Acropora turbinata*); northern Pacific (*Acropora coralD sp. undes.*); and the central Indo-Pacific respectively (*Acropora spicifera*), with the final lineage (*Acropora coralB sp. undes.*) occurring across the GBR, Fiji and Palau. Clade Hd contained the final eight PSHs which represented three

eastern Australian species (*Acropora pectinata* & three novel PSH being *A. sp1 '*aff. *conferta', A. sp.2* & *A. coralA sp. undes.*), a central Pacific species (*Acropora conferta*) and three (*Acropora bifurcata, A.* aff. *bifurcata 'Aceh'* & *A.* aff. *hyacinthus 'Aceh'*) central Indo-Pacific species (Supplementary Material, Fig S2.4). Clade Hc contained one outlier individual (A. sp.PN02, 2.3) that did not form any clear associations in the phylogeny or morphological analysis.

2.4.3 Species delimitation (PSH assignments, STRUCTURE, DAPC, t-SNE, SNAPP)

To improve species resolution, SNP data was categorized according to the recovered subclades (Ha – Hd) and filtered to remove any individuals and loci with >20% missing data. This resulted in two samples being removed (Hc = 29-8257; Hd = 19.GBR.112) and an average of 34 loci removed per dataset. An additional filtering step to detect and remove loci that were no longer polymorphic resulted in a final species delimitation dataset for each subclade as follows; 47 samples with 1,481 SNPs for Ha, 20 samples with 713 SNPs for Hb, 28 samples with 985 SNPs for Hc and 28 samples with 843 SNPs for Hd.

The genetic clustering resolved by STRUCTURE analysis was overall conservative in relation to initial PSH assessments with a clear indication of recent ancestry and admixture amongst populations in each subclade. Within clade Ha, the optimal $\Delta K = 2$ with *A. hyacinthus* and *A. tanegashimensis* displaying majority ancestry to one cluster, whilst *A. coralC* sp. undes. displayed majority clustering to the second population Fig. 2.4). Clade Hb resolved similar proportions amongst all individuals across $\Delta K = 2$ clusters with no distinction between individuals (Supplementary Material, Fig. S2.2). As the minimum

number of clusters ($K_{STRUCTURE}$) possible in STRUCTURE analysis is two it is likely this clade is represented by a single species, which is consistent with phylogenetic PSH assignments. Clade Hc individuals also resolved ΔK = 2, showing majority clustering split in congruence with the monophyletic clades in the ML phylogeny (Fig. 2.4), although interestingly *A. turbinata* & *A. coralB sp. undes.* each displayed >97% ancestry to distinct lineages showing high support for these two PSHs, whilst *A. anthocercis, A. coralD sp. undes.* and *A. spicifera* each displayed mixed ancestry amongst the two populations. The STRUCTURE analysis for clade Hd with ΔK = 3 resolved three clear lineages with the first containing the A. aff. bifurcata'Aceh' and A. aff. hyacinthus 'Aceh' specimens, the second combining *A. pactinata* and *A. bifurcata*, and the third containing *A. coralA sp. undes., A conferta, A. sp.1* 'aff. *conferta' and A. sp.2* (Fig. 2.4). Amongst all STRUCTURE analyses, a significant proportion of admixture was evident between PSH. Interestingly, where I identified a second most probable $K_{STRUCTURE}$ for subclade Ha and Hd – identified where Evanno plots displayed a second ΔK peak and/or an alternate high Mean L(K) score – I found clustering in alignment with initially identified PSH. For subclade Ha at $K_{STRUCTURE}$ = 5 I found *A. tanegashimensis* to contain a portion (>25%) of unique genotypes, and within *A. hyacinthus* I found the population from the central Pacific to also contain >20% of a distinct genotype not found in significant proportions in the GBR populations (Ha K = 5, Fig. 2.4). For subclade Hd at $K_{STRUCTURE}$=5 I resolved clear populations for *A. coralA sp. undes.* and *A. conferta* that were not identified in the ΔK=3 populations (Hd K = 5. Fig. 2.4)

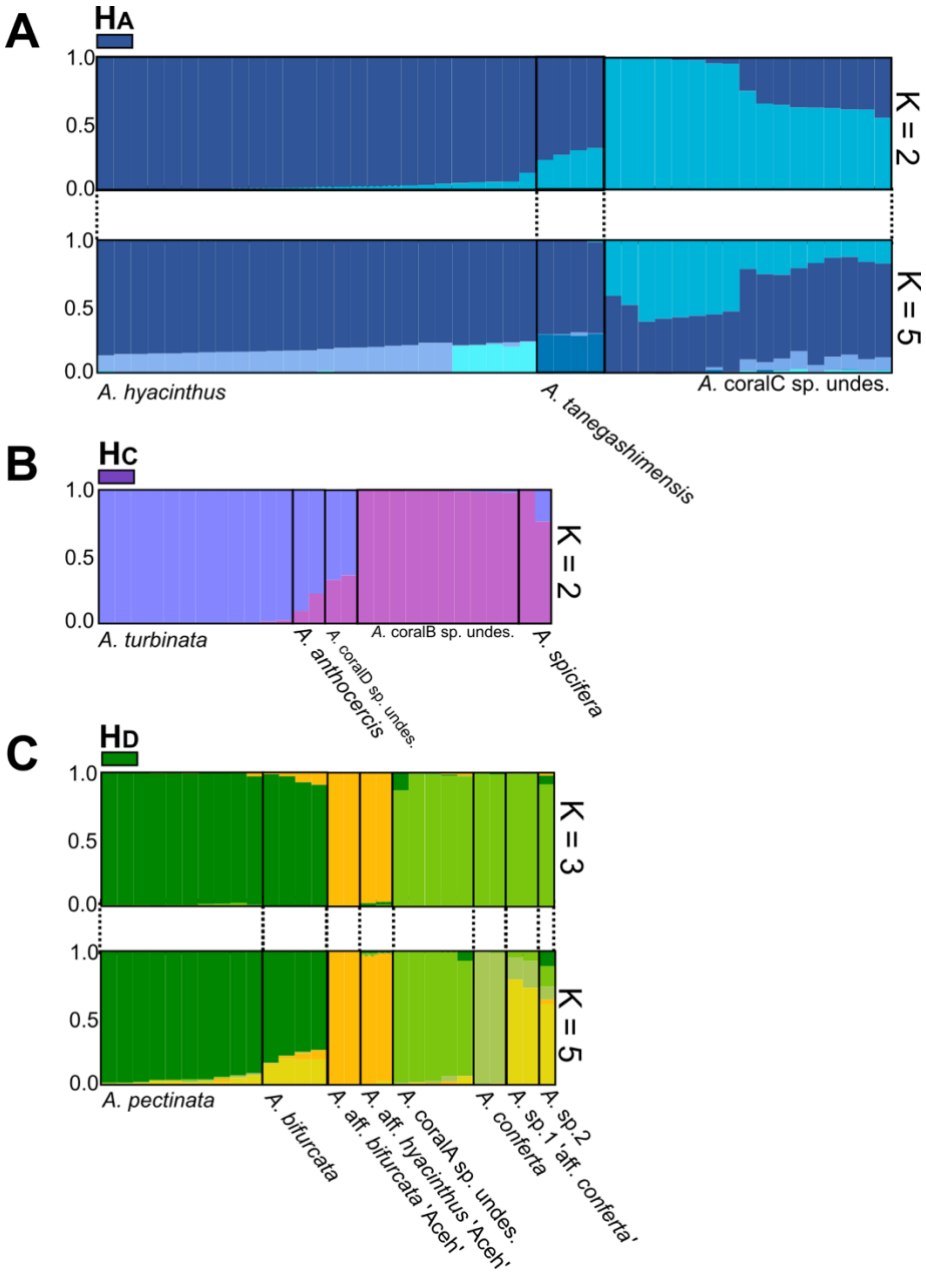

Figure 2.4 Results from STRUCTURE analysis for subclades A) Ha (K = 2 & K = 5), B), Hc (K = 2), and C) Hd (K = 3 and K = 5). Bars are coloured according to majority ancestry and PSH have been grouped and labelled.

The DAPC analysis followed a similar pattern to that of STRUCTURE, with conservative clustering determined by K_{DAPC} (Supplementary Material, Fig. S2.3). Both clade Ha and Hc resolved the same clustering determined by ΔK in STRUCTURE (K_{DAPC} = 2), while Hb DAPC analysis proposed a most likely

K_{DAPC} = 1 population, in line with PSH assessment of a single species as discussed above. This single genetic cluster delineation was echoed by subsequent t-SNE analysis (K_{gap} and K_{HCA}), so the following results will focus on the remaining three subclades where further species resolution was found. The DAPC analysis for Hd proposed a most likely K_{DAPC} = 1, which combined all PSH assignments into genetic cluster.

For the t-SNE analysis the general clustering of each subclade was concordant with initial PSH assignments and highlighted geographic patterns amongst the datasets (Fig. 2.5). For subclade Ha, *A. hyacinthus* formed three populations in HCA, roughly represented sampling efforts from the central Pacific, southern GBR and northern GBR (Fig. 2.5). Both clustering methods successfully resolved *A. tanegashimensis* as a single genetic entity, while *A. coralC sp. undes.* was again split into biogeographic clusters with groups representing sampling in Western Australia, the Coral Triangle and Okinawa in Japan (Fig. 2.5). For subclade Hc, HCA resolved *A. anthocercis* and *A. turbinata* as distinct clusters, while combining the remaining PSH under one cluster (Fig. 2.5). This pattern was mirrored by PAM clustering, with the distinction of *A. anthocercis* and *A. turbinata* forming one single cluster and the single specimen for *A. coralD sp. undes.* switching groups. For Hd, HCA designated five clusters successfully resolving *A. conferta* and *A. coralA sp. undes.*, and clustering *A.* aff. *bifurcata* 'Aceh' and *A*. aff. *hyacinthus* 'Aceh' as one population, although with visual distinctions between these PSH populations. The remaining PSH were all grouped into two populations with mixed alignments to the PSH designations (Fig. 2.5). These results were mirrored by PAM clustering.

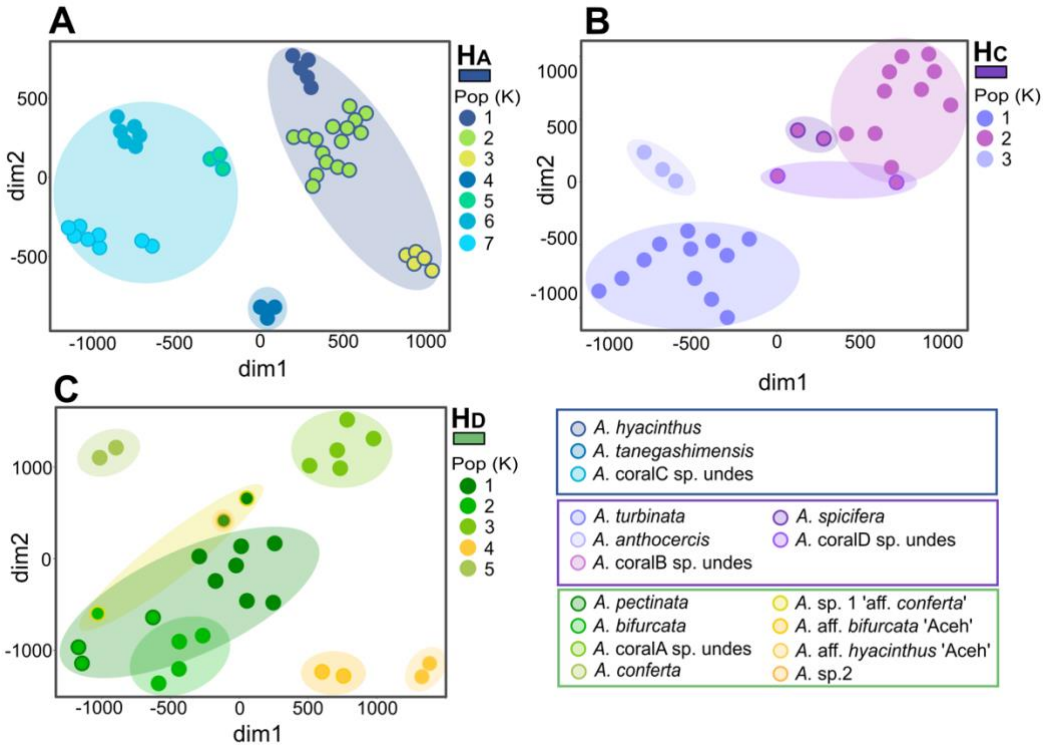

Figure 2.5 Results from the t-SNE analysis showing clustering of specimens according to most likely population (K) determined by Hierarchical Clustering Analysis for clade Ha (A), Hc (B) and Hd (C). Colours within circles represent HCA populations, whilst outlines represent PSH according to the species list in the bottom right. Ellipses are drawn around PSH clusters.

Amongst the BFD* analysis (Ha, Hc & Hd) the population models with the highest Bayes Factor (BF) support were those that represented the most diverse population models (Supplementary Material, Table S2.6), in congruence with PSH for Hc and Hd clades, although supporting a further split in PSH for clade Ha (Fig. 2.6). Support of a five species model in subclade Ha (MLE = -28797, BF= -1676) included splitting *Acropora hyacinthus* into separate central Pacific and Great Barrier Reef populations, reflecting the STRUCTURE (K = 5) results. Similarly, *A. coralC sp. undes.* was split geographically, with a distinction between the Western Australian population and the Coral Triangle and Japan

populations (*A. coralC 'WA'* & *A. coralC 'Okinawa'* respectively, Fig. 2.6).-The highest supported model for both clades Hc (MLE= -12733, BF= -899) & Hd (MLE= -13625, BF= -1783) were congruent with PSH designated from phylogenetic data.

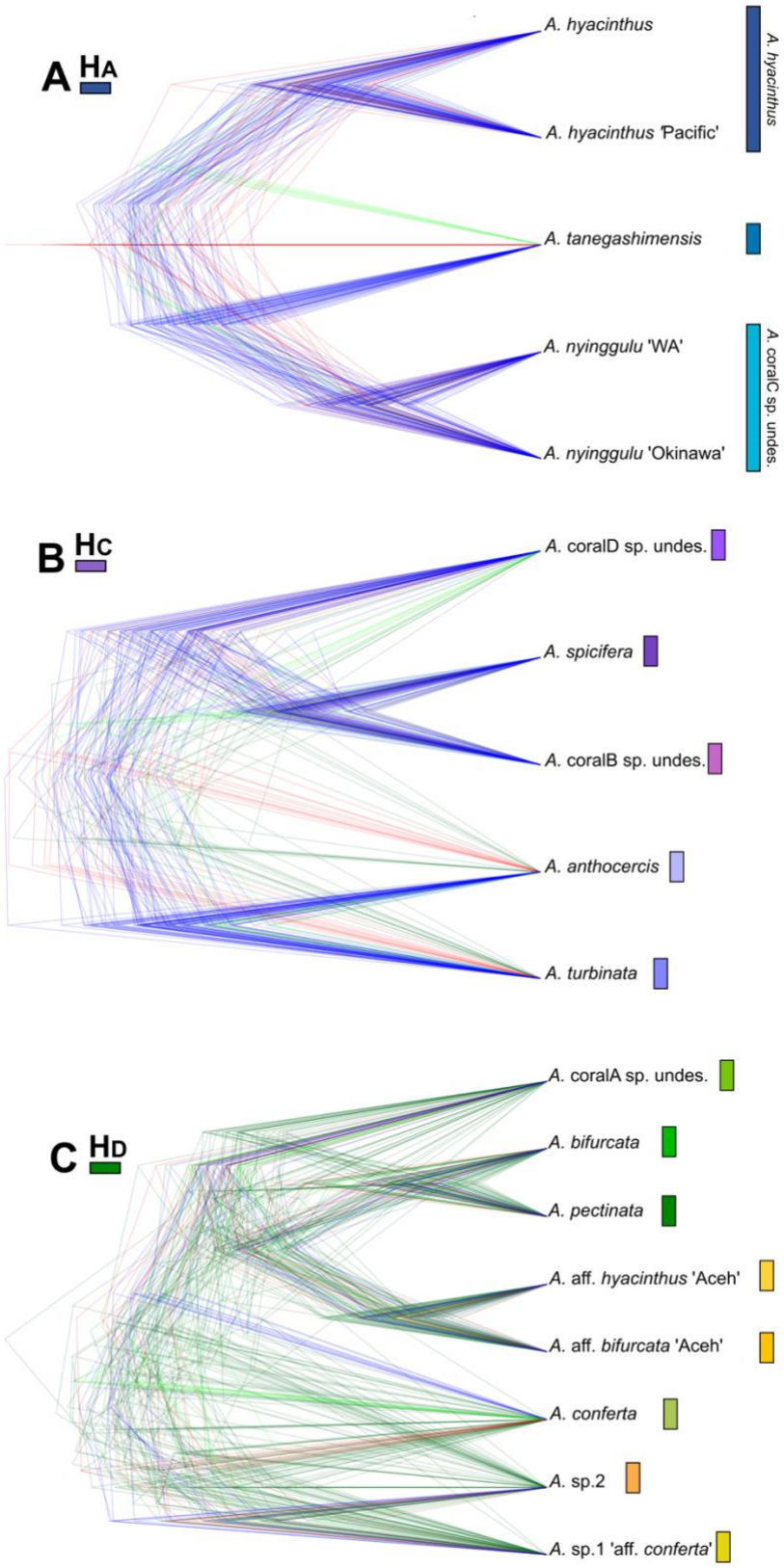

Figure 2.6 Results from Bayes Factor Delimitation with genomic data (BFD*). Topologies displayed represent the highest supported species model for subclade

Ha (A), Hc (B) and Hd (C). Branch colours represented the support of that topology with blue representing the most likely topology, red the second most likely and green the remaining topologies Branch tips are labelled according to the BFD* model, and coloured bars indicate PHS according to the ML phylogeny.

2.4.4 Morphology

We found intraspecific variability in morphology across geographic space with species clustering across all subclades representing regional morphological variability (Fig. 2.7). For the visual morphological traits, all subclades resolved clusters that strongly represented the variation in the data (agglomerative coefficient ≥ 0.82). While PCA results were less definitive and varied amongst the subclades with the first two PC explaining between 68.8% - 83.4% of the variation in the data.

For subclade Ha the morphological HCA resolved a monophyletic group for *Acropora tanegashimensis*, nested within a paraphyletic cluster of *Acropora hyacinthus* specimens from the Great Barrier Reef & central Pacific (Fig. 2.7). Interestingly, the four specimens of *Acropora hyacinthus* that did not cluster in this group were ones sampled from higher latitude reefs spanning the Capricorn Bunkers region down to Lord Howe Island. These high latitude specimens were all characterized by thicker branchlets, and nude or brown colony colour compared to the tropical counterparts. *A. coralC sp. undes.* was split across two clusters, generally representing regional diversity, congruent with molecular species delimitation (Fig. 2.5, 2.6 and 2.7). Specimen WA31 was identified as an outlier in the HCA analysis, which was verified with visual inspection of the specimen which appeared to display an unusual arborescent morphology, and not the standard bifurcating plate of tabulate *Acropora*. In the PCA matrix specimens

formed two broad clusters, split on the PC2 axis with overlapping trait space amongst PSH (Fig. 2.7).

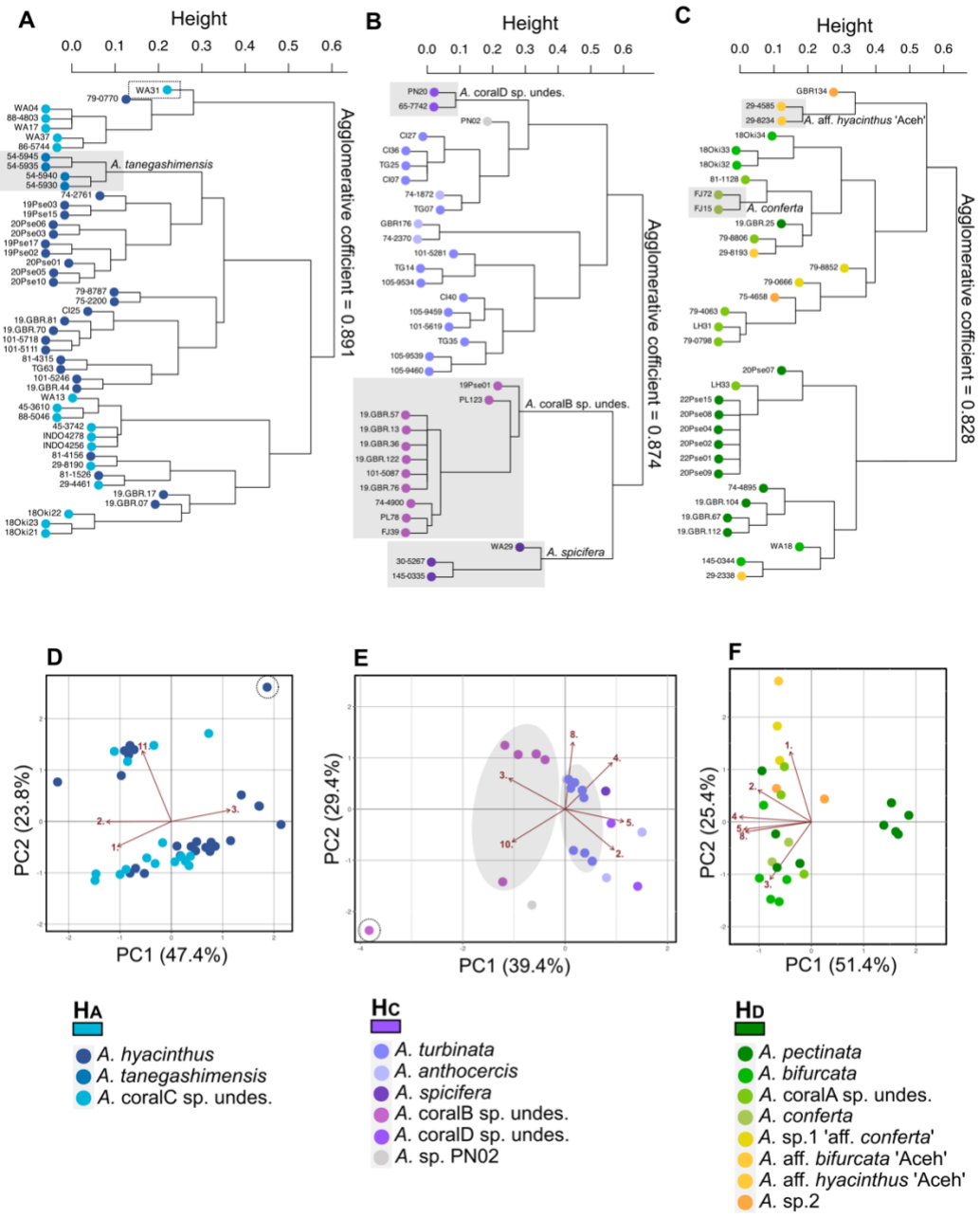

Figure 2.7 Results from morphological analysis showing Hierarchical Clustering Analysis of morphological traits for subclade Ha (A), Hc (B) and Hd (C). Below, results for Principle Component Analysis of morphometric traits for subclade Ha (D), Hc (E) and Hd (F). Colours indicate PHS assigned from the ML phylogeny.

No clear morphological patterns were evident amongst subclade Hb, with short branches and no consistent geographic clusters to aid in morphological delimitation amongst HCA. Similarly, although with high PC scores (PC1 & PC2 = 83.4%, Supplementary Material, Fig. S2.2), the PCA was noisy and resolved no further structure to the data. Morphological analyses of this subclade were incompatible with the phylogeny, showing no geographic structure, further blurring resolution of this species group.

Subclade Hc was the most resolved amongst both trait-based analysis, with morphological and morphometric traits showing affinity to the phylogenetic PSH assessments and the species delimitation analyses. Only one specimen was identified as an outlier in the PCA (19.Pse.01, Fig. 2.7), likely due to tight clustering of branchlets and small Axial corallite width. The HCA was congruent with the phylogeny and species delimitations, resolving *Acropora spicifera*, *A. coralB sp. undes.* & *A. coralD sp. undes.* each as monophyletic groups (Fig. 2.7), indicating these species were able to be distinguished from each other using the morphological traits measured. The PCA also resolved *A. coralB sp. undes.*, which clustered according to an affinity to higher branchlet density (Fig. 2.7, Supplementary Material, Table S4), which is a visually unique feature of this species. Across both analysis *A. turbinata* was split into two distinct clusters.

Low morphological resolution of species was found for subclade Hd. The PCA (78.2% variance explained, Fig. 2.7) displayed a tight cluster of all PSH overlapping in trait space, explained by an increase in the basal branch diameter and higher axial to radial ratio (Supplementary Material, Table S4). One unique cluster of *A. pectinata* from the Palm Islands on the Great Barrier Reef displayed a negative relationship to the six traits analysed in the PCA. The HCA resolved

two species (*A.* aff. *hyacinthus 'Aceh'* & *A. conferta*, Fig. 2.7.C,) each as monophyletic groups, and *A pectinata* as a paraphyletic group clustering with a single *A. coralA sp. undes.* specimen. The remaining PSH all formed paraphyletic clades, with clusters often representing intraspecific biogeographic groupings.

2.5 Discussion

This study is the first taxonomic revision of tabulate *Acropora* that utilizes an integrated approach combining both molecular and morphological data and demonstrates that the taxonomic diversity of the group is considerably higher than suggested by recent taxonomic revisions of the group based solely on skeletal morphology (Veron & Wallace 1984; Wallace, 1999). In contrast to previous research (Wallace 1999; Veron 2000) I show that none of the species in this clade are geographically widespread across Indo-Pacific. Some of the increase in taxonomic diversity is attributable to our findings of distinct sister species divided by the East or West coastlines of Australia (e.g. *A. hyacinthus* in Eastern Australia and Southern Pacific and *A. coralC sp. undes.* in Western Australia, Indo-Australian Archipelago north through the Philippines as far as the Ryukyu Islands). However, I also found extensive overlooked diversity within specific geographic regions. For example, what was previously considered a single species (*A. hyacinthus*) on the central GBR, is clearly at least three distinct species (*A. hyacinthus*, *A. pectinata* and *A. coralB sp. undes.*), while a fourth species (*A. coralA sp. undes.*) appears restricted to the subtropical coral communities of the Tasman Sea. Critically, I also show that co-occurring species can be distinguished using morphological characters both in the field and in Museum collections. Given the ecological dominance of tabular *Acropora* across the Indo-Pacific and

their widespread use in experimental research and reef restoration, our results have implications for a range of basic and applied research questions.

I found evidence for multiple species in all four subclades examined, with the exception of Hb, which included a wide range of morphologies and I consider unresolved. However, different species delimitation analyses were not always congruent in the number of species indicated. This is not surprising because each analysis uses a different method for identifying natural breaks in the data that could indicate species boundaries, and it is important to consider the potential for factors such as isolation by distance (IBD) to create the mirage of distinct species (e.g. Chan et al. 2017). These inconsistencies were largely driven by STRUCTURE analysis, in which I resolved conservative populations across subclades. This may have been a result of two factors known to influence STRUCTURE analysis, being i) uneven sample size across populations (Gilbert et al. 2016), and ii) analysing closely related populations. Firstly, I found that PSH with a greater sample size (n > 5) were more likely to resolve stronger population structure than PSH with less representation. For example, *A. turbinata* (n= 12) & *A. coralB sp. undes.* (n= 11) in subclade Hc both resolved clear genetic clusters, where *A. anthocercis* (n= 3), *A. coralD sp. undes.* (n = 2) and *A. spicifera* (n = 1) all displayed a portion of mixed ancestry to each of the forementioned taxa. This was evident again in some degree within subclade Hd where *A. pectinata* (n= 10) & *A. coralA sp. undes.* (n= 5) also formed majority ancestry to a single lineage, although I also found *A. conferta* (n= 2) to form a distinct lineage. Despite this, a large sample size for *A. hyacinthus* (n= 26) and *A. coralC sp. undes.* (n= 17) in subclade Ha did not yield these results, with a large degree of mixed ancestry evident across all individuals, although, the presence of the small *A.*

tanegashimensis (n= 4) population may have affected these results. Further, as indicated previously, where populations are closely related the Evanno method of calculating ΔK has been shown to favor K = 2, which is what was found in the current study (Evanno 2005).

Support for PSHs amongst all other SNP automated species delimitation methods varied depending on the subclade analysed. For subclade Ha, I found all other methods to support a higher number of species than proposed by PSH, generally splitting *A. hyacinthus* into distinct GBR and central Pacific populations. Similarly, *A. coralC sp. undes.* was consistently split into two populations representing the Western Australian lineage and the Indo-Australian Archipelago, Philippines and Ryukyu Islands lineage (Fig. 2.4, 5 and 6). Morphological analysis provided some support for a split of *A. coralC sp. undes.* into two distinct species, although with some incongruence with the molecular populations. Despite this support for distinct populations, I found individuals of *A. coralC sp. undes.* to switch placement in the phylogenetic reconstructions (Supplementary Material, Fig. S2.1). Based on this, along with visual morphological similarities between individuals and the presence of specimens that fit within the morphological range of this species in the coral collection at the Museum of Tropical Queensland from across the range sampled here (Western Australia, the Indo-Australian Archipelago and Japan - see taxonomic account below), I currently consider this a single species that exhibits population structure across its range. However, additional collections – particularly from Indonesia - and further analysis is required to confirm whether the northern population represents a distinct species.

Morphological identification of many coral species is considered difficult due to morphological plasticity (Kitahara et al. 2016); however, I found an integrated taxonomic approach combining molecular phylogenomics with quantitative morphology was capable of identifying taxonomically-informative morphological characters at the species level. The species examined in this study fit into four species considered valid by the taxonomic revisions of Veron & Wallace (1984) and Wallace (1999) based entirely on skeletal morphology: *A. hyacinthus*, *A. spicifera*, *A. anthocercis* and *A. tanegashimensis.* Of these, only *A. tanegashimensis* is not considered geographically widespread across the Indo-Pacific. However, our molecular phylogeny, unsupervised machine learning and BFD* analysis combined with morphological investigations provides multiple congruent lines of evidence to support our species delimitation, including the resurrection of species that were previously considered junior synonyms and the description of new species. Whilst not all methods individually were able to delineate all PSHs, these methods combined successfully identified most taxa, whilst SNP based BFD* and t-SNE also revealed geographic structure within *A. hyacinthus* and *A. coralC sp. undes.* populations. I also identified additional lineages that I was unable to resolve taxonomically (*A. sp.7, A.* aff. *hyacinthus 'Aceh', A.* aff. *bifurcata 'Aceh'* & *A. sp.2*), likely due to small sample size, that will require further investigation to resolve.

Numerous studies have used molecular data to show that the morphological species concepts of Veron & Wallace (1984), Wallace (1999) and Veron (2000) do not accurately reflect genetic diversity within tabular *Acropora* (e.g. Ladner & Paulmbi 2012; Suzuki et al. 2016; Sheets et al. 2018; Nakabayashi et al. 2019). However, molecular studies typically refer to the lineages identified

as ‘cryptic species’, without conducting any taxonomic examination of whether these lineages show morphological differences. The one notable exception (Ramirez-Portilla et al. 2021) examined three co-occurring tabular *Acropora* in the Ryukyu Islands, Japan (referred to as *A.* cf. *bifurcata*, *A.* aff. *cytherea* & *A.* aff. *hyacinthus*) and found that the three species could be delineated on the basis of molecular, morphological and ecological evidence (i.e. the species did not hybridise). Our study provides further evidence that many tabular *Acropora* species exhibit distinctive morphological characters that enable them to be identified on the basis of morphology, both in the field and in museum collections (Fig. 2.7). For example, in contrast to the species concepts of Wallace (1999), our results support the findings of Ramírez-Portilla (2021) that colour is an informative character for delineating several co-occurring species (Fig. 2.7).

I also found some geographic variation in morphology, with specimens of the same species collected in proximity (e.g. from the same reef) clustering together in the morphological HCA. For example, in *A. hyacinthus,* specimens from the north-central GBR formed a single cluster, while another cluster contained all four specimens from higher latitude reefs of south-eastern Australia along with *A. coralC sp. undes.* specimens from the central Indo-Pacific region (Fig. 2.7A). This result is attributable to the fact the specimens of *A. hyacinthus* from higher latitudes had thicker ‘robust’ branchlets and were commonly cream in colour =compared to those from further north. This pattern is repeated in other clades, illustrating that individual species can exhibit somewhat different morphologies in different regions. However, the fact that species within a given region tend to be conserved in morphology suggests that species can be accurately delineated within specific geographic regions, highlighting the importance of

becoming familiar with specific local faunas and the challenges of trying to delineate the same species across biogeographic regions.

Our results also show that the tabular *Acropora* species have much smaller geographic ranges than currently assumed. In contrast to the geographic ranges proposed by Wallace (1999), none of the 12 species examined here are geographically widespread across the Indo-Pacific. I found no specimens from this group further west than the eastern Indian Ocean (Western Australia and the Andaman Sea) despite extensive collections at locations including Christmas and Cocos (Keeling) Islands and the Red Sea. Examination of specimens in the Worldwide *Acropora* Collection at the Museum of Tropical Queensland also revealed no specimens that matched the morphology of species identified here in the Western Indian Ocean, although one species with morphological affinities to *A. coralB sp. undes.* does occur in the Maldives (figured in Wallace et al. 2012 under *A. hyacinthus*). Our results also reveal little overlap between the Pacific and Indian Ocean faunas, with only a single lineage (the unresolved *A. sp.7*) occurring across the Coral Triangle to the central Pacific (Supplementary Material, Fig. S2.4).

Introgression and hybridization are proposed mechanisms to explain the presence of cryptic speciation and diversity of coral reefs (Richards & Hobbs 2015; Mao et al., 2018), particularly in *A. hyacinthus*, which has been described as a 'pseudocryptic complex' that represents a global syngameon (Ladner & Palumbi 2012; Suzuki et al. 2016). However, more recent evidence using more advanced molecular methods and utilizing more updated species concepts (Ramírez-Portilla et al. 2021) suggest that this may be an artefact, and that hybridization in tabular *Acropora* is far less common than currently assumed. In this study, I found some

evidence for hybridization between species in the STRUCTURE analysis (Fig. 2.4) across all clades, with interspecific admixture present amongst multiple lineages. However, I did not find any outlier individuals showing intraspecific admixture and thus I cannot disregard that this admixture may have been caused by i) uneven sampling across populations (Gilbert et al. 2016), ii) or introgression caused by 'secondary genomic admixture' from a recent hybridizing ancestor (Mao et al. 2018). Further, this mixed signal in STRUCTURE analysis was not always congruent with automated species delimitation and BFD* analysis where species were delineated as distinct lineages; however, it does confer the low gene concordance factors resolved in the ML phylogeny (Supplementary Material, Fig. S2.1) which could indicate recent introgression (Mao et al. 2018). Further phylogenomic analysis specifically examining the question of hybridization, combined with other evidence such as breeding trials, are required to better understand both pre- and post-zygotic barriers to hybridization.

Our integrated approach, combining type material with topotype specimens, reveals that the taxonomic revisions of the late 20th century (Veron & Wallace 1984; Veron & Hodgson 1989; Wallace 1999) substantially underestimated the diversity of tabular Acropora. For example, at least four valid species were synonymized erroneously with *A. hyacinthus*, with differences in morphology between the type material attributed to environmental or geographic variation within widespread species. Our results indicate that not one of the synonymized taxa are sister to *A. hyacinthus* or fall within the same subclade in the molecular phylogeny. I did, however, discover some overlap in morphological characters for several species occurring in clade Hd (*A. pectinata, A. bifurcata* and *A. conferta*). This is not surprising because all three taxa exhibit an anastomosing

and bifurcating basal branching pattern with short vertical branchlets. Indeed, the close relationship between these taxa and the similarities in morphology require further investigation to resolve geographic range and species boundaries. Nonetheless, it is clear that neither of these species are synonyms of *A. hyacinthus*. I also found that some decisions on synonymy may be correct: *Madrepora recumbens* is retained as a junior synonym of *A. hyacinthus* based on specimen 19.GBR.44 (Supplementary Material, Table S1) because the specimen, collected from the type locality of the Great Barrier Reef, is morphologically similar to the type of *M. recumbens* (Lectotype: 1892.6.8.269 Natural History Museum, London) and clustered within the *A. hyacinthus* lineage in all molecular and morphological analysis. This highlights the necessity of taxonomic revisions to not only highlight inconsistencies between molecular and morphological based taxonomy, but to also verify past decisions to form an overall robust taxonomy for the future.

These results have clear implications for understanding extinction risk and informing conservation of tabular *Acropora*. For example, *A. hyacinthus* is currently listed as Near Threatened by International Union for Conservation of Nature (IUCN) Red List (Aeby et al. 2008). While its population trend is listed as decreasing, it is also considered to occur on most reefs across the Indian and Pacific Oceans (Supplementary Material, Fig. S2.5). Consequently, it is considered resilient to habitat loss and degradation due to an assumed large and connected population despite its susceptibility to bleaching and disease; however, our results show *A. hyacinthus* is restricted to the south-west Pacific. *Acropora anthocercis* and *A spicifera*, which are both listed as Vulnerable (Aeby et al. 2008), also have much more restricted geographic distributions than reported on

the IUCN Red List (Supplementary Material, Fig. S2.5), with *A. anthocercis* potentially endemic to the GBR and *A. spicifera* restricted to the western Coral Triangle and eastern Indian Ocean (Supplementary Material, Fig. S2.5). The increased species diversity and smaller geographic ranges of tabular *Acropora* species illustrated here suggests a high risk of 'silent extinction' for these key ecosystem engineers, and clearly illuminate the issues with conducting risk assessments with inadequate data. Therefore, our results support previous studies (e.g. Bridge et al. 2020; Raja et al. 2021) that find that the IUCN Red List does not accurately reflect species extinction risk in corals and should not be used as a basis for conservation prioritization.

Here, I examined species boundaries in tabular *Acropora* within the '*Acropora hyacinthus* complex' *sensu* Ladner & Palumbi, 2012 and more broadly as morphologies resembling the range of species, including synonymies, of *A. hyacinthus* sensu Veron & Wallace (1984) and Wallace (1999). By sampling broadly across the Indian and Pacific Oceans, targeting topotype specimens and collecting a range of morphologies I show that the true diversity of this clade is far greater than suggested by previous taxonomic revisions based solely on morphology.

2.6 Taxonomic Account

On the basis of both molecular and morphological analysis, I delineate seventeen lineages belonging to four molecular sub-clades. Clade-Ha contains three species (*A. hyacinthus, A. tanegashimensis* and *A. coralC sp. undes.*), clade-Hb contains just one unresolved lineage (*A. sp. 7*), clade-Hc contains five resolved species (*A. turbinata, A. anthocercis, A. coralB sp. undes., A. coralD sp. undes.*

and *A. spicifera*) and clade-Hd contained four resolved species (*A. pectinata, A. bifurcata, A. coralA sp. undes.* and *A. conferta*), and four unresolved lineages (*A. sp1 '*aff. *conferta', A. sp.2, A*. aff. *bifurcata 'Aceh'* and *A.* aff. *hyacinthus 'Aceh'*).

Of the seventeen lineages I was able to identify eight nominal species (Fig. 2.8), of which just four are currently accepted as valid species according to the most recent taxonomic revisions (sensu Wallace 1999, 2012) being: *Acropora hyacinthus* (Dana, 1846), *Acropora tanegashimensis* Veron, 1990a, *Acropora anthocercis* (Brook, 1893) and *Acropora spicifera* (Dana, 1846). The remaining four nominal species being: *Acropora turbinata* (Dana, 1846), *Acropora pectinata* (Brook, 1892), *Acropora bifurcata* Nemenzo, 1971 and *Acropora conferta* (Quelch, 1886) which were all previously synonymised with *A. hyacinthus*. Of the eight remaining species resolved in this study, four are described below (*Acropora coralC sp. undes., Acropora coralB sp. undes., Acropora coralD sp. undes.* and *Acropora coralA sp. undes.*), and four (*A. sp.1 '*aff. *conferta', A. sp.2, A. aff bifurcata 'Aceh'* and *A.* aff. *hyacinthus 'Aceh',* Fig. 2.9) are likely also undescribed species although with small sampling efforts (n = 2 each), poor molecular resolution and unusual morphological features these species all require further investigation and additional sampling to confirm species status. Additionally, as a result of the resurrection of *A. pectinata* (Brook, 1892) I also deemed *A. pectinata* Veron, 2000 to be an invalid homonym and have provided a *nomen novum* (replacement name) for the later species in the taxonomic account below. Finally, species Acropora sp. 7 (Fig. 2.9) which encompasses all clade-Hb provides an unusual case where a large sampling size (n = 22) resolved poor resolution across both molecular and morphological analysis. Specimens of this subclade display broad morphological variation both within and across geographic

regions, and across molecular species delimitation specimens were grouped as one population in all methods. It is possible that this subclade represents a single undescribed species with a broad morphological and geographic range, however, it is advised that further sampling and analysis be done to resolve boundaries and species delineation.


A. hyacinthus
A. tanegashimensis
A. turbinata
A. anthocercis
A. spicifera
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
20mm
101-5718
54-5935
105-9539
74-2370
30-5267

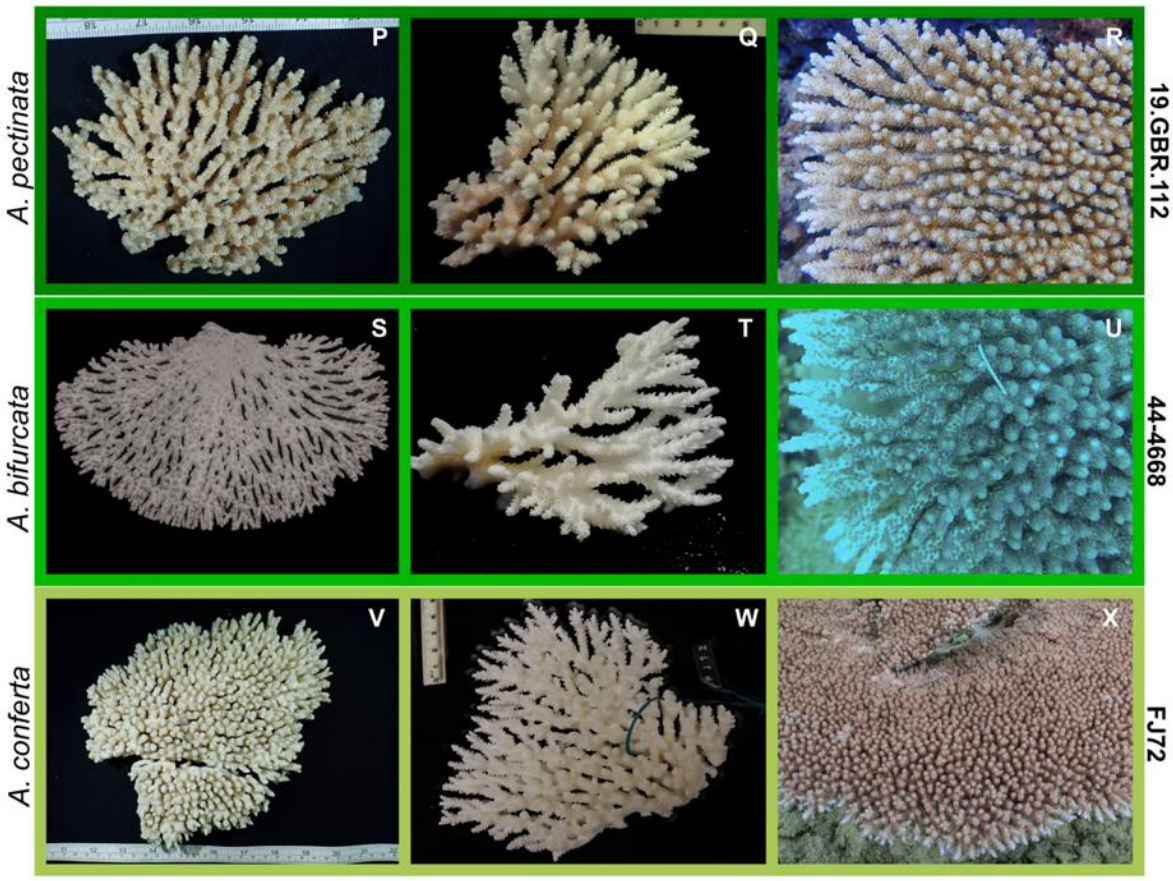

Figure 2.8 Nominal species of tabular *Acropora* resolved in the present study. A) *Acropora hyacinthus* (Dana 1846) syntype USNM 246, Fiji, B, C) and topotype specimen 101-5718, Fiji; D) *Acropora tanegashimensis* Veron 1990a, holotype MTQ G32477, Tanehashima, Japan, E, F) and topotype specimen 54-5935, Okinawa, Japan; G) *Acropora turbinata* (Dana 1846) holotype YPM: 2017, Tahti, H, I) and topotype specimen 105-9539, Society Islands, French Polynesia; J) *Acropora anthocercis* (Brook, 1893) lectotype NHM: 1892.6.8.235, Palm Island, Great Barrier Reef, Australia, K, L) and topotype specimen 74-2370, Palm Island, Great Barrier Reef, Australia; M) *Acropora spicifera* (Dana 1846) lectotype USNM: 244, Singapore, N, O) and topotype specimen 30-5267, Malacca Strait, Singapore; P) *Acropora pectinata* (Brook, 1892) lectotype, NHM: 1892.6.8.154, Thursday Island, Torres Straits, Q, R) and topotype specimen 19.GBR.112, 12-040 Reef, Far-North Great Barrier Reef, Australia; S) *Acropora bifurcata* Nemenzo, 1971 holotype UP: U.P.C.-1295, Mindoro, Philippines, T, U) and topotype specimen 44-4668, Philippines; V) type specimen for *Acropora conferta*

(Quelch, 1886) holotype NHM: 1885.2.1.12, Fiji, W, X) and topotype specimen FJ12, Fiji.

In resolving the '*Acropora hyacinthus* complex' in the current study I have found that the diversity of this group is much higher than previously thought and the geographic range of common species in this group is much smaller than accepted, both of which have implications on the taxonomic status on nominal species of tabulate *Acropora*. Firstly, by sampling over a broad area covering the Indian and Pacific Oceans I have shown that *Acropora hyacinthus*, first described by Dana in 1846 from a specimen collected in Fiji, has a range restricted to the central Pacific Ocean and eastern Australia (specimens in Fiji, Tonga, the Cook Islands & eastern Australia). This small range is wildly different to previous estimates which places this as a cosmopolitan and common species occurring in almost all tropical coral reefs spanning the Red Sea to the Pacific Ocean. Secondly, by sampling a broad range of morphologies I have been able to identify four species previously synonymized with *Acropora hyacinthus*, being: *Acropora bifurcata* Nemenzo 1971, *Acropora conferta* (Quelch, 1886), *Acropora pectinata* (Brook 1892) and *Acropora turbinata* (Dana, 1846). These species all resolved distinct molecular and morphological clusters in our analysis, with no one species occurring within the same subclade as *A. hyacinthus* (2.3) ultimately revealing that these species are not just distinct from *A. hyacinthus*, but also proving this to be a polyphyletic species grouping. I also deemed *Madrepora recumbens* as a valid synonym in the current study due to the resemblance of specimen 19.GBR.44 in our analysis (collected from Paul Reef, Great Barrier Reef, Australia) to the holotype specimen examined (NHM 1892.6.8.269., *Madrepora*

recumbens lectotype, Rocky Island, Great Barrier Reef, Australia), indicating that this may represent a true morphological variation of *A. hyacinthus* in this region. Although an effort was made to sample topotype specimens for all nominal species, I was unable to collect topotype specimens for the following species currently synonymized with *A. hyacinthus*: *Madrepora patella* Studer, 1879; *Madrepora surculosa* Dana, 1846 and *Madrepora sinensis* Brook, 1893. Of these, morphological comparison of type material shows clear variation to that of *A. hyacinthus*, especially considering the morphological range of *A. hyacinthus* I have captured in the current study (see *A. hyacinthus* taxonomic account below). As I did not collect topotypes of these nominal species, and the type material for each display clear morphological variation to that of *A. hyacinthus*, I decided to consider these species unresolved taxonomically, and not valid synonyms until further evidence (being topotype material and molecular phylogenomics) show these species to be true synonyms of *A. hyacinthus*, as was proven for *M. recumbens*.

A. sp. 7
A
B
22Pse25
A. aff. hyacinthus 'Aceh'
C
D
29-4585
A. aff. bifurcata 'Aceh'
E
F
29-8193
A. sp.1 'aff. conferta'
G
H
79-0666
A. sp.2
I
J
GBR134

Figure 2.9 Unresolved lineages of tabular *Acropora* from the present study. A, B) *Acropora sp7*, 22Pse25, Orpheus Island, Great Barrier Reef, Australia. C, D) *Acropora* aff. *hyacinthus* 'Aceh', 29-4585, Aceh, Indonesia. E, F) *Acropora* aff. *bifurcata* 'Aceh', 29-8193, Aceh, Indonesia. G, H) *Acropora sp1* 'aff. conferta', 79-0666, Fiji. I, J) *Acropora sp2*, GBR134, location GBR, Australia.

Due to the revised geographic range, there are two junior synonyms of *A. hyacinthus* that although are not represented in our study I have decided to resurrect on the basis that the location where the type specimen was collected is far outside the *A. hyacinthus* geographic range. The first of these is *Acropora flabelliformis* (Milne Edwards, 1860) which has a type locality of the Indian Ocean. The second species I are resurrecting on the grounds of biogeographic distinction is *Acropora sinensis* (Brook, 1893) which has a type locality of Taiwan. Below I provide a synopsis of all taxonomic changes to tabulate Acropora specimens investigated in the current study, including descriptions of new species and resurrections of previous synonymies.

When referring to specimens examined the institution abbreviations are:

MTQ: Museum of Tropical Queensland, Townsville, Queensland, Australia

NHM: Natural History Museum, London, United Kingdom

USNM: United States' National Museum of Natural History – Smithsonian Institute, Washington DC, United States

MNHN: Muséum national d'histoire naturelle, Paris, France

YPM: Peabody Museum of Natural History at Yale University, New Haven, Connecticut, United States

UP: University of the Philippines, Manila, Philippines

Order SCLERACTINIA Bourne, 1900

Family ACROPORIDAE Verrill, 1901

Genus *Acropora* Oken, 1815

***Acropora coralD* Bonito, Burdick & Randall, sp. undes.**

Material examined: HOLOTYPE: sp. undes. Guam. PARATYPES: sp. undes., sp. undes. Pohnpei, Micronesia (Fig. 2.10)

Type locality. Micronesia, Guam

Skeletal characteristics of holotype: Corymbose colony with finger-sized ascending branches that mostly have one to five or more distal subparallel appressed branch divisions that form a flat, slightly convex, or shallow vasiform upper surface. The horizontally spreading primary branches that form the basal plate of the colony are generally fused into a reticulum devoid of descending branches, or if present consists of very short branchlets and incipient axials. Axial corallites on the ascending branches are only slightly exsert, 1.7 to 2.2 mm in diameter, with calices 0.8 to 1.5 mm in diameter, and with two cycles of unequal septa deep in the calice. The radial corallites on the ascending branches are crowded, consisting mostly of appressed tubular forms with flaring lips on the upper stem parts, 1.1 to 1.7 mm in diameter, with dimidiate and gutter-shaped apertures 0.7 to 1.2 mm across, and with one variably developed cycle of septa. The directives of the first cycle are generally more prominent, and when the second cycle is present the septa are generally incomplete and rudimentary. Proximally from the ascending branch tips the radials become more appressed,

less exsert and less flaring, eventually becoming sub-immersed to immersed like those on the fused branches of the basal reticulum. Proximally from the branch tips the septa are better developed, generally consisting of two unequal cycles, sometimes with the second cycle being incomplete. The corallite walls are spinulo-costulate on the upper stem parts, becoming more spinulate lower down, and the intervening coenosteum is sparingly spinulo-reticulate to moderately spinulate on the upper branch parts, becoming densely spinulate on the lower branch regions.

Similar species: This species looks similar to *Acropora surculosa* (Dana, 1846), which forms similar shaped colonies and also usually has tentacles extended during the day, but forms less thick and shorter branches. Previously identified as *A. surculosa* (Dana, 1846) in Randall and Myers, 1983 and Randall, 2003.

Habitat: Forms sturdy, corymbose colonies on the upper seaward reef slope and reef margin habitats, and on reef flat platforms where there is good water circulation. Generally a lavender or beige color, commonly with sage colored tentacles greatly expanded during the daytime giving colonies a hairy appearance. The directive tentacle is conspicuously longer than the other tentacles. When the polyps are retracted the tentacular ring is conspicuously more darkly pigmented. The underside of the fused basal branches is pale lavender to pale tan.

Distribution: Currently recorded from Guam, Pohnpei, Palau and Wake Island and appears restricted to the northern Pacific Islands.

Phylogeny: Clade VI (Cowman et al. 2020).

Etymology: Named after the University of Guam (UOG) Marine Lab in recognition of its tremendous contribution to supporting marine research, conservation, and management in Micronesia for more than 53 years.

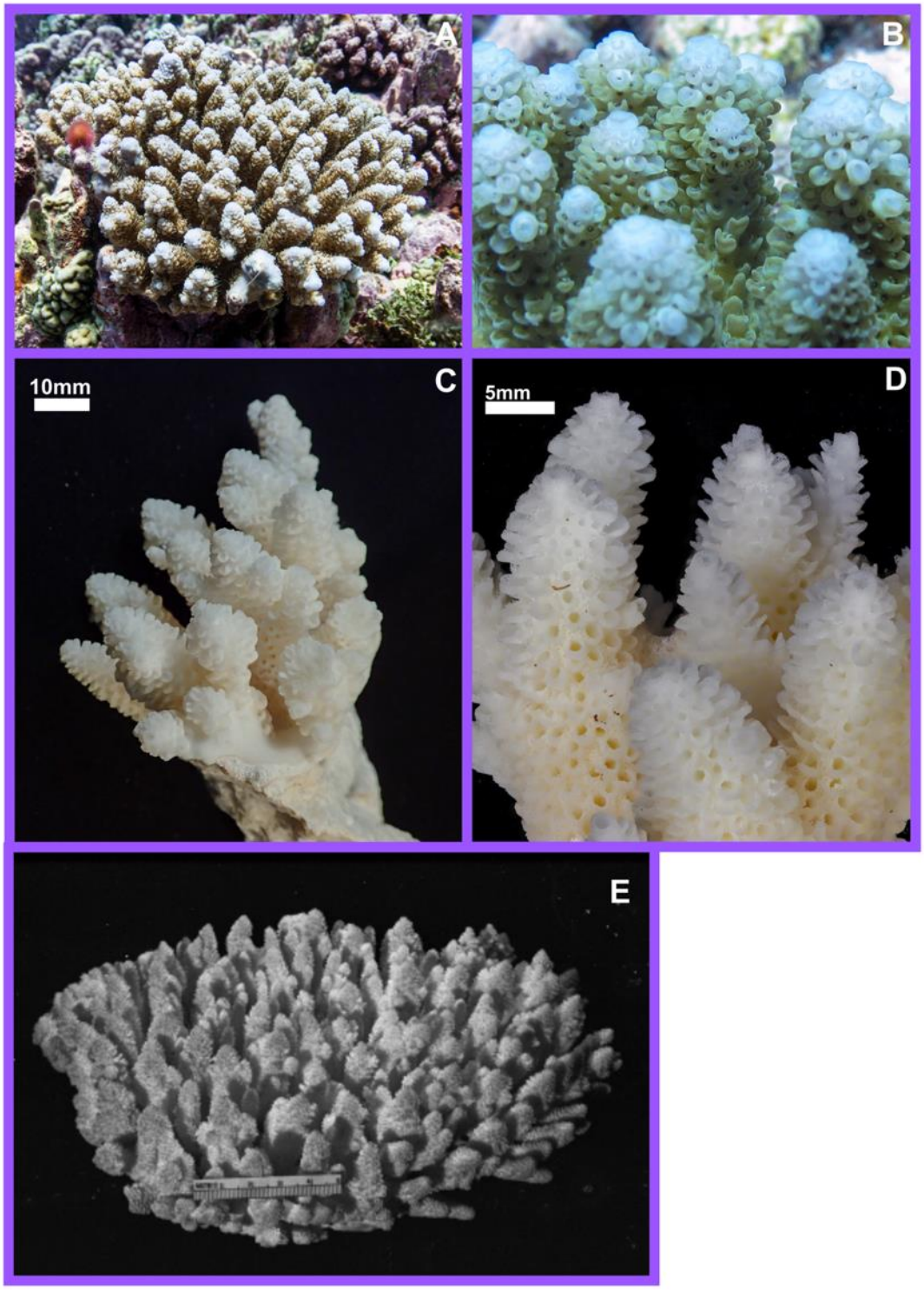

Figure 2.10 ***Acropora coralD*** **sp. undes.** E) Holotype sp. undes., Guam. A, D) Syntype sp. undes., Pohnpei, Micronesia. B, C, Syntype sp. undes., Pohnpei, Micornesia.

***Acropora coralC* Bridge & Rassmussen, sp. undes.**

Material Examined: HOLOTYPE MTQ sp. undes. PARATYPE: MTQ sp. undes.. Both specimens collected from 1 metre depth at Coral Bay, Western Australia (Fig. 2.11)

Type locality: Fringing reef of Bill's Bay, Coral Bay, CoralC (Ningaloo), Western Australia

Other Material: MTQ: G39762, G39764, G39770, G39771, G40470, G47008, G51538, G52447, G52449, G52450, G52452, G52454, G52456, G52457 Western Australia, Australia; G51269 Northern Territory, Australia; G46695, G46696 Bali, Indonesia; G48523 Alor Islands, Indonesia; G50170, G55429 Sulawesi, Indonesia; G36823, G47774 Akajima, Japan; G50065 Pratas, South China Sea

Skeletal characteristics of holotype: Colony fragment taken from the edge of the colony, diameter 100 x 100 mm, 30 mm in height. Branches: tertiary branching order absent, terminal branchlets 5 to 20 mm in length and 3 to 6 mm in diameter; axial dominated; predominantly terete; branchlet density 1.5 per cm^2. Corallites: axial corallites tubular, terete; outer diameter 2.0 to 2.4 mm; inner diameter 0.7 to 0.9 mm; 2 synapticular rings; porous; primary septa all present up to 1/2 R; secondary septa sometimes present up to ¼ R. Radial corallites: labellate, becoming immersed with increasing distance down axial corallite; relatively uniform in size; mostly touching; 6-8 radials on the branch circumference; primary septa vary between corallites, up to ¼ R in some corallites but absent in others; secondary septa poorly developed or absent. Coenosteum: the same on and between radial corallites; costate; no spinules.

Field characteristics of the holotype: Colony outline: determinate. Colony morphology: tabular, with multiple tiers. Colour: dark green-brown with yellow axials; directive tentacles extended during the day.

Variations shown in paratypes: sp. undes.: Upright branchlets much shorter than holotype (5 – 10 mm in height), and lower walls of radial corallites generally less developed.

Habitat: Intertidal reef flats, subtidal upper reef slopes, back reef margins and shallow lagoons.

Distribution: Specimens included in our phylogeny were collected from the Houtman Abrolhos Islands and Ningaloo Reef, Western Australia, northwards through the Indo-Australian Archipelago (Gulf of Tomini, Sulawesi and Luzon, Philippines) as far as Okinawa, Japan, and west to the Andaman Sea, north Sumatra.

Molecular phylogeny: *A. coralC* **sp. undes.** is recovered as a monophyletic clade within Acropora Clade VI sensu Cowman et al. (2020). It is sister to *A. tanegashimensis*, with both of these species forming a clade that is sister to *A. hyacinthus*.

Etymology: This species is named after the local indigenous name – CoralC (Ningaloo) - for the region where the type material was collected. To the traditional owners this word means ‘deep water’, and the species is particularly abundant in the region. The Traditional Owners, the Baiyungu and Yinnigurrura people, occupied the region for over 30,000 years. We thanks the Traditional Owners and the Nganhurra Thanardi Garrbu Aboriginal Corporation for allowing us to work on their Country. We acknowledge that their country was never ceded,

and pay our respects to the traditional owners of the land and sea country past, present and emerging.

Remarks: This species has previously been recorded in Western Australia as *A. spicifera* (Dana, 1846), initially by Veron (1986) and then by other scientists working on Western Australian reefs. The images listed as *A. spicifera* in Veron (1986) are clearly *A. coralC*, who states that the species is not found on the east coast but, somewhat intriguingly, extends eastwards to Fiji. This may be due to the fact that Dana's original description of *A. spicifera* includes material from both Singapore and Fiji, rather than any specific records of the species from the South Pacific. Wallace (1999) discussed the taxonomic uncertainties surrounding *A. spicifera* and concluded that Dana's types from syntypes from Fiji (USNM 234) and Singapore (USNM 244) were different species, designating USNM 244 as the lectotype. The species we identify as *A. spicifera* in this study, which we have sampled from the east coast of Malaysia as well as the type locality of Singapore, occurs in Clade Hc, sister to *A. coralB* **sp. undes.** and relatively distant from *A. coralC*, and demonstrating that the species is distinct from *A. spicifera*. The specimens figured under A. spicifera by Wallace (1999) vary widely in gross morphology and likely include numerous distinct species, although one of these specimens (G51538) is *A. coralC*.

The species dominates intertidal reef flats and back-reef margins at Ningaloo Reef and the Houtman Abrolhos Islands, Western Australia, where it can form very large colonies >3 m in diameter and large monospecific stands, as illustrated by Veron (1986). Examination of specimens in the MTQ collections indicate the species is also common on reefs elsewhere in Western Australia. While it is most common in shallow depths, it can occur to depths of at least 8 m.

At these depths, the species can develop an unusual morphology where branches become more sparse and project upwards rather than in a flat plane. In isolation these colonies could easily be confused for a different species; however, it is possible to observe the morphological transition from the unusual deep-water morphology to the standard tabular morphology at sites where the species is abundant throughout the depth range. These observations are confirmed by our phylogeny, which shows WA31 is a deep-water morph of *A. coralC*. Interestingly, we have not observed this morphology outside of the Houtman Abrolhos Islands.

Molecular species delimitation analyses indicate some genetic structure separating populations in Western Australia from those in Indonesia, the Philippines and Japan, suggesting these may be two distinct species. However, neither molecular nor morphological analyses consistently delineated two distinct groups; moreover, over the 2,500 km gap in sampling between subtropical Western Australia and equatorial Sulawesi means we cannot reliably delineate population structure from species-level divergence. Additional sampling in the intermediate locations (e.g. northern Western Australia, the Lesser Sunda Islands and the Makassar Strait, Indonesia) is required to resolve this issue.

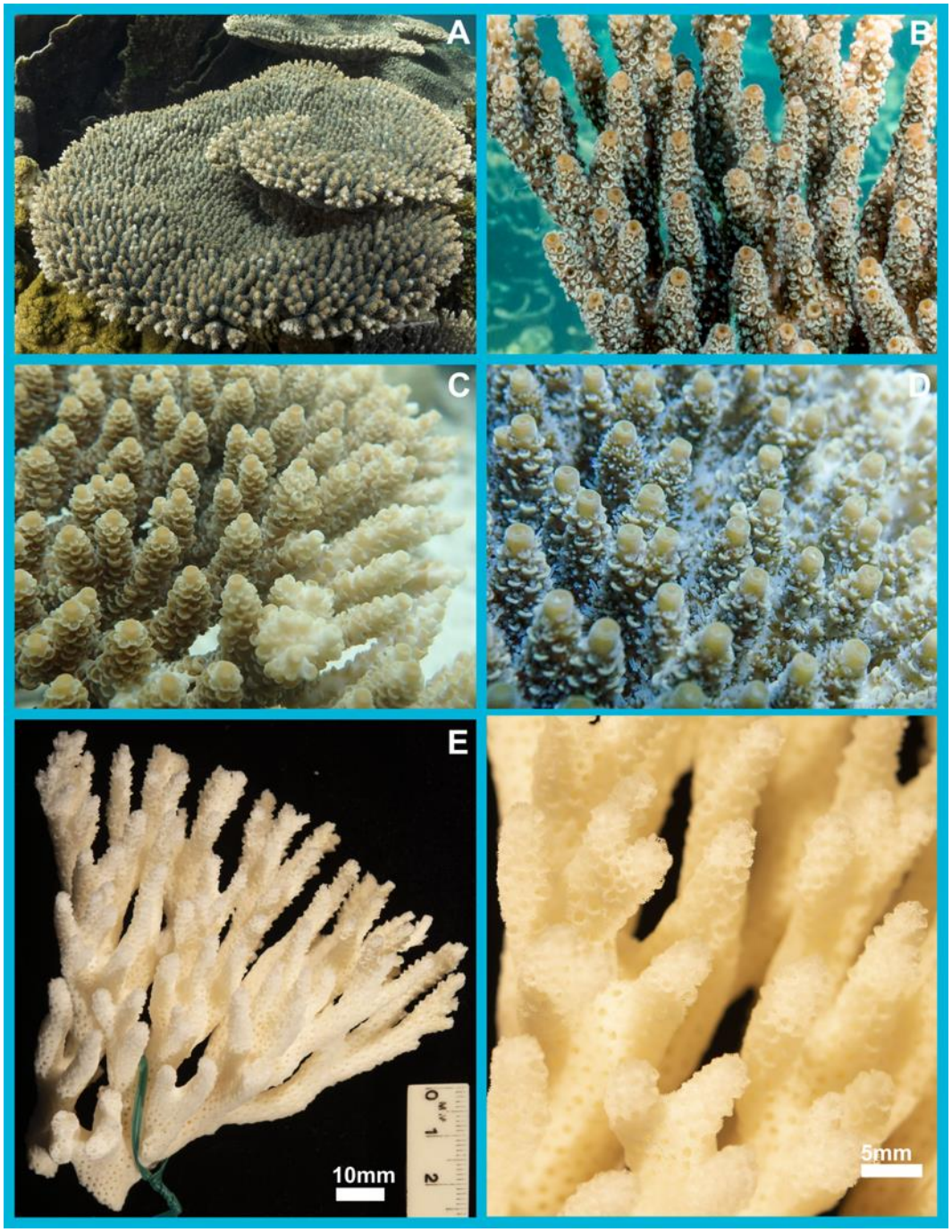

Figure 2.11. ***Acropora coralC*** **sp. undes.** A, B, E, F) Holotype sp. undes.; Ningaloo Reef, Western Australia, Australia. C) variety 18Oki23; Okinawa, Japan. D) variety 45-3610; northern Philippines.

Acropora coralA Baird, sp. undes.

Material examined: HOLOTYPE: sp. undes.; North Solitary Island. PARATYPES: sp. undes. North Solitary Island; sp. undes. Lord Howe Island (Fig. 2.12)

Type locality. Australia: New South Wales: North Solitary Island

Other Material: MTQ: G7359 Moreton Bay, Queensland, Australia; G24194, LH31, LH33 Lord Howe Island, New South Wales, Australia; 79-8806, 79-4063 North Solitary Island, New South Wales, Australia

Skeletal characteristics of holotype: Part of colony, 11 cm greatest length and 7 cm wide and 3 cm high. Branches: tertiary branching order absent; final branch length 5 to 12 mm; 3 to 5 mm in diameter; axial dominated; terete Corallites: axial corallites tubular, some with a slight taper; outer diameter 1.5 to 2.0 mm; inner diameter 0.8 to 1.0 mm; height 2.0 to 2.5 mm; 2 synapticular rings; porous; primary septa all present up to 1/4 R; secondary septa absent. Radial corallites: labellate with flaring lips; mixed sizes; mostly touching; 4-6 radials on the branch circumference; primary septa absent; secondary septa absent. Coenosteum: the same on and between radial corallites; costate; no spinules.

Field characteristics of holotype: Colony outline: indeterminate; colony morphology: a side-attached plate; colour: grey, with a white growing margin.

Variations shown in paratypes: sp. undes.: the final branches are tapered; radial corallites are more regularly distributed than in the holotype, the colony colour is dark brown. 81-1128: colony is a centrally attached table, axial corallites no larger than 1.5 mm in height; colour is light brown with pinkish margin.

Habitat: Subtidal, growing on rocks in the Solitary Island to 8 m depth, or in the lagoon on Lord Howe Island.

Distribution: This species has only been documented to occur at Lord Howe Island, the Solitary Islands, Moreton Bay and Great Keppel Island in south-east of Australia.

Phylogeny: Clade V1 (Cowman et al 2020)

Etymology: Named for the late Dr. Vicki Harriott, in recognition of her significant contributions to coral reef ecology, in particular, her research on the subtropical reefs of Australia's east coast.

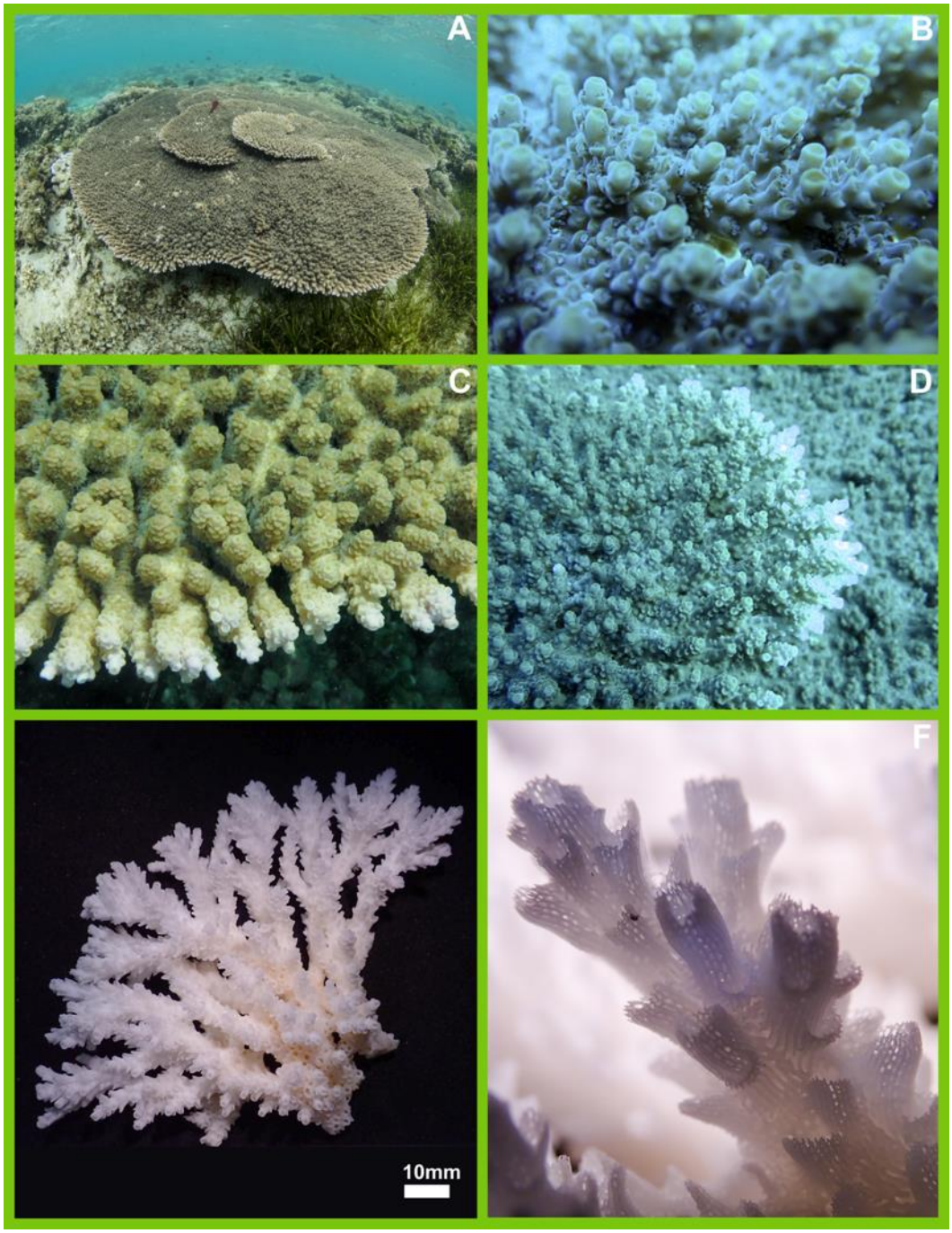

Figure 2.12 ***Acropora coralA*** **sp. undes.** B, D, E, F) Holotype sp. undes.; North Solitary Island, Australia. A) LH31; Lord Howe Island, Australia, C) Paratype 81-1128; Lord Howe Island, Australia.

***Acropora coralB* Rassmussen & Bridge, sp. undes.**

Material examined: HOLOTYPE: MTQ sp. undes. collected from 5 m depth at the southern end of Little Stevens Reef, Great Barrier Reef, Australia (Fig. 2.13)

Type locality. Australia: Great Barrier Reef, Little Stevens Reef

Other Material: MTQ: G43548, G43550 Southern GBR; G27616, G32749, G46057, GBR695, GBR696, GBR697, GBR698 Central GBR; G27619, G43502, G43520, G43530, G435325 Northern GBR; 35634, 53594 Papua New Guinea; G61865 Palau

Skeletal characteristics of holotype: A vase-shaped table with tightly reticulate basal branches. Basal branches are < 8mm in diameter. Vertical branchlets are < 3mm in diameter and scarcely more than 10mm in length, neatly compact with 3 – 8mm spaces between branchlets at the Axial tip. Axial corallites are tubular, inner diameter 0.6 - 0.8mm, outer diameter 1.2 - 1.8 mm. Radial corallites are flaring labellate with uniformly smooth square lips, sometimes slightly appressed towards the axial tips, inner diameter 0.4 – 0.8mm, outer diameter 0.6 – 0.10mm, often crowded and touching forming a neat rosette around the Axial. Axial corallites show six-star septa with two prominent directives half the radius of the calyx. The septa in radial corallites are weakly developed and often absent, occasionally a single or two prominent directives apparent less than a quarter of the radius of the calyx. Coenosteum is costate on the axial and radials, whilst becoming reticulate along the branches in between corallites. The neat arrangement and growth of the vertical branchlets of this colony form a smooth and consistent plane on the tops

of these colonies, with no axials or corallites extruding from the surface (Fig. 13B).

Field characteristics of holotype: This species is often confused with *A. hyacinthus* on the Great Barrier Reef and is a common species where tabulate Acropora are present, and in the MTQ collections I found this species to be identified as *A. hyacinthus*. The molecular phylogeny, however, places this species as a sister lineage to *A. spicifera* in clade Hc, clearly distinct from *A. hyacinthus* in clade Ha. In the field this species is easy to identify due to the compact and orderly arrangement of the branchlets and radial corallites, as well as the often-pastel like colony colours which can range from pinks, purples, blues and greens. In shallow and exposed reefs this species has been observed to form fused encrusting plates, whilst in deeper colonies the branchlets become less compact and the basal branches form a wider mesh, making it harder to distinguish from *A. hyacinthus* or *A. pectinata* where it co-occurs.

Etymology: Colloquially referred to as 'Acropora neat' upon first collection, *coralB*, Latin for 'neat', refers to the orderly, compact and neat morphological features of the branchlets of this species, compared with the often irregular branchlet morphology of *A. hyacinthus* of which it co-occurs.

Phylogeny: Clade V1 (Cowman et al 2020)

Distribution: This species is confirmed in the present study to have a broad distribution across the Pacific Ocean, with specimens collected from Fiji, eastern Australia, New Guinea and Palau. Further sampling is required to resolve the full range of this species.

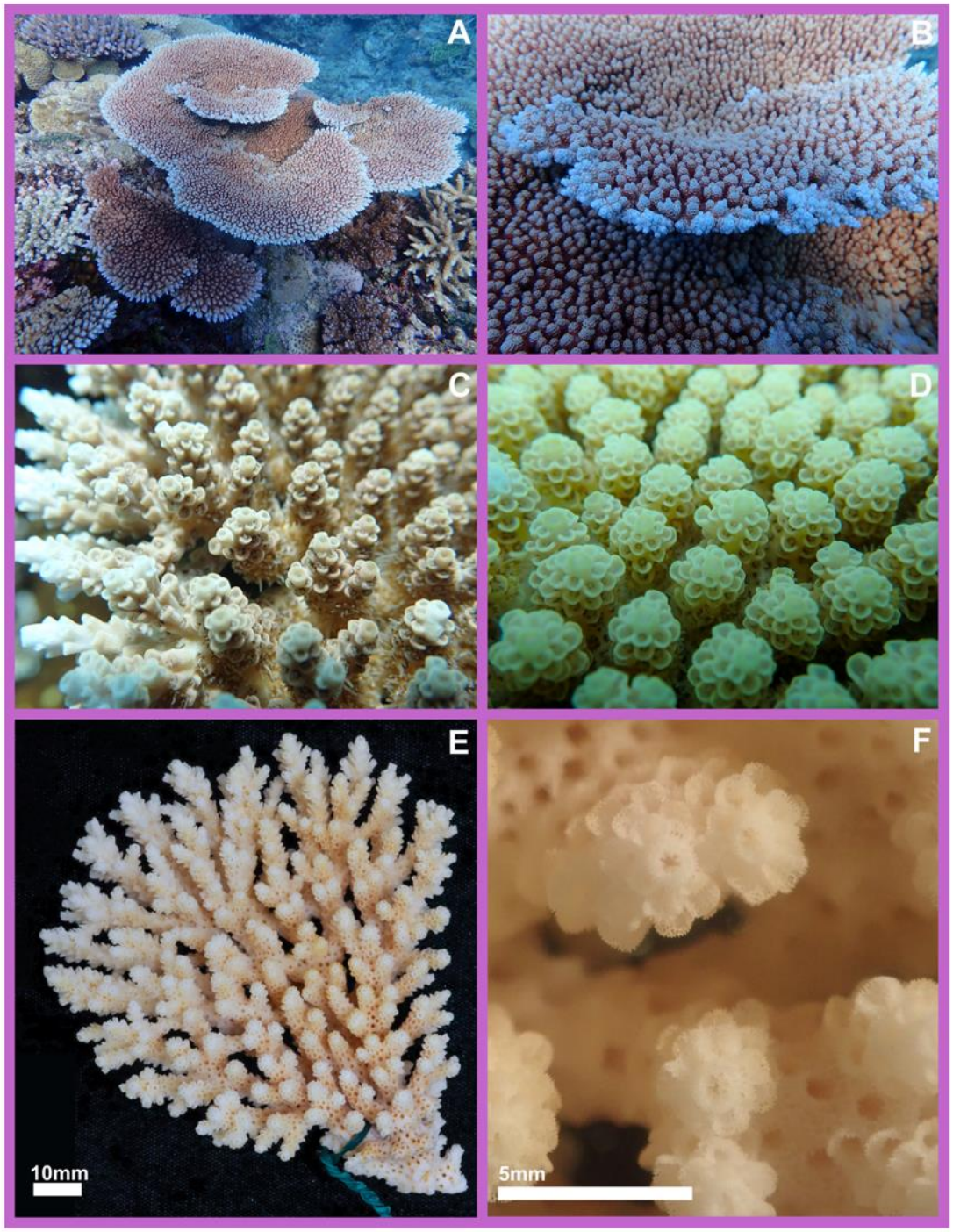

Figure 2.13. ***Acropora coralB*** **sp. undes.** A, B, E, F) Holotype sp. undes.; Little Stevens Reef, Great Barrier Reef, Australia. C) variety 74-4900; central Great Barrier Reef, Australia. D) variety 19.GBR.122; Great Detatched Reef, Great Barrier Reef, Australia.

Acropora hyacinthus (Dana, 1846)

Madrepora hyacinthus Dana, 1846: p. 444, Plate 32, fig. 2

Acropora hyacinthus (Dana): Verrill, 1902: p. 216

Madrepora recumbens Brook, 1892: p. 461.Plate XXVII. fig. F

Madrepora turbinata Dana, 1846. Here removed from synonymy with *Acropora hyacinthus* (Dana) contra Wallace (1999) p. 256.

Madrepora conferta Quelch, 1886. Here removed from synonymy with *Acropora hyacinthus* (Dana) contra Veron & Wallace (1984) p. 310.

Madrepora pectinata Brook, 1892. Here removed from synonymy with *Acropora hyacinthus* (Dana) contra Veron & Wallace, 1984: p. 310

Madrepora sinesis Brook, 1893. Here removed from synonymy with *Acropora hyacinthus* (Dana) contra Veron & Wallace, 1984: p 310.

Acropora bifurcata Nemenzo, 1971. Here removed from synonymy with *Acropora hyacinthus* (Dana) contra Veron & Hodgson, 1989: p. 247.

Material examined: USNM 246, *Madrepora hyacinthus* syntype, Fiji; NHM 1892.6.8.269., *Madrepora recumbens* lectotype, Rocky Island, Great Barrier Reef; MTQ: 101-5718, topotype, Fiji; MTQ G27595, GBR702 Central GBR; G28686, G32756, G43499, G43503, G43506, G43509, G54325, G54355 Northern GBR; G34973, G58738, G61032 New Caledonia; G37560 Tuvalu

Remarks: Although the specimens in the current study display some variations in gross morphology there are some discernible features that can identify this species. Recent revisions describe *A. hyacinthus* as being tabulate corals with short rosette-like branches, formed of labellate radials with a square or rounded

lips (Wallace 1999), although I have found here that this description alone could describe almost all the species examined in the current study. The original description from Dana (1846) as well as the type material show this species to be a vasiform plate with tubo-labellate (or square labellate lipped) radial corallites, with sometimes proliferous vertical branches (Fig. 2.7A). An important feature for this species – and noted by Dana (1846) – is the presence of a 'violet tinge' to the tips of specimens when fresh. Similarly, I have found to be a recognizable feature of this species *in situ* across both the central Pacific and central to northern GBR specimens is the distinct colour of the axial tip which is often darker than the branchlet below (Fig 7C). This feature, however, in often lost amongst southern GBR specimens where whole colonies tend to take on a cream or brown colour. In museum collections and in the field, this species can be distinguished by the almost pleiotropic vertical branchlet growth and incipient axials giving this species a 'messy' appearance which is apparent in the type material (USNM 246). Additionally, the presence of the labellate radials with a square lip that somewhat form a rosette around the Axial tip is a defining feature, although once more the specimens in south-eastern Australia tend to display a more uniform rosette making this species harder to identify in this region (19.GBR.07, 19.GBR.17). In some specimens, vertical branchlets appear to fan out towards the edge of the colony (19.GBR.70, 19.GBR.81) This species tends to occur in shades of pink, unless in the south-east of Australia where individuals are often uniformly cream. With mounting evidence over the past decade that *A. hyacinthus* forms a 'species complex' across the Indo-Pacific region containing a minimum of six genetically distinct species across this range (Ladner & Palumbi 2012; Suzuki et al. 2016) I here find evidence that the true range for *A. hyacinthus* is likely restricted to the

central Pacific Ocean – with a type locality of Fiji - and across to eastern Australia. With this revised species range, as well as clear morphological differences amongst type material for *A. sinensis* (Brook, 1893) stat. rev. from Taiwan I formally resurrect *A. sinensis* from synonymy (discussed below) although with further sampling and taxonomic investigations required to resolve the true range and status of *A. sinensis*. Further, due to sampling of topotype material, distinct lineages in the ML phylogeny (2.3) and morphological differences amongst type material for *A. turbinata* (Dana, 1846) stat. rev. from Tahiti, *A. conferta* (Quelch, 1886) stat. rev. from Fiji, *A. pectinata* (Brook, 1892) stat. rev. from the Torres Strait, and *A. bifurcata* Nemenzo, 1971 stat. rev. from the Philippines I formally resurrect these species from synonymy. As discussed, morphological similarities between *A. pectinata* and *A. bifurcata*, the close relations of these taxa in the ML phylogenies and the proximity of species ranges warrant further investigations into the boundaries between *A. pectinata* and *A. bifurcata*.

As discussed above, two species that have been considered in the past as synonyms for A. hyacinthus – being *A. patella* and *A. surculosa* – have been omitted as synonyms in this revision. Investigations of the type material show these nominal species to not fall within the morphological variation of *A. hyacinthus*, however, I was unable to locate topotype material to investigate these species in the current study. Past revisions have also noted on the morphological differences between the type material of *A. surculosa* and *A. hyacinthus* in particular (Veron & Wallace 1984), and further investigations are warranted to resolve the status of these species.

Distribution: Confirmed in the present study to occur in Fiji, Tonga & the Cook Islands in the south Pacific Ocean and throughout the Great Barrier Reef, Coral Sea, Solitary Islands & Lord Howe Island along the East coast of Australia.

Acropora spicifera (Dana, 1846)

Madrepora spicifera Dana, 1846: p. 442, Plate 33. Fig. 2.4a, 4b, 5 & Plate 31. fig. 6a, 6b, 6c.

Acropora spicifera (Dana): Verrill, 1902: p. 218

Material examined: USNM: 244, *Madrepora spicifera* lectotype, Singapore; MTQ: 145-0335, Sunda Shelf, Malaysia; 29-8257, Aceh, Indonesia; 30-5267, Malacca Strait, Singapore; G48739 Western Australia; G50033 Bali, Indonesia; G50055, G50179, G50049 East Kalimantan, Indonesia; G53742 Halmahera, Indonesia; G71688 Raja Ampat, Indonesia; G49836 Riau, Indonesia; G50062 Seribu Islands, Indonesia; G50086, G50176, G50053 Sulawesi, Indonesia; G59144 Malaysia; G41022 Singapore; G50063 Pratas, South China Sea

Remarks: This species was first compared with *A. surculosa* by Wells (1964) however, validity of the species was retained. Veron & Wallace (1894: p. 310) then listed as a potential synonym of *A. hyacinthus* (Dana) although it has since been retained as a valid species (Veron 1986; Heron & Hodgson 1989; Wallace et al. 2012) although with questioning of its similarity to *Acropora millepora* (Wallace 1999). The specimens in the current study vary in morphology, with a specimen from Western Australia (WA29) having poorly formed corallites and broader spacing of branchlets, somewhat resembling the branchlets of Dana's

syntype of *A. spicifera var. abbreviata* (USNM 235) from Singapore, whilst a specimen from Malaysia (145-0335) resembles the type material although with widely reticulating basal branches, and from Aceh in Indonesia (29-8257) the basal branches are almost fused, again resembling the syntype series of *A. spicifera var. abbreviata* (USNM 235, 245).

Description: This species forms a table with closely reticulate basal branches. Vertical branchlets often fork with numerous incipient axial corallites branching from the one base. The type material show the radial corallites to be labellate with a square lip, proliferating in size and number down branchlet, often poorly formed at the branch tip.

Distribution: Confirmed in the present study to occur in Malaysia, Singapore, Indonesia and Western Australia. This species was not identified in Fiji or surrounding Pacific Ocean islands and suspect the variation from Fiji identified by Dana (1846) was a distinct species.

Acropora anthocercis (Brook, 1893)

Madrepora anthocercis Brook, 1893: p. 106, Plate XIII. fig. C.

Specimens examined: NHM: 1892.6.8.235, *Madrepora anthocercis* lectotype, Palm Island, Great Barrier Reef, Australia, 1892.6.8.236, *Madrepora anthocercis* syntype, Rocky Island GBR, 1892.6.8.237, *Madrepora anthocercis* syntype, Rocky Island GBR; MTQ: GBR176, 74-2370, 74-1872, Palm Island, Great Barrier Reef, Australia. G28417, G48299, G29890, G29897 northern GBR

Remarks: The three specimens in the current study, all from the Palm Islands group in the Great Barrier Reef bear resemblance to the type specimen (NHM: 1892.6.8.235).

Description: This species is distinct from all other lineages in this tabulate Acropora clade in that the colonies are cespitose clumps instead of plating or tabulate forms, a feature shared by the specimens in the current study as well as the types. Another distinguishing feature are the crowded and acervate branchlets, which are top-heavy with densely crowded radial corallites. Axial corallites are exerted, 1.5 – 2.5 mm in diameter, although often indistinguishable from numerous incipient axial-radial corallites. Radial corallites display numerous forms throughout the colony, with some nariform, and tubular dimidiate, and occasionally labellate.

Distribution: Here confirmed to occur in the northern Great Barrier Reef, with specimens in the current study collected from the type locality of Palm Island. While further sampling and study is required to confirm the full range of this species, our extensive sampling efforts deem it unlikely that it occurs in the full range of the Red Sea to the south Pacific Ocean as previously accepted (previously documented in the Red Sea by Antonius et al., 1990; Seychelles by Selin et al. 1992; Phillipines by Veron, 1990a & Vanuatu by Veron, 1990b).

Acropora tanegashimensis Veron, 1990a

Acropora tanegashimensis Veron, 1990a: p. 109, Figs. 13, 14 & 73

Specimens examined: MTQ: G32477, *Acropora tanegashimensis* holotype, Tanegashima, Japan; 54-5930, 54-5935, 54-5940, 54-5945, Okinawa, Japan;

G62308, G62312, G62315 Tanegashima, Japan; G62321, G62322 Kochi, Japan; G62347, G62349, G62996 Wakayama, Japan

Remarks: Veron (1990a) noted similarity to *A. hyacinthus*, although remarked on the distinguishing features of the crowded radial corallites, indistinct axial corallite and the colour of the colony *in situ*, being greenish-grey. *A. tanegashimensis* was considered a valid species by Wallace (1999) and Wallace et al. (2012), although the IUCN Red List assessment for *A. tanegashimensis* indicates that Wallace considered the species a junior synonym of *A. hyacinthus* (Richards et al. 2008). The specimens in the current study were cream coloured *in situ*, which may indicate regional colour variation given they are from lower latitudes than the holotype, and subtropical *Acropora* are often darker in colour than tropical counterparts. In our phylogeny I find this species to occur in the same subclade as *A. hyacinthus* across the edge trimmed alignments with strong node support, although with irregular placement amongst the internally trimmed phylogenetic alignments where this this species falls in a sister clade to *A. hyacinthus* (Supplementary Material, Fig S1). Overall, the species delimitation shows this species to be distinct, supported by a visually distinct morphology.

Description: Of the specimens in the current study, 54-5940 closely resembles the *in situ* image in Veron, 2000 (Fig. 73, p. 169), while the remaining three specimens match that of the description of flat corymbose plates. A distinguishing feature of this species seems to be the crowded radial corallites both on the branchlets and along the upper surface of the main branches as noted by Veron (2000).

Distribution: In addition to the type locality of Tanegashima in Japan, in the present study specimens were collected from the island of Okinawa, Japan, extending the range of this species further south in the Japanese archipelago. Museum material also places this species as occurring along the coastal mainland of Japan.

Acropora flabelliformis (Milne Edwards, 1860)

Madrepora flabelliformis Milne Edwards, 1860: p. 156

Specimens examined: MNHM: 329a (407), *Madrepora flabelliformis* holotype, Indian Ocean.

Remarks: While researching it became evident that the status of *A. flabelliformis* is unclear, with the species commonly believed to be a junior synonym of *A. hyacinthus* (Dana) our research uncovered no evidence that it was ever formally synonymised. The most recent taxonomic revision of *Acropora* (Wallace et al., 2012) omitted this species completely, while prior revisions (Wallace, 1999) indicated that this species was in fact *A. hyacinthus* (p. 258), although not formally synonymized pending further analysis of the type specimen, placing this species in a status limbo. In 1984, Veron & Wallace listed this as a nominal species while omitting this from the taxonomic account. This species was last recorded as valid by Sheppard (1987) as an Indian Ocean species and earlier as occurring in Rodriguez of the Mascarene Islands in the Western Indian Ocean by Bruggemann (1879). Here, I formally recognize this as a valid species with further sampling required to establish range and lineage.

Distribution: Species is only known to occur in the Indian Ocean according to the original description and type material, with no indication of the exact collection locality.

Acropora turbinata (Dana, 1846) status revised

Madrepora surculosa var. turbinata Dana, 1846: p. 445, Plate 32. fig. 5.

Acropora turbinata (Dana): Verrill, 1902: p. 219, 242

Madrepora turbinata Dana, 1846. Here removed from synonymy with *Acropora hyacinthus* (Dana) contra Wallace (1999) p. 256.

Specimens examined: YPM: 2017, *Acropora turbinata* (Dana) Verrill, 1902, holotype, Tahiti; MTQ: G33080, G53584, G54696 Tahiti; G44044 Mo'orea; G30102, G36017 Austral Islands; G40713 Cook Islands; G36021, G36023, G36025 Niue; G63113 America Samoa; G57878 GBR

Remarks: Dana (1846) first described this species as a variety of *Madrepora surculosa* (Dana, 1846) with the distinction that the branching pattern formed obliquely upward instead of forming a reticulate plate. This distinct long and obliquely vertical branchlet pattern – clear in the holotype (YPM 2017) - is a clear morphological distinction of this species compared to the horizontal plates with short branchlets of the *A. hyacinthus* holotype (Fig. 2.7). Verrill (1902: p. 242.) accepted this as a distinct species, although remarked on the similarity to *A. surculosa* (Dana) whilst also noting that it was likely indistinct from *A. armata* (Brook). Veron & Wallace (1984) included this species in their list of nominal *Acropora*, while omitting it from any taxonomic account. Wallace (1999: p. 256.,

2012) then included this species in the list of synonymy with *A. hyacinthus* (Dana) with no remarks as to this decision. Of the specimens in the current study, our topotype from the Society Islands (105-9539) closely resembles the holotype, and throughout the material examined it became clear that the solid base and long branchlets were a common feature amongst this species.

Description: This species tends to form a solid base with long branchlets growing obliquely upward instead of forming a reticulate horizontal mesh which is common amongst this tabulate *Acropora* clade. Branches have some incipient axials, and radial corallites form elongated labellate lips, sometimes tubular-dimidiate in appearance.

Distribution: Confirmed in the present study to occur throughout the South Pacific Ocean, specifically French Polynesia, Tonga, Cook Islands and Fiji.

Acropora conferta (Quelch, 1886) status revised

Madrepora conferta Quelch, 1886: p. 164, Pl. X. figs. 3-3c.

Acropora conferta (Dana): Verrill, 1902: p. 213

Specimens examined: NHM: 1885.2.1.12, *Madrepora conferta* holotype, Fiji; MTQ: FJ15, FJ72, Fiji. G41296, America Samoa

Remarks: Brook (1893: p.109) first questioned the validity of *Madrepora conferta* (Quelch), noting the only distinctive factor separating this species from *Madrepora hyacinthus* (Dana) was the shape of the colony. Wells (1956) accepted this species as valid and extended the range to include much of the Pacific Ocean and potentially across to the Indian Ocean. Veron & Wallace (1984) then

synonymized this citing clear resemblance to *A. hyacinthus*. Here, I find this species to occur in a separate clade to *A. hyacinthus* in our molecular phylogeny, presenting clear evidence of the validity of this species.

Description: Specimen FJ72, being a topotype specimen for *A. conferta* strikes a fantastic resemblance to Quelch's type (NHM 1885.2.1.12). As per the original description, this specimen displays a densely intricate coalescent mesh of the main branches, with some small sections fusing together completely. Branchlets are short and grow at right angles to main branches, becoming longer and thinner at the edge of the colony. Axial corallites are no more than 2 mm wide, scarcely prominent, with a distinct 6-star septa. Radial corallites small, with a curved labellate lip. Quelch (1886) remarked on the less distinct rosette formation of the corallites towards the colony edge, which is also apparent in the current study specimens, which also display irregular size and shape of corallites towards the colony edge. Both colonies sampled (FJ15 & FJ72) in the current study were rose-pink in colour *in situ* with distinct Axial tip that shared the same dark ring around the axial prominent in *A. hyacinthus* (Fig. 2.7).

Distribution: Confirmed in the present study to occur in Fiji, being the type locality for this species. Further sampling and investigation are required to determine the full range of this species which currently presents as a south Pacific endemic.

Acropora pectinata (Brook, 1892) status revised

Madrepora pectinata Brook, 1892: p. 460., Brook, 1893: p. 95, Plate XXVII. fig. D,E

Specimens examined: NHM: 1892.6.8.154, *Madrepora pectinata* lectotype and 1892.6.8.155, *Madrepora pectinata* syntype, Thursday Island, Torres Straits; 1892.6.8.156, *Madrepora pectinata* syntype, Capricorn Islands GBR; MTQ: G37400, G46428, G59024 Central GBR; G37400, G46428, G59024, G28691, G54342, G54348, G54359 Northern GBR

Remarks: This species was synonymized by Veron & Wallace (1984), where the authors cited that this was a "very clear" synonym of *A. hyacinthus* (Dana), with later works of Wallace (1999, 2012) also retaining this synonymy. Earlier revisions (Wells, 1954: p. 420.) however indicated that this species was likely *A. corymbosa* (Lamarck, 1816). Here, contra Veron & Wallace (1984) p. 310 I remove this species from synonymy with *A. hyacinthus* (Dana 1846).

Description: All colonies in the current study strongly resemble the type specimens and original description from Brook (1892), forming open branching networks where individual branches are distinct, a feature that becomes more prominent in deeper specimens with wider branching patterns. Branches are between 5-12 mm diameter. Branchlets are short (< 14 mm length) often clustered in groups of 2 - 5 and are no more than 6 mm apart along the branch. Axial corallites are rarely exert and are no more than 2 mm in diameter. Radial corallites are labellate, curved, and generally with a rounded or square lip. Radials are tightly clustered forming a neat rosette around the Axial corallite. At the edge of the colony, branches extend outward continuing anastomosing mesh. Where this species co-occurs with *A. hyacinthus* and *A. coralB sp. undes.* on the Great Barrier Reef, Australia it can be easily distinguished *in situ* by the colony colour, which often has a cream skeletal tissue and brown polyps, compared with the often-

bright pinks, blues, and greens of the forementioned species. This species, however, can be confused with *A. coralB sp. undes.* in museum collections where a lack of diagnostic *in situ* traits can confuse boundaries between the two when *A. pectinata* is collected from shallow high wave exposure environments. Colonies also often fan out in horizontal plates with irregular boundaries.

Distribution: Confirmed in the present study to occur along the central and northern Great Barrier Reef. One specimen (19.GBR.25) collected from the southern GBR in the Pompey & Swains region showed affinity with this lineage in the phylogeny, see notes below. Holotype specimen was collected from Thursday Island in the Torres Strait.

Notes: Brook (1892) remarks of the similarity of this species colony form to that of *M. conferta* & *M. hyacinthus*. In 1893 (Brook), included a variety from the Capricorn Islands, Australia with shorter branchlets which strongly resembles specimen 19.GBR.25 which was collected from Paul Reef, just north-east of the Capricorn Islands. This specimen had some molecular affinity with the specimens of *A. pectinata* in the current study, however, with irregular placement amongst phylogenetic reconstructions. This specimen also fell as an outlier in the SNP and morphological analysis (Fig. 2.4 & 6) causing some questioning of the validity of this identification. Further sampling and study are required to resolve this outlier.

Acropora florensis (Veron, 2000) nom. n.

Acropora pectinatus Veron 2000 (incorrect spelling)

Acropora pectinata Veron: ICZN, 2011: p. 163

Material Examined: MTQ: HOLOTYPE G55801 *Acropora pectinatus* Flores, Indonesia.

Remarks: This species was first described by Veron (2000) as *Acropora pectinatus*, although due to incorrect spelling was corrected as *Acropora pectinata* Veron, 2000 through a request by The International Commission on Zoological Nomenclature (2011). Consequently, by resurrecting *A. pectinata* (Brook, 1892) in the current study Ifind the later species from Veron to be an invalid homonym, as the Principle of Priority (Article 23. Ref) deems the earlier name from Brook to take precedence. A *nomen novum* of *A. florensis* has been provided in place of *A. pectinata* Veron, 2000.

Etymology: I have chosen the name to reflect the Indonesian Lesser Sunda Island of Flores of which the holotype specimen was collected.

Acropora sinensis (Brook, 1893) status revised

Madrepora sinensis Brook, 1893: p. 114, Plate XXXIII. fig. C

Specimens examined: NHM: 1870.5.9.12., *Madrepora sinensis* syntype, Taiwan

Remarks: See remarks under taxonomic account (above). Species resurrected from synonymy with *A. hyacinthus* contra Veron & Wallace (1984).

Distribution: Species is only known to occur in Taiwan & Southern China according to the original description, with further research required to establish environmental and geographic range.

Note: Original description indicates some affinity of this species to *Madrepora spicifera* group, while Veron & Wallace (1984) synonymized based on clear resemblance to *Acropora hyacinthus*.

Acropora bifurcata Nemenzo, 1971 status revised

Acropora bifurcata Nemenzo, 1971: p. 147, Plate 2, fig. 1 & 2.

Specimens examined: UP: U.P.C.-1295, *Acropora bifurcata* holotype, Mindoro, Philippines; MTQ: G38015 Akajima, Japan; G53822 Tukangbesi Is, Indonesia; G47300, G50038 Sulawesi, Indonesia; G50045, G50050 Kalimantan, Indonesia; G41545, G48512 Sumatra, Indonesia

Remarks: This most recent reference of this species as a synonym of *A. hyacinthus* was from Veron & Hodgson (1989), while earlier revisions listed this as a nominal species (Veron & Wallace 1984: p. 141) while omitting this species from the taxonomic account. In later (1999) revisions of *Acropora* Wallace indicated uncertainty about the species status and listed this species as a potential junior synonym of *A. hyacinthus* (Wallace 1999: p. 14 "?j.s. *A. hyacinthus*"), citing 'insufficient available information to resolve' the species status. No clarification was provided by the 2012 revision of *Acropora* (Wallace, Done & Muir) where this species was omitted from the publication. Then, Veron (2000) published this species in the popular book Corals of the World, citing Wallace (1999) as a source of taxonomic confusion. Here, contra Veron & Hodgson (1989) p. 247 I remove this species from synonymy with *A. hyacinthus* (Dana 1846).

Description: The specimens in the current study from Okinawa in Japan most closely resemble the holotype specimen, displaying the slightly terete branchlets with blunt axial tip, bifurcating lip of the radial corallites and tight anastomosing mesh of the branches which fan out horizontally. Specimens from Western Australia (WA18) and Malaysia (145-0344) differ only by the colony morphology, being a wide anastomosing branching pattern instead of the tight mesh described by Nemenzo (1971). This wide branching pattern is a common feature of deep colonies throughout the tabulate *Acropora* clade.

Distribution: Here confirmed to occur in Western Australia, Malaysia, Phillipines and Okinawa Japan.

Notes: The original description remarks on resemblance to *A. pectinata* (Brook, 1892), which occurs as a close relative of this species with a similar morphology in the current phylogeny. While it is clear that neither of these nominal taxa are *A. hyacinthus* of which they were synonymized, I do encourage further investigations into the boundaries – if evident – between *A. pectinata* and *A. bifurcata*. Additionally, specimens 18Oki32, 33 & 34 in the current study were initially included in Ramírez-Portilla et al. (2021) and identified as *A.* cf. *bifurcata* by the authors due to morphological assessment. Here, I verify their assessment by confirming the identification of these specimens in the current study as *A. bifurcata* (Nemenzo 1971).

2.7 Acknowledgements

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2.8 Supplementary Material

2.8.1 Supplementary Figures

Figure S2.1 Maximum Likelihood Phylogenetic reconstructions of both edge and internally trimmed alignments at both 50% and 75% complete matrices each. Node support displays Ultra-Fast bootstrap results, gene concordance factors and site concordance factors respectively. Node tips are labelled with the Primary Species Hypothesis of each specimen, and the individual voucher numbers. **Note:** Figure S2.1 can be found in **Appendix Chapter 2 Figures.**

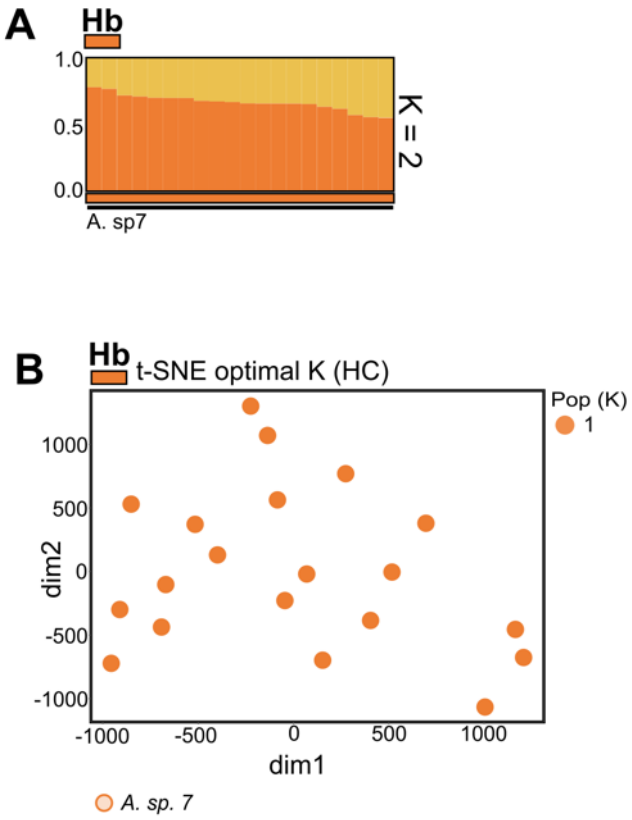

Figure S2.2 Results from SNP species delimitation analysis for clade Hb showing A) STRUCTURE analysis (K = 2) Bars are coloured according to majority ancestry. As cluster cannot resolve K = 1 Ifind these results to depict a single population. And B) Results from the t-SNE analysis showing clustering of specimens according to most likely population (K = 1) determined by Hierarchical Clustering Analysis.

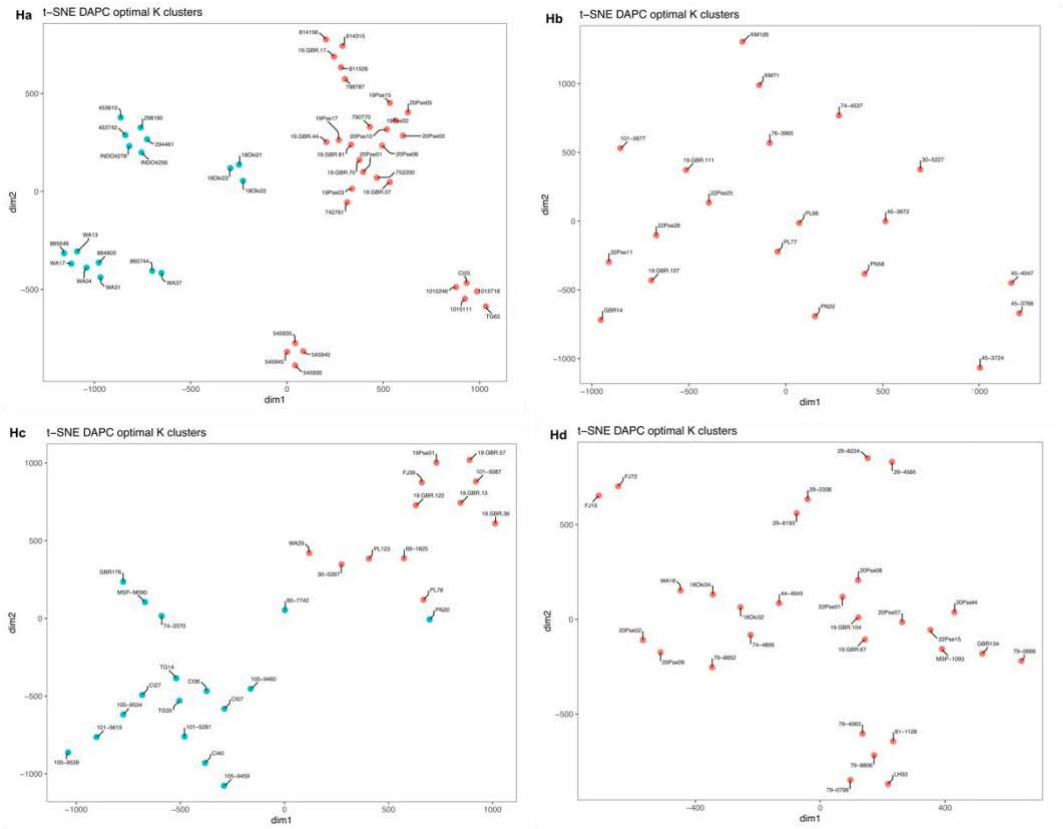

Figure S2.3 t-SNE plots showing results of DAPC analysis for all four subclades (Ha – Hd). Each circle represents one specimen, labelled.

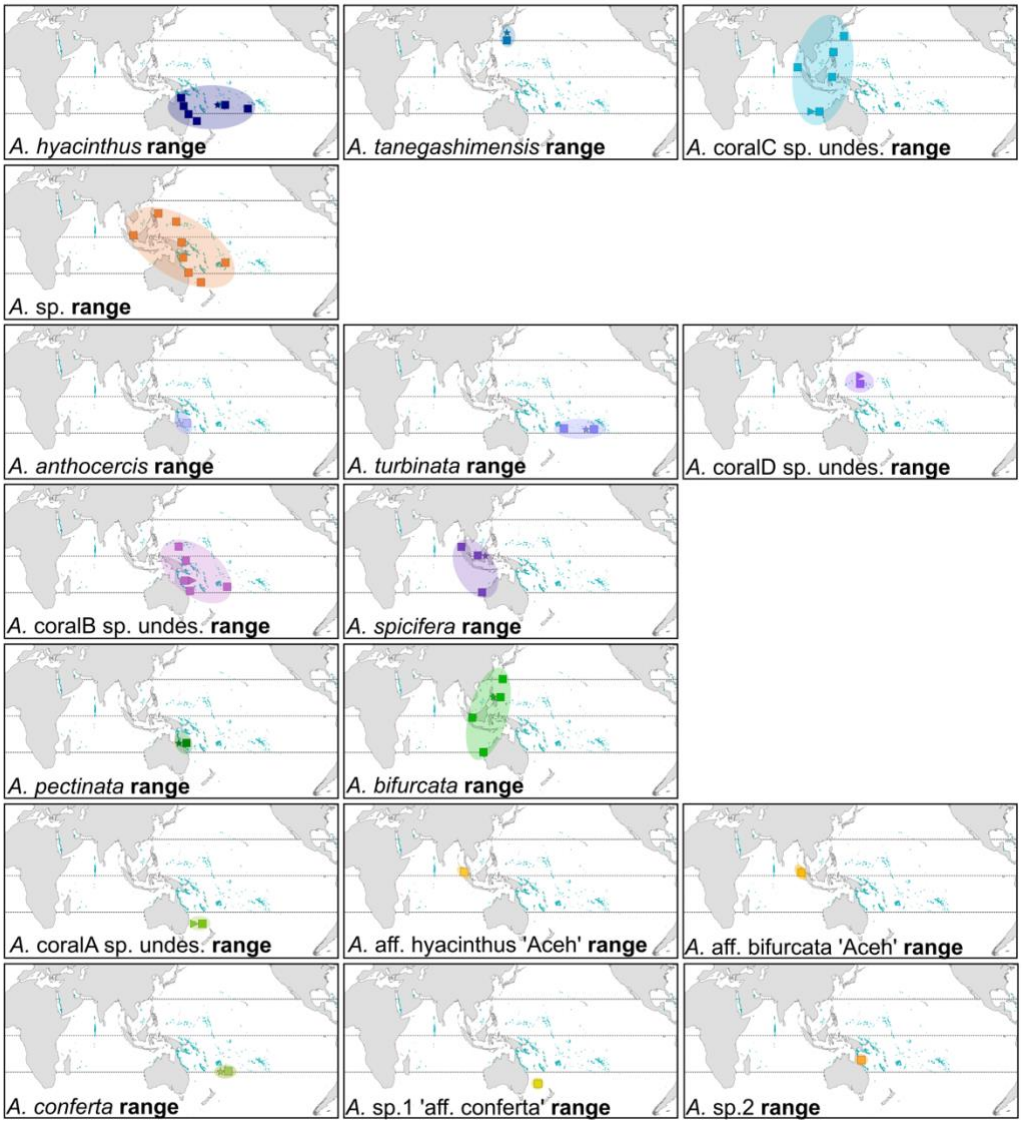

Figure S2.4 Individual global ranges for each of the species in the current study. Coloured squares indication locations where specimens were sampled from, and highlighted area depicts hypothesised range based on sampling effort.

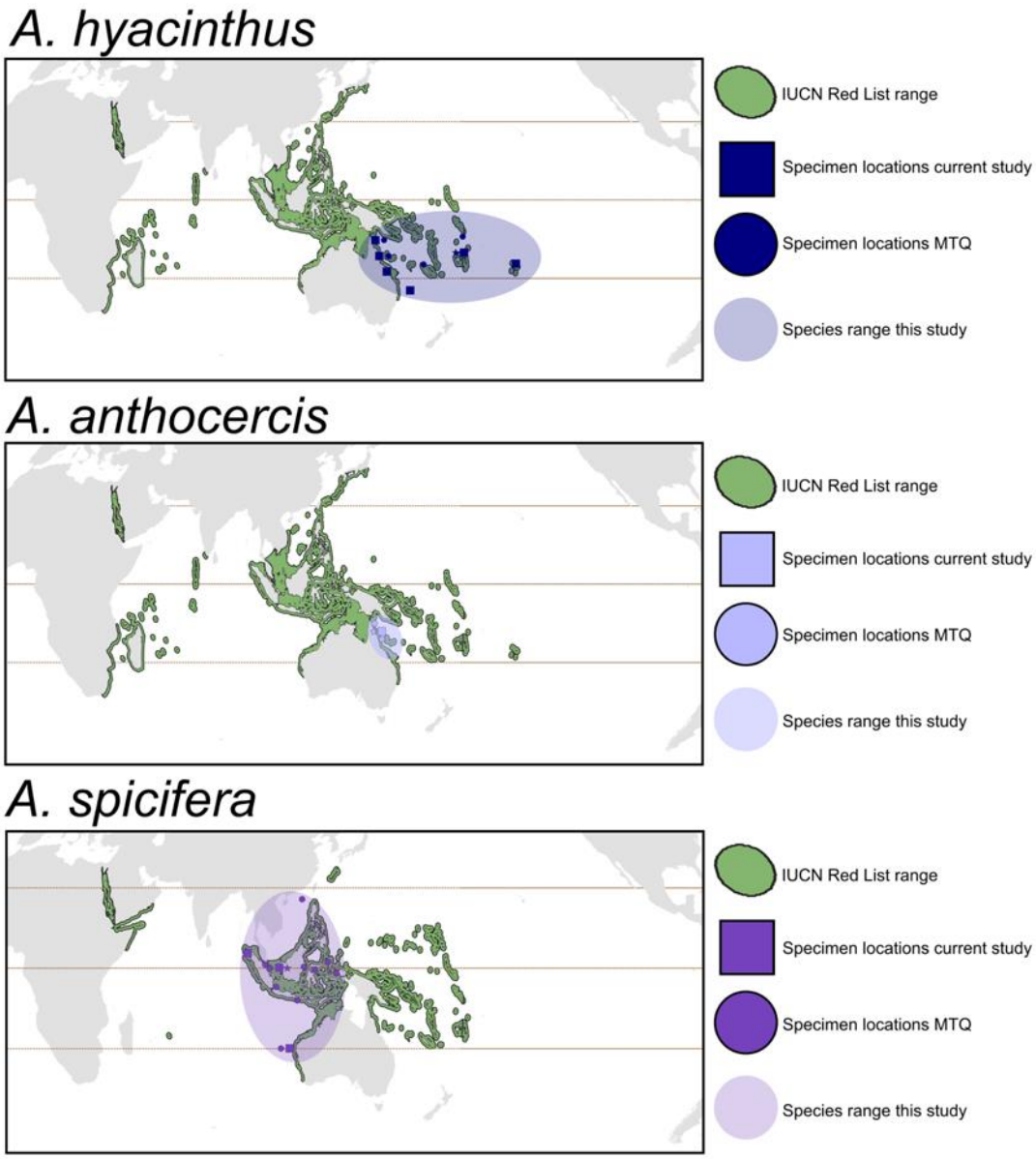

Figure S2.5 Distribution maps showing the species ranges according to the current study, and the assumed ranges from the International Union for the Conservation of Nature (IUCN) Red List. The three *Acropora* species listed are the only species from the current study with IUCN Red List assessments.

2.8.2 Supplementary Tables

Table S2.1 Nomenclature for all nominal species of *Acropora* with tabular morphologies or which have been included in the present study due to

morphological or phylogenetic similarities to *A. hyacinthus*. **Note:** Table S2.1 can be found in **Appendix Chapter 2**.

Table S2.2 Specimen collection metadata and species status according to the most recent taxonomic revisions for all specimens included in the current study. **Note:** Table S2.2 can be found in **Appendix Chapter 2.**

Table S3 Population Models used for input into for Bayes Factor Delimitation with genomic data analysis for clades Ha, Hb and Hc.

subclade Ha		
pA	K = 5	populations assigned according to STRUCTURE (K = 5) results.
pB	K = 4	populations assigned according to Primary Species Hypothesis. A. coralC sp. undes. split into geographic regions.
pC	K = 3	populations assigned according to Primary Species Hypothesis.
pD	K = 2	populations assigned according to STRUCTURE (K = 2) results.
subclade Hc		
pA	K = 5	populations assigned according to Primary Species Hypothesis. (A. anthocercis, A. turbinata, A. coralB sp. undes., A. spicifera, A. coralD sp. undes.)
pB	K = 2	populations assigned according to STRUCTURE results. A. spicifera & A. coralB sp. undes. combined, A. turbinata, A. anthocercis & A. coralD sp. undes. combined
pC	K = 4	populations assigned according to Primary Species Hypothesis (pA), with A. turbinata & A. anthocercis combined into one.
subclade Hd		
pA	K = 8	populations assigned according to Primary Species Hypothesis.
pB	K = 3	populations assigned according to STRUCTURE (K = 3) results.
pC	K = 5	populations assigned according to STRUCTURE (K = 5) results.

Table S2.4 Glossary of all morphological colony traits that were analysed in the current study. **Note:** Table S2.4 can be found in **Appendix Chapter 2.**

Table S2.5 Raw data from target capture of UCE/exon loci for each specimen in the current study.

Specimen ID	# trimmed reads	contigs	total bp	mean length
FJ15	5,538,512	1157	1676585	1449.07952
FJ72	5,613,146	1122	1283073	1143.55882
79-0666	7154693	1139	1620409	1422.65935
79-8852	4535473	1409	1706843	1211.38609
75-4658	3961066	1230	1134355	922.239837
GBR134	7701335	1039	1067420	1027.35322
LH33	9805287	1085	1349805	1244.05991
81-1128	5199320	1169	1493962	1277.98289
LH31	1597550	1177	907609	771.120646
79-0798	7067258	1060	1222220	1153.03774
79-4063	5689027	1235	1602354	1297.45263
79-8806	7360504	1305	1656598	1269.42375
29-8234	2919620	1178	1113861	945.552632
29-4585	971251	1298	1347332	1038.00616
29-2338	7366760	1245	1542744	1239.15181
29-8193	4369337	1545	1398986	905.492557
WA18	6141769	1233	1436078	1164.70235
145-0344	1049535	1407	1074939	763.993603
44-4668	4945636	1174	1357985	1156.71635
18Oki32	6511584	1246	1370824	1100.17978
18Oki34	2813047	1102	1152691	1045.99909
18Oki33	1168557	1289	1049689	814.343677
19.GBR.25	1000507	1205	972033	806.66639
20Pse07	7158774	1110	1390358	1252.57477
22Pse01	7566658	1143	1277803	1117.93788
20Pse09	5471583	1141	1690711	1481.78002
20Pse02	9501244	1072	1324510	1235.55037
19.GBR.67	6465750	1119	1254419	1121.01787
74-4895	823857	1161	1017429	876.338501
20Pse04	9812235	1155	1966617	1702.6987
19.GBR.104	6183459	1105	1350930	1222.56109
20Pse08	7538215	1094	1366558	1249.13894
22Pse15	8139382	1137	1186720	1043.72911
19.GBR.112	2012746	1034	1028619	994.795938
WA29	9426900	1086	1639939	1510.07274
PN02	738358	1397	911919	652.769506
145-0335	2432280	1220	1100359	901.933607
29-8257	3707855	1409	1185481	841.363378
30-5267	5462998	1406	1196225	850.800142
19.GBR.57	8727682	1198	1563515	1305.10434
19.GBR.13	7161463	1228	1598304	1301.55049
19.GBR.36	3213951	1086	1222365	1125.5663
19Pse01	8470084	1130	1352521	1196.92124
69-1825	960252	1203	1198933	996.619285
19.GBR.122	6841639	1184	1628116	1375.09797
74-4900	373617	1506	1232993	818.720452

19.GBR.76	1622363	1045	995663	952.78756
101-5087	8390173	1216	1655039	1361.05181
FJ39	6393372	1190	1440460	1210.47059
PL123	7256642	1117	1218327	1090.71352
PL78	783587	1201	1072706	893.177352
65-7742	10157099	1165	1251510	1074.25751
PN20	2979891	1204	1267007	1052.3314
GBR176	11646606	984	1050444	1067.52439
74-2370	10178964	1133	1369420	1208.66726
74-1872	593430	1274	1101491	864.592622
TG14	6675857	1032	944549	915.260659
CI36	6438860	1108	1361481	1228.77347
105-9534	14105724	974	1150856	1181.577
CI07	6069559	1063	1181257	1111.24835
101-5281	4896802	1035	977007	943.968116
105-9539	11374413	1028	1269014	1234.44942
105-9460	8921756	1011	1154108	1141.55094
CI40	9789013	1032	1156409	1120.55136
CI27	6359108	1072	1313389	1225.17631
105-9459	9320857	1002	1293381	1290.7994
TG35	8835720	1110	1378021	1241.46036
101-5619	8903396	1035	1389922	1342.91981
TG25	379136	1511	1136641	752.244209
TG7	2330869	1149	991664	863.067015
LH29	1860755	1139	953109	836.794557
KM126	13298097	1044	1360610	1303.26628
KM71	12476777	1088	1559996	1433.81985
101-5677	4635109	940	1028873	1094.54574
30-5227	5437177	1329	1042400	784.349135
45-4047	10052658	1075	1182810	1100.28837
45-3768	7629319	1123	1323808	1178.81389
45-3672	8420262	1070	1400914	1309.26542
45-3724	8222008	1107	1323873	1195.91057
PN22	4454507	1254	1463925	1167.40431
PN58	8911613	1168	1357239	1162.01969
PL77	3587097	1095	1123227	1025.77808
PL66	3860628	1080	1305862	1209.13148
76-3965	6628643	1088	1232027	1132.37776
75-2321	644517	1281	1085359	847.274785
22Pse25	9132337	1066	1292972	1212.91932
74-4537	3900633	1364	1163915	853.310117
19.GBR.111	5277918	1223	1677677	1371.77187
GBR14	10611409	1115	1353436	1213.84395
19.GBR.107	5405114	1106	1388492	1255.41772
22Pse28	8293809	1059	1184415	1118.42776
22Pse11	7145495	1122	1213580	1081.6221
54-5930	6239435	1151	1356832	1178.82884

54-5935	5135551	1157	1295168	1119.41919
54-5940	6615113	1163	1470783	1264.64574
54-5945	5319144	1168	1463884	1253.32534
18Oki21	3102051	1079	1084329	1004.93883
18Oki22	3127926	1127	1137447	1009.26974
18Oki23	1683133	1280	1119018	874.232813
INDO4278	10415623	1090	1220495	1119.72018
INDO4256	7499420	1108	1185467	1069.91606
45-3742	9500850	1113	1305340	1172.81222
29-8190	3988157	1226	1368873	1116.53589
29-4461	2307579	1115	1132294	1015.51031
45-3610	8120946	996	1021385	1025.48695
WA13	9216946	1190	1495187	1256.45966
88-5046	14997213	1106	1615307	1460.49458
WA17	6483505	1148	1308443	1139.75871
88-4803	3539198	1375	1405704	1022.33018
WA04	2283438	1160	1082262	932.984483
WA31	622561	1458	1031225	707.28738
86-5744	856693	1332	1073315	805.792042
WA37	1701698	1171	1034350	883.304868
19.GBR.17	5645922	1153	1471998	1276.66782
19.GBR.07	6157796	1071	1389380	1297.27358
81-1526	5241293	1174	1368041	1165.28194
81-4156	7305712	1144	1283402	1121.8549
81-4315	5977964	1127	1386930	1230.63886
101-5111	6833306	1181	1586820	1343.62405
101-5246	5118201	1117	1237293	1107.69293
101-5718	7338912	1070	1246097	1164.57664
TG63	629303	1367	1216434	889.85662
CI25	4655299	1179	1341653	1137.95844
79-8787	7294062	1182	1672401	1414.89086
19.GBR.44	6382790	1163	1506858	1295.66466
19.GBR.70	7145098	1083	1192204	1100.83472
19.GBR.81	6550949	1086	1248185	1149.34162
79-0770	7947207	1022	1136487	1112.0225
75-2200	1456984	1065	1091148	1024.55211
74-2761	6697543	1062	1256861	1183.48493
19Pse03	9458399	1072	1182889	1103.44123
19Pse15	3959191	1070	1261203	1178.69439
20Pse06	10063943	1101	1324260	1202.77929
20Pse03	10357147	1049	1136355	1083.27455
19Pse17	9920289	1103	1312658	1190.07978
20Pse01	9383687	1075	1220196	1135.06605
19Pse02	10464384	1065	1390593	1305.72113
20Pse05	9019443	1051	1282731	1220.4862
20Pse10	8449632	1100	1307147	1188.31545
KA0025	9290980	1266	1725295	1362.79226

KA0089	10270637	796	961694	1208.15829
05-1449	6192671	1216	1348681	1109.11266
05-1453	6266162	1388	1652649	1190.66931
29-2324	7433393	746	789578	1058.41555

Table S2.6 Results for Bayes Factor Delimitation with genomic data (BFD*) for subclades Ha, Hc & Hd. Models with the highest support, indicated by the highest Bayes Factor (BF) are highlighted in green.

	MODEL	#_species	Marginal_L_Estimate	MLE_Rank	BF
Ha	**pA**	5	-28796.8759	1	-1675.586781
	pB	4	-28970.98098	2	-500.6267441
	pC	3	-29221.29435	3	
	pD	2	-29634.66929	4	826.7498819
	MODEL	**#_species**	**Marginal_L_Estimate**	**MLE_Rank**	**BF**
Hc	**pA**	5	-12733.88838	1	-899.0868915
	pB	2	-13183.43182	3	899.0868915
	pC	4	-12813.41699	2	159.0572269
	MODEL	**#_species**	**Marginal_L_Estimate**	**MLE_Rank**	**BF**
Hd	**pA**	8	-13625.71839	1	-1782.654273
	pB	3	-14517.04552	3	1782.654273
	pC	5	-14369.15934	2	1486.881898

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Chapter 3

Biogeography of tabular *Acropora*-Symbiodiniaceae associations along the Great Barrier Reef

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3.1 Abstract

Aim: Rapidly advancing molecular technologies are evolving our understanding of coral-algal symbiosis, leading to enhanced resolution of both coral taxonomy and that of their endosymbiotic algae of the family Symbiodiniaceae. In this study, our aim was to provide a first look into the patterns of host-Symbiodiniaceae specificity along the length of the Great Barrier Reef, investigating associations amongst closely related tabular *Acropora* coral species and testing for host, reef, and environmental factors in driving biogeographical patterns. **Location:** Offshore reefs along the entire latitudinal gradient of the Great Barrier Reef. **Taxon:** Tabular morphology *Acropora* corals and their associations with endosymbiotic algae of the family Symbiodiniaceae. **Methods:** Specimens of tabular *Acropora* representing six closely related coral taxa were sampled from thirteen reefs spanning the latitudinal gradient of the Great Barrier Reef. Next-generation sequencing of the ITS2 marker was performed and through the *SymPortal* analytical framework we assessed diversity of Symbiodiniaceae associations across host taxa, latitudinal gradient, and environmental variables. **Results:** Amongst the six closely related coral taxa we found diverse Symbiodiniaceae associations, with patterns of dominant *Cladocopium* and rarer *Symbiodinium* and *Durusdinium* ITS2 Type Profiles. Host specificity was low, with common ITS2 Type Profiles occurring across multiple host taxa. We revealed a geographic-specific algal association in the southern Great Barrier Reef, in line with cooler temperature maximums in these higher latitude regions. **Main conclusions:** Our study represents the first large scale study of closely related tabular *Acropora* species according to the new taxonomic framework. We discovered a unique symbiont community common in the southern region of the

Great Barrier Reef, revealing biogeographic structuring of tabular *Acropora*-Symbiodiniaceae associations. Patterns of symbiont associations across taxa also highlighted the potential for different thermal thresholds, an important factor for management and intervention when considering future predicted reef conditions.

3.2 Introduction

Advances in molecular-based taxonomy have transformed our understanding of the diversity of both reef corals (Kitahara et al. 2016) and their algal endosymbiont (Family: Symbiodiniaceae LaJeunesse et al. 2018) in terms of resolved genera (LaJeunesse et al. 2018, 2022; Nitschke et al. 2020) and species (e.g. Hume et al. 2019; Turnham et al., 2021; Butler et al, 2023). Improved resolution of Symbiodiniaceae diversity is enhancing our understanding of their ecology and physiology and in turn how this relates to host specificity (Johnston et al. 2022; Davies et al. 2023). Notably, the specificity – or not – of symbiont associations continues to prove central in regulating the severity with which corals can resist anomalously stressful conditions, including heat stress susceptibility of adults (Berkelmans & van Oppen 2006; Sampayo et al, 2008; Claar et al, 2020) and larvae (Gómez-Cabrera et al, 2008; Matsuda et al. 2021; Yoshioka et al, 2022), but also persistence of corals across different reef environments (Camp et al., 2019). Thus, changes in symbiont associations likely facilitate long term persistence of coral populations across gradients of environmental conditions and represent fine-tuning of host-symbiont metabolic compatibility (e.g. Haydon et al. 2023) where environments begin to limit emergent fitness properties such as growth (Turnham et al. 2023).

Patterns in Symbiodiniaceae species distribution are driven by host taxa (LaJeunesse et al. 2004; Hume et al. 2020; Osman et al., 2020; Turnham et al, 2021), geographic location (LaJeunesse et al. 2004; Reimer et al., 2017; Terraneo et al., 2019), and environmental conditions (Camp et al. 2020; Haydon et al, 2021; Leinbach et al, 2023). For example, the conditions of the Red Sea – a region characterised by extreme temperature and salinity gradients (Terraneo et al. 2019) – drive shifts in symbiont communities across temperature gradients for some (but not other) zoanthids (Reimer et al. 2017) and hard and soft coral (Terraneo et al. 2019, Osman et al. 2020) species, often attributed to the cooler temperature maximums in the northern regions of this system. In the northwest Pacific, a longitudinal gradient across the Micronesian archipelago identified unique *Cladocopium* (formerly clade C; LaJeunesse et al. 2018) communities both within and across islands for two *Acropora* coral species (Davies et al. 2020), revealing both host specificity and local scale structure which may improve host fitness along environmental gradients. Similarly, symbiont identity varied for the coral *Favia gravida* Verrill 1868 across the species range in the Atlantic (Teschima et al. 2019), a region of relatively low coral diversity by comparison to the Indo-Pacific.

The Great Barrier Reef (GBR) spans a latitudinal distance of ~2,000km (a range of 14^{o} latitude) and represents an ideal study system for exploring coral-algal symbiosis specificities (e.g., Quigley et al. 2022). Relatively early studies exploring symbiosis for corals and/or other marine invertebrates across the GBR (van Oppen et al. 2001; LaJeunesse et al., 2004, Cooper et al. 2011) and within regions of the GBR (Tonk et al. 2014, 2017) highlighted the roles of geography, host species, sea surface temperature (SST) or water quality in explaining

Symbiodiniaceae community compositions. However, most studies since have focused on more locally specific coral-Symbiodiniaceae associations, e.g. specific reef sites (Quigley et al. 2019; Damjanovic et al. 2020; Grima et al. 2022) or ecological niches (Camp et al. 2019; Haydon et al. 2021). Recent work from the central to northern region of the GBR (~900 km) pre- and post-bleaching for three *Acropora* species identified restructuring of symbiont composition in response to bleaching episodes (Quigley et al. 2022); specifically, finding some taxa are more adaptive in symbiont uptake and recovery than others after these stressor events. Collectively, these data highlight the importance of individual taxa, spatial and temporal scales, and environmental 'stability' in structuring Symbiodiniaceae communities for GBR corals – and hence how understanding symbiont association flexibility is critical to inform future species prevalence in the face of continually changing environments and climates (Ainsworth et al. 2016; Quigley et al. 2022).

Acropora is the most abundant coral genus on the GBR, with tabular *Acropora* identified as disproportionately key taxa for maintaining ecosystem function (Linares et al. 2011; Oritz et al., 2021). However, tabular *Acropora* species are amongst the most susceptible to stressor events, including heat-induced mass bleaching mortality (Hughes et al. 2017; Brodnicke et al. 2019; Sakai et al. 2019). Tabular *Acropora* show spatially variable bleaching patterns on the GBR (Marshall & Baird 2000; Hoogenboom et al. 2017; Brodnicke et al. 2019) and elsewhere in the Indo-Pacific (Gold & Palumbi 2018; Morikawa & Palumbi 2019; Cornwell et al. 2021). Patchiness in bleaching response has been linked to host-Symbiodiniaceae associations and abundance (Cornwell et al. 2021) as well as micro-environments and thermal refuge (Baird et al. 2018; Gardner et

al. 2019; Cheung et al. 2018) and has persisted in the recovery response of tabular *Acropora* across the GBR (Linares et al 2011; Johns et al. 2014). Indeed, recent reports on post-bleaching responses of *Acropora* found that *A. hyacinthus* (Dana, 1846) was less flexible in shuffling or switching symbiont communities in response to environmental changes compared to *A. millepora* (Ehrenberg, 1834), indicating that these adaptive mechanisms may be taxa specific and not as common as prior indications (Quigley et al. 2022).

Threats to ecologically important tabular *Acropora* has led to a call for focused management and protection of this group on the GBR (Oritz et al., 2021). Their high ecosystem value, as well as their high perceived aesthetic value for tourism often places them as key targets for active management approaches such as coral restoration (Morikawa & Palumbi 2019), including on the GBR following the 2016/17 mass bleaching event (Howlett et al. 2021, 2022). However, as with other *Acropora,* the taxonomy of this group remains in a state of revision, confounding effective study and integration of data for these taxa across space and time.

To date, studies of tabular *Acropora* symbioses across the Pacific tend to focus on *A. hyacinthus*, which is now known to represent a complex of different species lumped together on the basis of morphological characters (Ladner & Palumbi 2012; **Chapter 2**). Whilst recent taxonomic work has begun to resolve individual species of tabular *Acropora* and is leading to new species being described from the GBR (e.g., *A. coralB* **Chapter 2**), we are yet to resolve host-symbiont specificity of these corals and explore the influence of closely related host taxa on Symbiodiniaceae distribution. As such, it is plausible that the inability to identify individual coral species within this complex may explain past

patchiness observed in bleaching response (Marshall & Baird 2000; Hoogenboom et al. 2017; Brodnicke et al. 2019; Morikawa & Palumbi 2019; Cornwell et al. 2021) and recovery (Linares et al 2011; Johns et al. 2014; Quigley et al. 2022) with at least five morphologically similar and closely related tabular *Acropora* species of the 'hyacinthus' group occurring on the GBR (**Chapter 2**). Many acroporid corals obtain their symbionts from the surrounding environment, i.e., 'horizontal transmission' (Baird et al. 2009; Nitschke et al. 2016; Davies et al. 2020; Leinbach et al, 2023), and form stable associations across time (Epstein et al, 2019; Quigley et al. 2022). In turn *Acropora* tend to be symbiont generalists (Lewis et al. 2022; Leinbach et al, 2023), with environment and geography playing an important role in structuring the symbiont composition (LaJeunesse et al, 2003; Kriefal et al. 2022; Leinbach et al, 2023). Given the recent advances in the capacity to delineate species of tabular *Acropora* hosts previously lumped as a single species across the GBR (**Chapter 2**), there is now a clear gap in knowledge in the intra- and inter- specific coral-Symbiodiniaceae associations of these keystone taxa.

Here, we explored the coral-symbiont associations of closely related tabular *Acropora* species according to the new taxonomic framework (Cowman et al, 2020; **Chapter 2**) along the entire latitudinal gradient (14^{o} latitude, > 2,000km) of the GBR. Samples of tabular *Acropora* (n = 91) were collected from 13 reefs along the gradient and the Internal Transcribed Spacer 2 (ITS2) region of the algal symbionts was analysed to identify Symbiodiniaceae type sequences and profiles. ITS2 type profiles indicative of Symbiodiniaceae species were explored to determine symbiont associations amongst *Acropora* species and along a geographical gradient. In addition, environmental regimes from the site of sample

collection were analysed to resolve patterns across taxa and geographic region. In doing so, we show that spatial scales – aligning with temperature gradients - are important in driving coral-algal symbiosis and discuss the importance of understanding host taxa flexibility to associate both across and within reefs when considering future reef conditions and management approaches.

3.3 Materials and Methods

3.3.1 Sampling and Species Identification

Colonies (n= 91) of tabular *Acropora* were sampled by SCUBA across 13 reefs along the latitudinal gradient of the GBR between August and December 2019 (n = 66) and February 2020 (n = 25; Supplementary Material, Table S3.1). Sampling in 2019 involved collecting representatives of the tabular diversity encountered at each location, thus, resulting in an uneven sample size across sites and species (Fig. 1). Sampling in 2020 was performed at Opal Reef in the Northern GBR (16° 12′37.62″ S, 145° 52′52.752″ E) with sampling performed to capture a minimum of 20 colonies of tabular *Acropora* at a single reef site that were >5cm in diameter. All colonies were photographed *in situ* (Olympus TG5) to aid in later taxonomic assignments. In 2019, colonies were sampled by collecting an approximately $5cm^2$ voucher fragment from each colony, of which a 1-2cm tissue fragment was further taken and stored in 99% ethanol at a minimum 4^oC for later molecular analysis. The remaining voucher fragment from each colony was bleached in sodium hypochlorite for a minimum for 36h to remove remaining tissue and subsequently air dried. These voucher fragments were photographed and deposited to the coral collection at the Museum of Tropical Queensland, Townsville Australia (MTQ). In 2020, colonies were sampled by collecting a

~10cm fragment which was immediately frozen in liquid N_2 and stored in a dry shipper. Due to permitting restraints on this expedition, voucher fragments were unable to be collected and *in situ* images were utilised for species identification.

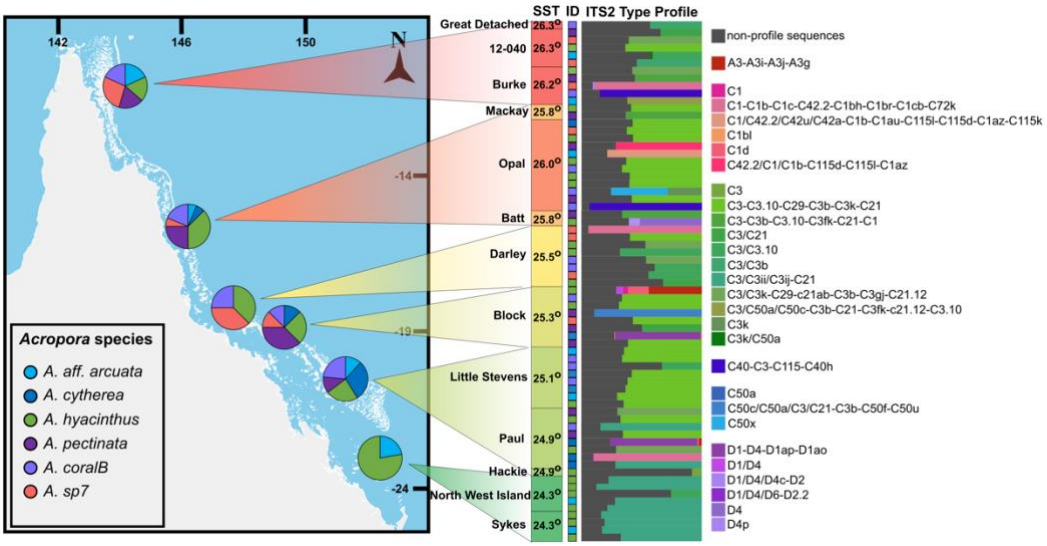

Figure 3.1 Map of Great Barrier Reef with sampling locations and proportion of each tabulate *Acropora* species sampled per Ecoregion. Starting from the northernmost locations, we sampled in the following Ecoregion; Lockhart (n = 11), Cairns (n = 16), Townsville & Cape Upstart (n= 8), Whitsundays & Townsville (n = 8), Pompey & Swains (n = 17) & Capricorn Bunkers (n = 9). Expanded to the right is the name of each individual reef sampled and the 2019 average Sea Surface Temperature (SST) derived from the National Aeronautic and Space Administration (NASA) Giovanni website (see methods). Columns to the right represent species identification (ID) and the ITS2 Type Profiles obtained from Symportal, with each row representing a single colony.

Due to known cryptic morphologies and unresolved taxonomy for tabular *Acropora* (Palumbi 2012; **Chapter 2**), colonies were identified to species level based on the current taxonomic knowledge. An open nomenclature system was utilised to identify colonies that had morphologies with affinity to (aff.) known species where taxonomic identity remained uncertain (*sensu* Cowman et al., 2020). A subset of the samples previously identified by molecular and taxonomic

assessment in **Chapter 2** (n = 15, Table 1.) were used as reference individuals for species identification.

3.3.2 DNA Extractions and Sequencing

To explore genetic diversity and identity of Symbiodiniaceae associated with host corals we extracted algal DNA and sequenced the internal transcribed spacer 2 (ITS2) region of the genome (Arif et al. 2014). DNA was extracted using the Qiagen DNeasy Plant Pro Kit (Qiagen, Germany) following the manufacturers protocol. Ethanol preserved samples were prepared for DNA extraction by lightly brushing the fragments in a 5mL vial filled with ethanol to free the tissue from the skeleton. The skeletal fragment was then removed from the vial and the remaining solution centrifuged to separate the tissue from the ethanol, which was discarded leaving a cell pellet remaining for the proceeding extraction. Frozen samples were prepared by air picking fragments in 10µl of Phosphate-Buffered Saline (PBS) buffer (see Grima et al., 2022) and DNA extraction followed the Qiagen DNeasy Plant Pro Kit (Qiagen), with minor modifications as previously described in Grima et al. (2022).

Amplification of the ITS2 regions was performed using primers ITS2intfor2 (LaJeunesse et al. 2000) and ITS2-reverse (Coleman et al. 1994) following the PCR conditions of Arif et al., (2014). PCR products were run on a 1% agarose gel to visualise successful amplifications. Samples were sent to the Australian Genome Research Facility (AGRF) for sequencing of the ITS2 region on the Illumina MiSeq (2 x 300 bp) Next Generation Sequencing (NGS) platform.

Resulting NGS ITS2 sequences were processed through the *SymPortal* analytical framework (Hume et al., 2019). The *SymPortal* framework resolves

putative Symbiodiniaceae taxa using the NGS sequencing data of the ITS2 amplicon. Briefly, the framework identifies informative intragenomic sequences referred to as 'defining intragenomic variants' (DIVs) which are used to identify 'ITS2 type profiles' which represent putative Symbiodiniaceae species (Hume et al., 2019). Initial quality control of sequences was performed within the *SymPortal* pipeline using Mothur 1.39.5 (Schloss et al. 2009) the BLAST+ suite of executables (Camacho et al. 2009) and Minimum Entropy Decomposition (Eren et al. 2015). *SymPortal* provides both sequence and ITS2 type profile count tables with absolute and relative abundances. These data matrices were filtered for quality control to remove samples that failed to meet a minimum sequence threshold (> 1,000 sequences) and to remove outlier sequences that associated with Symbiodiniaceae genera *Effrenium* and *Breviolum*. The genus *Effrenium* is currently only known to contain one free-living non-symbiotic species (LaJeunesse et al., 2018) and was only found here to associate with one host coral at low sequence depth we ascribe its presence as an artefact of sampling contamination. Similarly, the genus *Breviolum* is uncommon in Pacific acroporid corals (LaJeunesse et al, 2018) and was only found to associate in low sequence depth with two host corals (19.GBR.108 & 51, Supplementary Material, Table S3.1) indicating this too may be present due to sampling contamination.

3.3.3 Environmental Data

To determine if different environmental regimes explained shifts in coral-algal symbiosis associations, we explored three abiotic variables shown to influence coral-algal physiology; night-time SST to track the thermal temperature ranges and anomalies at each reef, Diffuse attenuation at 490nm (K_d490) which

provides a measurement of light penetration through the water column (an essential property for biogeochemical processors of coral reefs and key for maintained photosynthesis of Symbiodiniaceae (Hochberg et al. 2020)), and Chlorophyll-*a* concentration (Chl-*a*), which is used as a proxy for water quality (Hughes et al. 2017). Satellite-derived data was downloaded in July 2021 from the National Aeronautic and Space Administration (NASA) Giovanni website (Acker & Leptoukh, 2007), developed and maintained by the NASA Goddard Earth Sciences Data and Information Services Centre (NASA GES DISC) for each sampled reef between January 2003 – December 2020 with an 8-day temporal and 4km spatial resolution from the MODIS-Aqua database. To determine the prevailing environmental conditions at each reef over this 18-year period we computed monthly averages for each year and extracted the 18-year combined averages and standard deviations for each month allowing us to examine the distribution of the three abiotic variables (Supplementary Material, Fig. S3.1-S3.3). Delta variables and regressions were computed to compare data for 2019 – i.e., the year immediately preceding the sample acquisition here and therefore the immediate environmental history – relative to that for the combined 18-year average for each reef to ensure there was no significant difference in the 2019 data compared to the 18-year mean conditions. This was to ensure that the conditions present in the year leading up to sampling was comparable to standard environmental conditions over the 18-year period. As no significant deviations in 2019 conditions – when compared to the prevailing 2003-2020 conditions – were identified, we continued with the 2019 data for our analysis. Raw environmental data can be found in the Supplementary Material (Table S3.3).

3.3.4 Data Analysis

To determine the factors that influence the Symbiodiniaceae community distribution amongst tabular *Acropora* corals in the GBR we performed several statistical analyses across geographic parameters, host species diversity, and abiotic environmental measures. As our dataset involved an uneven number of samples per location throughout the latitudinal gradient, we categorised and statistically analysed our samples according to two different scales. The first scale was Reef, being the location where samples were collected (13 reefs, Supplementary Material, Table S3.1) and the second scale was Ecoregion, which combined reefs within marine management area boundaries as defined by the Great Barrier Reef Marine Park Authority (GBRMPA) which aggregated the 13 reefs into six Ecoregions (Supplementary Material, Table S3.1).

All statistical analyses were performed in RStudio v4.2.3 (R Core Team 2020). To explore the significance of latitude on Symbiodiniaceae composition along the length of the GBR, and to test for Symbiodiniaceae-host specificity we performed a Permutational Multivariate Analysis of the Variance (PERMANOVA) on Bray-Curtis distance matrices using the R package vegan v2.6-4 (Oksanen et al., 2022). Specifically, we input the datasets for the ITS2 sequence and ITS2 type profiles (relative abundances for each obtained from *SymPortal*) against three metrics, being Reef (n= 13), Species (n= 6) or Ecoregion (n= 6). All factors in the analysis were fixed and orthogonal. As our dataset involves an uneven number of samples and a range of species collected from each location, PERMANOVA was chosen for this analysis as it is relatively robust to such limitations (Anderson 2017). Where significant associations were found we performed a post-hoc Similarity Percentage (SIMPER) analysis (Clarke 1993) to

identify which ITS2 Type Profiles were responsible for the observed dissimilarity between groups. SIMPER calculates the differences in taxa abundances across groups and computes the percentage contribution of each taxonomic unit (ITS2 type profile) to the dissimilarity observed and this significance of this effect.

To further explore the effects of environmental variables on Symbiodiniaceae type profile and sequence diversity across latitude we performed a canonical correspondence analysis (CCA: ter Braak 1986). Specifically, environmental variables were input as 2019 yearly mean from each reef for SST, Chl-*a* and K_d490. Results of the CCA analysis were tested for significance with an analysis of variance (ANOVA) test. An initial visual assessment of the *SymPortal* output indicated a unique coral-algal association in the southernmost region of the GBR (detailed in "Results"); therefore, to further test if latitude was significant in explaining variation along the entire gradient, and not just in the highest latitude locations, we discarded the Capricorn Bunkers Ecoregion from the *SymPortal* dataset and re-performed PERMANOVA, post-hoc SIMPER and CCA analysis on this filtered data. Finally, to explore whether host species exhibited symbiont specificity regardless of latitude, we extracted the *SymPortal* data for the most sampled taxa, *Acropora hyacinthus* (n = 24, sampled 12/13 reefs; Supplementary Material, Table S3.1) and again re-ran the above statistical analysis (PERMANOVA, post-hoc SIMPER & CCA) on this species independently.

3.4 Results

3.4.1 Host Species Identification

From the initial 91 colonies sampled across 2019 and 2020, 21 failed to meet minimum sequencing thresholds (> 1,000 sequencing depth) and were

subsequently discarded from our analysis (Supplementary Material, Table S3.1). We also discarded one colony that hosted only *Breviolum* sequences (ID = 51, see methods). All subsequent results focus on the remaining 69 host samples that passed filtering and quality control. Of these (n= 69; Table 1), samples represented 13 individual reefs spanning 12° latitude, from Sykes Reef in the Capricorn Bunkers Group of Islands in the south to the Great Detached Reef in the far north (ranging -23 to -11° S; Fig. 1). Of these coral host specimens, 15 had been previously identified in **Chapter 2**. Using these specimens as a morphological guide, specimens were categorised as *A. hyacinthus:* n= 24, *A. pectinata* (Brook, 1892): n= 11, *A. coralB:* n= 12, *A. sp. 7:* n= 8, *A. cytherea* (Dana, 1846): n= 7 and *A.* aff. *arcuata:* n= 7. These six taxa (Fig. 2) represent the known diversity of tabular *Acropora* on the GBR (Cowman et al. 2020; **Chapter 2**). The only species present in all six GBR Ecoregions was *A. hyacinthus* (Fig. 1).

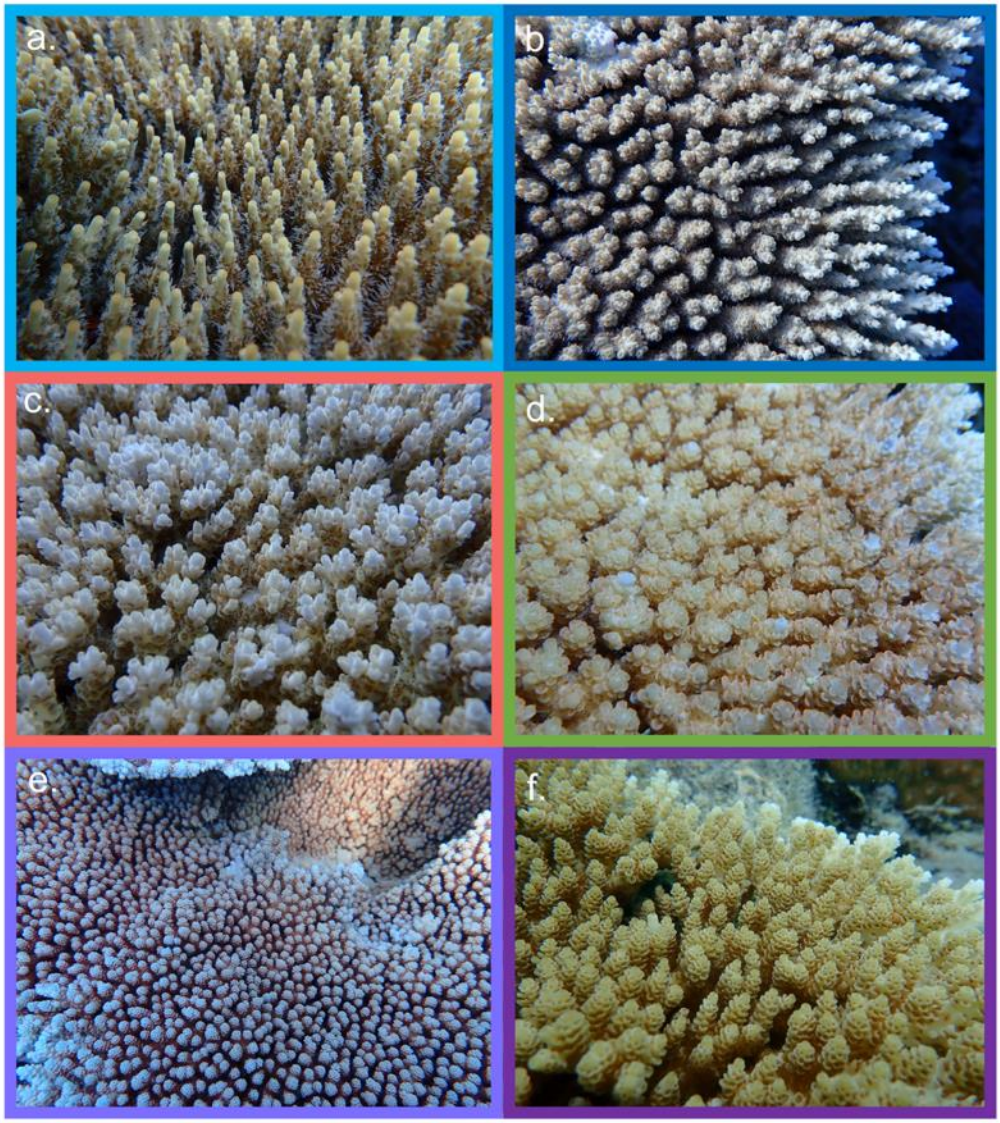

Figure 3.2 Macro *in situ* colony photographs showing the morphological variety of tabular *Acropora* species in the current study. Species represented are: **a**. *A*. aff. *arcuata* (19.GBR.98), **b.** *A. cytherea* (19.GBR.53), **c.** *A. sp.7* (19.GBR.107), **d.** *A. hyacinthus* (19.GBR.70), **e.** *A. coralB* (19.GBR.57) & **f.** *A. pectinata* (19.GBR.104).

3.4.2 Symbiodiniaceae Diversity

Next generation sequencing of the ITS2 region produced 5,748,359 raw sequences, with a final 4,520,429 sequences passing SymPortal analysis and filtering steps. This resulted in a total of 242 defining intragenomic variants

(DIV's). Across the 69 samples, three Symbiodiniaceae genera were recovered (S*ymbiodinium, Cladocopium* & *Durusdinium*), representing 28 distinct ITS2 type profiles. The most abundant genus was *Cladocopium*, which comprised 94.19% of the total Symbiodiniaceae community and was present in 68 of the 69 colonies sampled (absent in 19.GBR.84, *A. hyacinthus*) and was the dominant genus for 64/68 colonies sampled (column 'ITS2 Type Profile'; Fig 1.). *Symbiodinium* was present in just three samples and was the dominant profile for just one of those (19.GBR.72, *A. hyacinthus*, 42.0%; Fig 1), which also hosted *Cladocopium* (20.35%) and *Durusdinium* (5.59%). The one sample that did not host *Cladocopium* (19.GBR.84, *A. hyacinthus*) contained only *Durusdinium*. Two additional specimens hosted majority *Durusdinium* (19.GBR.28 = 72.76% & 19.GBR.62 = 70.78%, both *A. cytherea*), and three samples hosted a small population of *Durusdinium* (< 2%).

Overall, the most abundant ITS2 Type Profile was C3-C3.10-C29-C3b-C3k-C21 (21.93% relative abundance, Supplementary Material, Fig. S3.4) which was recovered in 24 host corals and formed a monospecific relationship with all 24 of these hosts (Fig. 1). This ITS2 Type Profile, however, did not show any host specificity and was found in hosts across all six tabular *Acropora* species. The second most abundant ITS2 Type profile was C3/C3ii/C3ij-C21 (11.46% relative abundance, Supplementary Material, Fig. S3.4) which was unique to the highest latitude Ecoregions (Capricorn Bunkers and Pompey & Swains). Similarly, this ITS2 Type Profile formed a monospecific relationship with the 10 samples it was recovered from (Fig. 1) and exhibited no host specificity, being present in hosts across four tabular *Acropora* species (absent from *A. cytherea* & *A. sp. 7,* Supplementary Material, Fig. S3.4). In total, 51.73% of individual sequences did

not contribute to type profiles ("non-profile sequences" Supplementary Material, Fig. S3.4). ITS2 sequence profiles of the C1 lineage occurred in six host corals, with co-communities of *Durusdinium* and *Symbiodinium*, or just *Durusdinium* symbionts present in five of these host corals (Fig. 1). Interestingly, both *Cladocopium* and *Durusdinium* profiles were also present in all three hosts that contained *Symbiodinium* (ITS2 Type Profile A3-A3i-A3j-A3g).

Correlation analysis of all samples indicated an association between ITS2 Type Profile and latitude (PERMANOVA, Factors = Ecoregion & Reef, $p < 0.05$, Table 1), which was further retained when the Capricorn Bunkers region was filtered out (see Methods; $p < 0.05$, Table 1). When considering just *A. hyacinthus* samples, only Ecoregions ($p = 0.001$, Table 1) but not individual reefs ($p = 0.225$, Table 1) explained distribution of algal associations; ITS2 sequence profiles were similarly only explained by Ecoregion ($p < 0.05$ Table 1), which was retained for the *A. hyacinthus* only dataset and no factors were significant when filtering out the Capricorn Bunkers region.

Table 3.1 Permutational Multivariate Analysis of Variance (PERMANOVA) results of *SymPortal* ITS2 Sequence and Type Profile relative abundances to identify associations with Great Barrier Reef Ecoregion, Reef or host coral Species. Analysis was performed on three datasets, being i) All data retrieved from *SymPortal* (n = 69), ii) A. hyacinthus dataset (n = 26), and iii) All data excluding the Capricorn Bunkers (No CB) Ecoregion (n = 60). Results shown are F-statistic (F) and P-value with significant results ($p < 0.05$) shaded.

	All		***A.hyacinthus***		**No CB**	
	F	P-value	F	P-value	F	P-value
ITS2 Type Profile						
Ecoregion	3.655	0.001*	3.702	0.001*	1.984	0.004*
Reef	1.449	0.017*	1.181	0.225	1.519	0.022*
Species	1.503	0.083			1.432	0.069

ITS2 Sequence						
Ecoregion	2.514	0.001*	4.697	0.002*	1.098	0.274
Reef	1.413	0.070	1.270	0.277	1.473	0.102
Species	1.250	0.164			1.195	0.204

Significant associations were consistent across ITS2 Type Profile and Ecoregion for all datasets, and so we subsequently performed post-hoc Similarity Percentage (SIMPER) analysis to extract the ITS2 Type Profiles and Ecoregions that were driving the differences observed. When considering all Ecoregions, the ITS2 Type Profile C3/C3ii/C3ij-C21 contributed most to the dissimilarity observed, only found in the Capricorn Bunkers region and in two specimens from Paul Reef in the Pompey & Swains region in the southern GBR (Fig. 3). This Type Profile accounted for half (SIMPER analysis, 48% - 52% cumulative dissimilarity (CD), $p < 0.05$, Supplementary Material, Table S3.2) of CD between the Capricorn Bunkers and all other Ecoregions when compared (Fig. 3). For all other Ecoregion comparisons, profile C3-C3.10-C29-C3b-C3k-C21 contributed to CD (SIMPER, 27% - 37%, Supplementary Material, Table S3.2), although most outcomes were not significant (Supplementary Material, Table S3.2) with each region containing some proportion of this Type Profile (SIMPER, ava & avb, Supplementary Material, Table S3.2). Type Profile C3/C3.10 appeared to differentiate Townsville & Cape Upstart from all other Ecoregions (Fig. 3). We found a similar pattern for the *A. hyacinthus* dataset, where ITS2 Type Profile C3/C3ii/C3ij-C21 was driving the majority of the significant differences in Ecoregions observed (46% - 55% CD, $p < 0.05$, Supplementary Material, Table S3.2), whilst profile C3-C3.10-C29-C3b-C3k-C21 contributed the most to comparisons of all other Ecoregions (30% - 57% CD) with mostly insignificant outcomes ($p > 0.05$, Supplementary Material, Table S3.2). When the Capricorn

Bunkers Ecoregion was filtered out, differences between Ecoregions were driven by profile C3/C3.10 which accounted for 17% - 21% of the dissimilarity between Townsville & Whitsundays and all other Ecoregions ($p < 0.05$, Supplementary Material, Table S3.2).

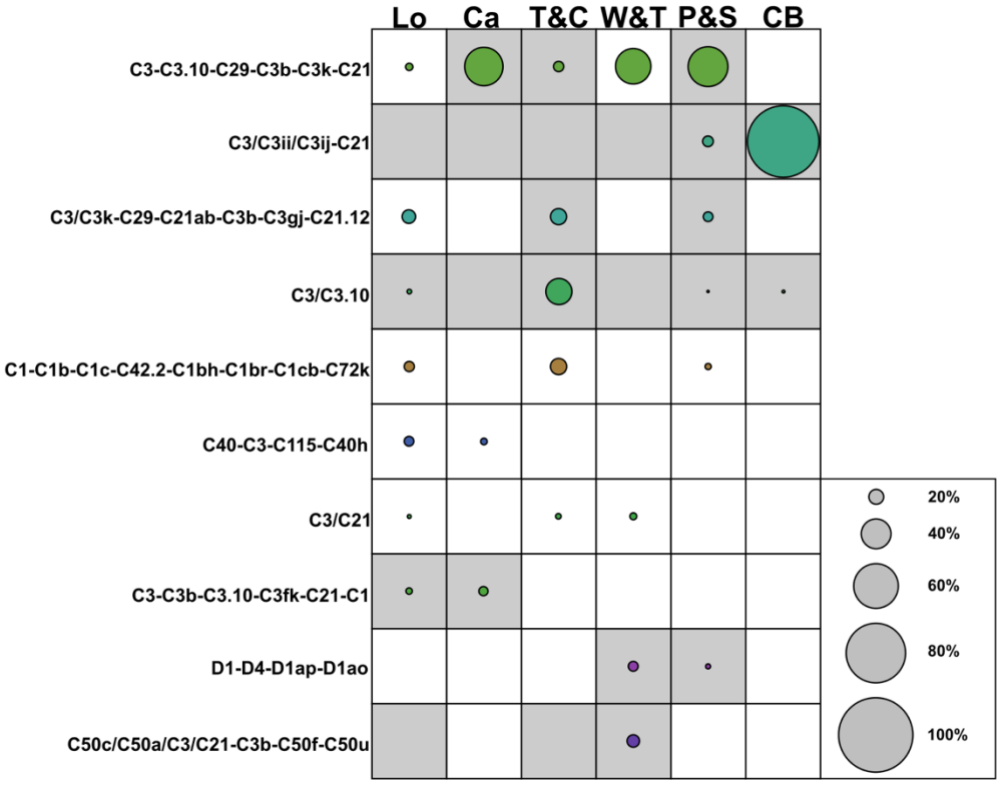

Figure 3.3 Similarity Percentages (SIMPER) results displaying average abundances of the 10 most abundant ITS2 Type Profiles that contributed to the dissimilarity between Ecoregions. Circle sizes according to percent abundance of ITS2 Type Profile in Ecoregion & colours represent distinct ITS2 Type Profiles. Shaded squares indicate significant ($p < 0.05$) comparisons. Ecoregions across the top are in order from lowest latitude to highest: Lockhart (Lo), Cairns (Ca), Townsville & Cape Upstart (T&C), Whitsundays & Townsville (W&T), Pompey & Swains (P&S), and Capricorn Bunkers (CB).

3.4.3 Environmental Factors in Symbiodiniaceae Distribution

Mean annual SST (2003-2020) from satellite derived data resolved a temperature gradient on the GBR with a mean 24.19°C ± 1.96 in the southernmost

reef sampled (Sykes Reef) and 26.50°C ± 1.29 in the northernmost reef sampled (Great Detached Reef, Fig 5). 2019 mean SST deviated within ±0.38°C of these means at each reef (Fig. 4). The southernmost reefs, being below -22 latitude were characterised by annual SST averages < 25°C. As expected, the temperature gradient followed a gradual decline from north to south. Mean annual Chl-*a* concentrations (2003-2020) followed unpredictable trajectory across the reefs sampled, with no clear pattern in relation to latitude (0.06 – 0.63 mg/m^3, Supplementary Material, Fig. S3.5). 2019 mean Chl-*a* followed a similar pattern, falling within ±0.074 mg/m^3 of the 18-year mean values. Mean values for K_d490 (2003-2020) for each reef exhibited little variation (0.05 – 0.08 m^{-1}, Fig 5) with 2019 mean values falling within ±0.01 m^{-1} of the 18-year mean (Supplementary Material, Fig. S3.5) indicating uniform deep light penetration at these sites.

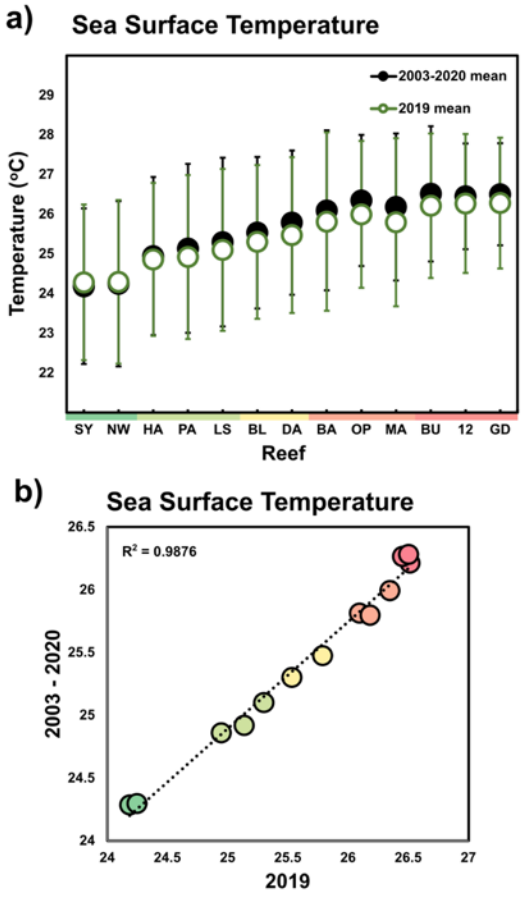

Figure 3.4 Graphs of 2019 and combined 18-year (2003-2020) average Sea Surface Temperature (SST) for each reef sampled in the current study in order from the highest to lowest latitude along the GBR with **a)** coloured bar indicating Ecoregion where reefs are located being the Capricorn Bunkers: Sykes (SY) & North West Island (NW); Pompey & Swains: Hackie (HA), Paul (PA) & Little Stevens (LS); Townsville, Capy Upstart & The Whitsundays: Block (BL) & Darley (DA); Cairns: Batt (BA), Opal (OP) & Mackay (MA); & Lockhart; Burke (BU), 12-040 (12), & Great Detached (GD). Error bars represent the standard deviations & **b)** Regression plots show the correlation of 2019 and combined 18-year mean values, with data points coloured according to Ecoregion (as above). Data was sourced from the MODIS-Aqua database, obtained from the National Aeronautic and Space Administration (NASA) Giovanni website. See methods for calculations of each datapoint.

Overall, environmental conditions in 2019 represented prevailing reef conditions (and not anomalous for the year immediately prior to sampling, Fig 3.4., Supplementary Material Fig. S3.1-S3.3). Mean 2019 SST and Chl-*a* were positively correlated with ITS2 Type Profiles and ITS2 sequence composition (CCA, $p \leq 0.018$ Table 3.2) When accounting for just *A. hyacinthus* only mean SST (2019) positively correlated with the distribution of ITS2 profiles and sequences ($p \leq 0.002$ Table 3.2). With the exclusion of the Capricorn Bunkers Ecoregion, we found that no environmental factors (2019) retained a significant relationship with the data (Table 3.2).

Table 3.2. Analysis of Variance (ANOVA) results on Canonical Correspondence Analysis (CCA) performed to determine significance of environmental factors: Sea Surface Temperature (SST), Chlorophyll-a concentration (Chl-a), & Diffuse Attenuation (K_d490) on distribution of *SymPortal* ITS2 Sequence and Type Profile relative abundances. Analysis was performed on three datasets, being i)

All data retrieved from *SymPortal* (n = 69), ii) *A. hyacinthus* dataset (n = 26), and iii) All data excluding the Capricorn Bunkers (No CB) Ecoregion (n = 60). Results shown are F-statistic (F) and P-value with significant results ($p < 0.05$) shaded.

	All		***A.hyacinthus***		**No CB**	
	F	P-value	F	P-value	F	P-value
ITS2 Type Profile						
SST	2.722	0.001*	2.746	0.002*	1.315	0.103
Chl-a	1.932	0.011*	1.863	0.130	1.569	0.063
K_d490	1.457	0.066	1.238	0.278	1.361	0.094
ITS2 Sequence						
SST	2.657	0.001*	2.682	0.001*	1.269	0.142
Chl-a	1.946	0.018*	2.729	0.087	1.586	0.068
K_d490	1.527	0.057	1.080	0.222	1.514	0.054

3.5 Discussion

Taxonomic resolution of both host taxa and algal symbiont is central to understanding the specificity of association, and the role this plays in facilitating coral adaptation to changing environments. Whilst such advances have been achieved for certain key coral taxa (e.g. *Pocillopora*, Johnston et al. 2022), they remain elusive for tabular *Acropora* despite their ecological importance for reefs on the GBR and wider Indo-Pacific. Here we establish Symbiodiniaceae community associations of closely related tabular *Acropora* species across a latitudinal gradient of the GBR for the first time according to the new tabular *Acropora* taxonomic framework. We show latitude strongly influences ITS2 Type Profile, highlighting the uniqueness coral-algal associations on the southern GBR where cooler temperature maximums are correlated with distinct symbiotic associations. Such an outcome aligns with patterns observed both on the GBR and in other global reef regions across large latitudinal gradients (e.g., Cooper et al. 2011; Terraneo et al. 2019; Osman et al. 2020). We also highlight the flexibility

of the associations between tabular *Acropora* and their symbiotic algae, where both common and relatively rare ITS2 Type Profiles were found both within host species and within reefs. By sampling corals over a latitudinal gradient and investigating ITS2 type profiles alongside several environmental gradients we observed biogeographic patterns of tabular *Acropora* in their endosymbiotic algae associations.

3.5.1 Tabular *Acropora* are Largely Generalists with their Symbiodiniaceae Associations

As a group, the six closely related species of tabular *Acropora* examined in this study conform to the notion of 'generalist hosts' (Putnam et al. 2012) that share common (e.g. C3 & C1 lineages, Fig. 1) and relatively rare (e.g. A & D lineages, Fig. 1) symbiont associations across a broad environmental gradient. Species-specific symbiont associations have been proposed for a range of coral taxa, particularly when comparing distantly related species or distinct genera of Scleractinia (Tonk et al. 2017; Osman et al. 2020; Grima et al. 2022), however, our results are consistent with previous findings that Pacific *Acropora* are generalists (Putnam et al. 2012; Davies et al. 2022). Importantly, we identify some rare associations that could indicate species specificity or environmental adaption (Ulstrup & van Oppen 2003; Kriefall et al. 2022), highlighting the need to resolve host taxonomies as well as of exploring associations between both distantly and closely related taxa when investigating symbiont specificity and distribution. That said, it is plausible to expect more species-specific associations that have not yet been detected as resolutions in Symbiodiniaceae taxonomy continue to improve (e.g. Turnham et al. 2021; Davies et al. 2023). In addition, a relatively high

portion of ITS2 sequences did not contribute to the ITS2 type profiles ("non-profile sequences" Supplementary Material, Fig. S3.4), which represent non-defining intragenomic variants (non-DIV) in the Symportal pipeline (Hume et al. 2019). These non-DIV sequences, when combined with a greater number of samples, may in future studies reveal finer-scale patterns of specificity not detected in the current study.

The majority of our tabular *Acropora* associated with Symbiodiniaceae of the C3 radiation regardless of latitude or coral taxon. Where a colony associated with a C3 Type Profile we found no secondary ITS2 Type Profiles in the colony, apart from one host colony from the northern Lockhart region hosting both C3/C.10 & C51a ITS2 Type Profiles (19.GBR.108). However, this latter association was not found elsewhere in colonies hosting C3 algal symbionts, and the high levels of non-profile sequences amongst most samples may indicate unknown symbiont communities. *Acropora* are well known to associate with *Cladocopium*, particularly the C3 lineage which contains a significant diversity of host generalist symbionts amongst tabular *Acropora* and scleractinia in general on the GBR (LaJeunesse et al. 2003, 2004; Tonk et al. 2014; Epstein et al. 2019; Butler et al. 2023) and the wider Indo-Pacific (Morikawa & Palumbi 2019). Importantly, the C3 lineage generally appears more sensitive to thermal stress than other *Cladocopium* and *Durusdinium* symbionts (Baker et al. 2004), placing corals that host C3 lineages at higher risk from stress events. This is particularly concerning when corals form associations with a single lineage, as observed here for tabular *Acropora*, as they may lack the adaptive capacity afforded by harbouring additional, more thermally tolerant species when faced with stressor events (Berkelmans & van Oppen 2006). Tabular *Acropora* species (*A.*

hyacinthus, Quigley et al. 2022) on the GBR have a relatively stable symbiont community characterised by the loss of symbiont ITS2 sequence richness post the 2016 bleaching event, with little evidence of uptake - or 'switching' - to new taxa. Although we found 2019 to represent prevailing reef conditions over an 18-year period, arguably, our sampling time frame may still only be capturing these lower diversity post-bleaching symbiont communities, since the time frame to re-organise symbiont taxa when faced with future stressors remains largely unknown. Overall, 88.4% of colonies formed single profile associations, though recent evidence shows that exhaustively characterising symbiont communities likely requires intra-colony sampling, whereas in the present study samples were only taken from the outer branches of the colony (Lewis et al. 2022). Ultimately, the low diversity of symbiont types suggests tabular *Acropora* remain highly susceptible to predicted future stress events.

Cladocopium C1 was the second most abundant lineage associated with tabular *Acropora*, occurring as the dominant lineage in four colonies and in background levels for three additional colonies. For five of these colonies, C1 sequences were present alongside *Durusdinium* and three of these colonies also hosted *Symbiodinium*. Presence of symbionts from the C1 radiation in association with GBR Scleractinia is common and may be either the most abundant (LaJeunesse et al. 2004; Tonk et al. 2014; Tonk et al. 2017) or second most abundant (LaJeunesse et al. 2003) taxon found. Indeed, a culture isolated from an *A. tenuis* (now *A. kenti,* Bridge et al. 2023) colony from Magnetic Island in the central GBR was formally described recently (*Cladocopium proliferum*, Butler et al. 2023), and is reported as a common symbiont amongst Scleractinia of the southern West Pacific, particularly along the central to northern GBR.

Intriguingly, profiles of the C1 lineage have been observed across a range of extreme reef environments and host taxa, from warm, acidic, and low oxygen mangrove lagoons in New Caledonia (*A.* cf. *muricata*, Camp et al. 2020) to multiple scleractinian taxa from marginal reefs in Hong Kong characterised by cool winter maximums (Ng & Ang 2016; Saad et al. 2022). The existence of this taxon in such extreme environments suggests that they have the capacity to adapt to a range of thermal and environmental regimes. In our study, the co-occurrence of C1 with *Durusdinium* and *Symbiodinium* suggests this species may be amenable to symbiont community formation, providing hosts with a high adaptive ability when faced with future stressor events, particularly thermal anomalies (van Hooidonk et al. 2014). What was surprising in our study was the lack of species, reef, or environmental specificity in which we found colonies to associate with either C3 or mixed C1 profiles. For example, at Paul Reef we sampled two adjacent colonies of *A. cytherea* which were found to host completely different ITS2 Type Profiles: the first hosting only C3/C3k-C29-C21ab-C3b-C3gj-C21.12 and the second hosting a majority D1 profile, with low levels of D1, C50a, A3 and C1d profiles. Both *A. cytherea* colonies exhibited identical morphology yet distinctly different colony colours (Supplementary Material, Fig. S3.6). Similarly, at Block Reef 2 *A. hyacinthus* colonies growing in close proximity hosted different profiles (only C3-C3.10-C29-C3b-C3k-C21 versus a mix of A3, C1d, C1 and D1 profiles), and at Darley Reef where 2 *A. sp7* colonies hosted the C3-C3.10-C29-C3b-C3k-C21 profile versus the C1-C1b-C1c-C42.2-C1bh-C1br-C1cb-C72k profile. This seemingly sporadic nature in which tabular *Acropora* associate with algal symbionts may explain patchiness and variability in response to past bleaching events (Marshall & Baird 2000; Hoogenboom et al. 2017;

Brodnicke et al. 2019; Morikawa & Palumbi 2019; Cornwell et al. 2021), and hence spatial and temporal influences that contribute to tabular *Acropora* symbiosis (Quigley et al. 2022). Understanding the causes of such variation is important for management, particularly with respect to interventions aimed at modifying symbiosis specificity (Lawson et al. 2022; van Oppen & Nitschke 2022; Peixoto & Voolstra 2023).

Symbionts of the genus *Durusdinium* are often characterised by the ability of species within this group – particularly *D. trenchii* (Rosset et al. 2019) – to provide high thermal tolerances to host taxa (LaJeunesse et al. 2018) especially when compared with *Cladocopium* symbionts that are commonly associated with *Acropora* (Berkelmans & van Oppen 2006; Morikawa & Palumbi 2019). Here, we detected six colonies with *Durusdinium* symbionts, three of which were dominated by this genus. We observed some evidence of species-specific associations with profile D1-D4-D1ap-D1ao with *A. cytherea*, where two colonies from different reefs hosted similar abundances of this Type Profile alongside low levels of profile C1d and other background types. This D1-D4-D1ap-D1ao profile was not found amongst any other host taxa. In addition, we also found profile D4 to be the dominant type for just one *A. hyacinthus* colony. Overall, *Durusdinium* associations in our study were uncommon. *Durusdinium* has been detected in *Acropora* corals in extreme environments across the GBR (Berkelmans & van Oppen 2006; Camp et al. 2019) with evidence of hosts shuffling their dominant community to *Durusdinium* when faced with stressor events. However, given the scarcity of *Acropora- Durusdinium* associations found in the current study, the thermal tolerance of other symbiont types, such as *Cladocopium*, will be crucial to

holobiont stability, and ultimately efforts to facilitate tolerance maybe key (Quigley et al. 2021).

Taxa from the genus *Symbiodinium* (LaJeunesse et al. 2018), known to tolerate high thermal stress and solar irradiance, are commonly found in juvenile *Acropora* corals but relatively rare in adult colonies (Quigley et al. 2016; Yoshioka et al. 2022). We detected only background levels of *Symbiodinium* (Type Profile A3-A3i-A3j-A3g) in two southern GBR *A. cytherea* colonies, however, we also detected this A3 Type Profile as the dominant community in one central GBR *A. hyacinthus* colony. All three of these colonies also hosted mixed abundances of C1 and D1 lineages. Such observations are consistent with previous findings where *Symbiodinium* lineages – specifically the A1 lineage - have been detected alongside *Cladocopium* and *Durusdinium* for *Acropora* corals (e.g *A. longicyathus* from the southern GBR, Gómez-Cabrera et al. 2007; *A. pulchra* in New Caledonia, Camp et al. 2020; A. *hyacinthus* in Mo'orea, Leinbach et al. 2023). Interestingly, we also detected the A3 lineage of *Symbiodinium*, which has been suggested as essential for early life stages of *Acropora* (Quigley et al. 2016) but uncommon in adult colonies, and suggesting this is a truly rare occurrence.

Whilst overall we found no specificity with host taxa, two colonies of *A. coralB* from the central and northern GBR hosted C40 Type Profiles (C40-C3-C115-C40h), which was not found in association with any other taxa. C40, a generalist symbiont recently formally described as *Cladocopium madreporum* (Butler), was recently found in association with *Acropora digitifera* colonies with the same ITS2 profile found here (Lachs et al. 2023), whilst Davies et al. (2019) found colonies of *A. digitifera* & *A. hyacinthus* to each associate with C40 and/or

C21 *Cladocopium* lineages across several Micronesian islands, with dominant types changing across Islands. Interestingly, recent work on delineating species of tabular *Acropora* found *A. hyacinthus* to be limited to the southern Pacific region, with the newly described species *A. coralB* occurring in Palau (Micronesian archipelago) and the GBR (**Chapter 2**) indicating that the corals sampled by Davies et al. (2019) may in fact be the recently described *A. coralB.* If this is the case, our findings would suggest that *C. madreporum* is common amongst the newly described coral species *A. coralB* in both the GBR and Micronesia (Davies et al. 2019), whilst also common amongst other *Acropora* taxa (Lachs et al. 2023). Further, Butler et al. (2023) provides indication that *C. madreporum* may have a higher thermal tolerance than *C. proliferum/vulgare* (C1 lineages). Association of this putative taxon with *A. coralB* on the GBR may again explain past patchiness in bleaching responses in tabular *Acropora*, with some coral hosts – which could represent distinct species previously lumped together as *A. hyacinthus* - withstanding higher thermal anomalies (Marshall & Baird 2000; Morikawa & Palumbi 2019). These findings suggest *A. coralB* could be more thermally resistant to thermal stress on the GBR than other similar taxa, a hypothesis that clearly warrants further targeted investigation but also underscores the need for future work examining thermal sensitivity of tabular *Acropora* to ensure robust host level identification.

3.5.2 Cooler Temperatures are Associated with Shifts in Symbiodiniaceae Community

Environmental gradients are correlated with shifts in dominant Symbiodiniaceae genera hosted by corals in the Red Sea (Reimer et al. 2017;

Terraneo et al. 2019; Osman et al. 2020) and the Caribbean (Eckert et al. 2020), where latitude or depth gradients have been used to explore shifts in algal symbiosis for a range of host taxa. In sampling along gradient spanning 13° of latitude along the GBR, we observed cooler waters in the high latitude reefs of the Capricorn Bunkers region to coincide with a unique Symbiodiniaceae community in tabular *Acropora*.

Mean SST on the GBR declines by ~2.31^{o}C (2003 - 2020) from north to south. The Capricorn Bunker region, which has an average SST of < 25 ^{o}C, was associated with a significant shift in the dominant Symbiodiniaceae community. Specifically, whilst tabular *Acropora* in general associated with C3 ITS2 profiles across latitude, the southern GBR C3 lineage (C3/C3ii/C3ij-C21) differed to the rest of the GBR (dominated by C3-C3.10-C29-C3b-C3k-C21). Indeed, to test this notion we removed the Capricorn Bunkers region from our dataset and found no significance in the distribution of ITS2 type profiles or sequences. Our observations are therefore consistent with those previously reported from the Red Sea, where cooler temperatures in the Gulf of Aqaba align with shifts in Symbiodiniaceae association across a range of cnidaria (Reimer et al. 2017; Terraneo et al. 2019). Similarly, SST, along with other environmental factors, appear important drivers of symbiont communities across latitude in *A. millepora* on the GBR (Cooper et al. 2011). A significant effect of Chl-*a* on symbiont diversity was detected ($p < 0.018$, Table 3.2) across the data set, but not when *A. hyacinthus* was examined independently, and therefore warrants further investigation into the influence of this variable on the coral symbiosis. Further, correlations between symbiont communities and environmental variables independent of temperature have often found associations to be strongest between

inshore and offshore reefs (Cooper et al. 2011), however this design was not repeated in the sampling strategy of the current study and as such may reflect the insignificant results observed.

The C3/C3ii/C3ij-C21 Type Profile was dominant in the Capricorn Bunkers Ecoregion (occurring in just 2 specimens outside of this region from the adjacent Pompey & Swains Ecoregion) and was the primary symbiont of 10 host colonies. Previous investigations in the Capricorn Bunkers region have identified symbionts of the C3 lineage as associating with *Acropora* (LaJeunesse et al. 2003), but our data identifies that a specific C3 ITS2 profile found in this region may be distinct from the C3 profile common to more northern regions of the GBR. It is plausible that the C3/C3ii/C3ij-C21Type Profile could represent the newly described *Cladocopium sodalum* (Butler et al. 2023), a species previously referred to as a member of the C3 lineage (or subclade C2 *sensu* van Oppen et al. 2001) with a type locality of Heron Island in the Capricorn Bunker region. However, with the known diversity of "C3" ITS2 sequence types in this region (Fujise et al. 2020), it is also plausible that this type profile represents an undescribed species of *Cladocopium*. Indeed, this is the first report of this specific ITS2 type profile in the *SymPortal* database deeming it a putative taxon with further investigations recommended to explore systematics and biogeography of this taxa.

Molecular phylogenomics is resulting in substantial changes to the taxonomy of *Acropora* at the species level (Cowman et al. 2020; Bridge et al. 2023; **Chapter 2**). Here we provided the first insight into Symbiodiniaceae communities diversity and distribution for closely related but taxonomically-resolved tabular *Acropora* species along the entire latitudinal gradient of the GBR.

These taxa have been particularly impacted by past stress events (Hoogenboom et al. 2017; Brodnicke et al. 2019; Morikawa & Palumbi 2019; Cornwell et al. 2021) and therefore the focus of recent propagation efforts for restoration (Morikawa & Palumbi 2019; Howlett et al. 2021, 2022) and understanding symbiont specificity is critical to support on-going microbial based interventions (Lawson et al. 2022; Peixoto & Voolstra 2023). Our approach reveals substantial 'generalism' and lack of host specificity in Symbiodiniaceae association between closely related tabular *Acropora* species along a large latitudinal gradient. However, we also detected potential for resilience amongst some host taxa, highlight the need for further study incorporating larger datasets and focused sampling on lesser studied – or newly discovered – species which may be key to surviving future stressor events. Our data also highlight the uniqueness of the Symbiodiniaceae communities of southern GBR, particularly the Capricorn Bunker region. The distinct communities in this region may be driven by a cooler temperatures, but further investigation into connectivity and environmental conditions of this symbiont community across regions is required. Given the importance of tabular *Acropora* to ecological function of coral reefs, this knowledge provides a new baseline for understanding the mechanisms behind their survival and drives the need for continued study and focused management of these keystone taxa.

3.6 Acknowledgements

Thank you to all crew of the respective vessels for the 2019 and 2020 Great Barrier Reef expeditions where samples were collected. Thank you to the crew of the Kalinda as well as Coral Project Phoenix researchers on board who aided in specimen collection being Prof Andrew Baird, Ass Prof Francesca

Benzoni, Dr Jeremy Horowitz, Augustine Crosbie & Hanaka Mera. We also thank John Edmondson and the crew of Wavelength Reef Cruises as well as researchers from the Climate Change Cluster (C3) from the University of Technology Sydney (UTS), Paige Strudwick, Isabel Nunez Lendo and Lorna Howlett, for their help during 2020 fieldwork. Fieldwork was conducted on the Great Barrier Reef under permits from the Great Barrier Reef Marine Park Authority (GBRMPA) permits G19/39364.1 (T. Bridge, Museum of Tropical Queensland) and funded by the ARC Discovery (DP180103199) awarded to Andrew Baird (James Cook University) for 2019 sampling. Sampling in 2020 was performed through GBRMPA permit G18/40023.1 (E. Camp & D. Suggett).

3.7 Supplementary Material

3.7.1 Supplementary Figures

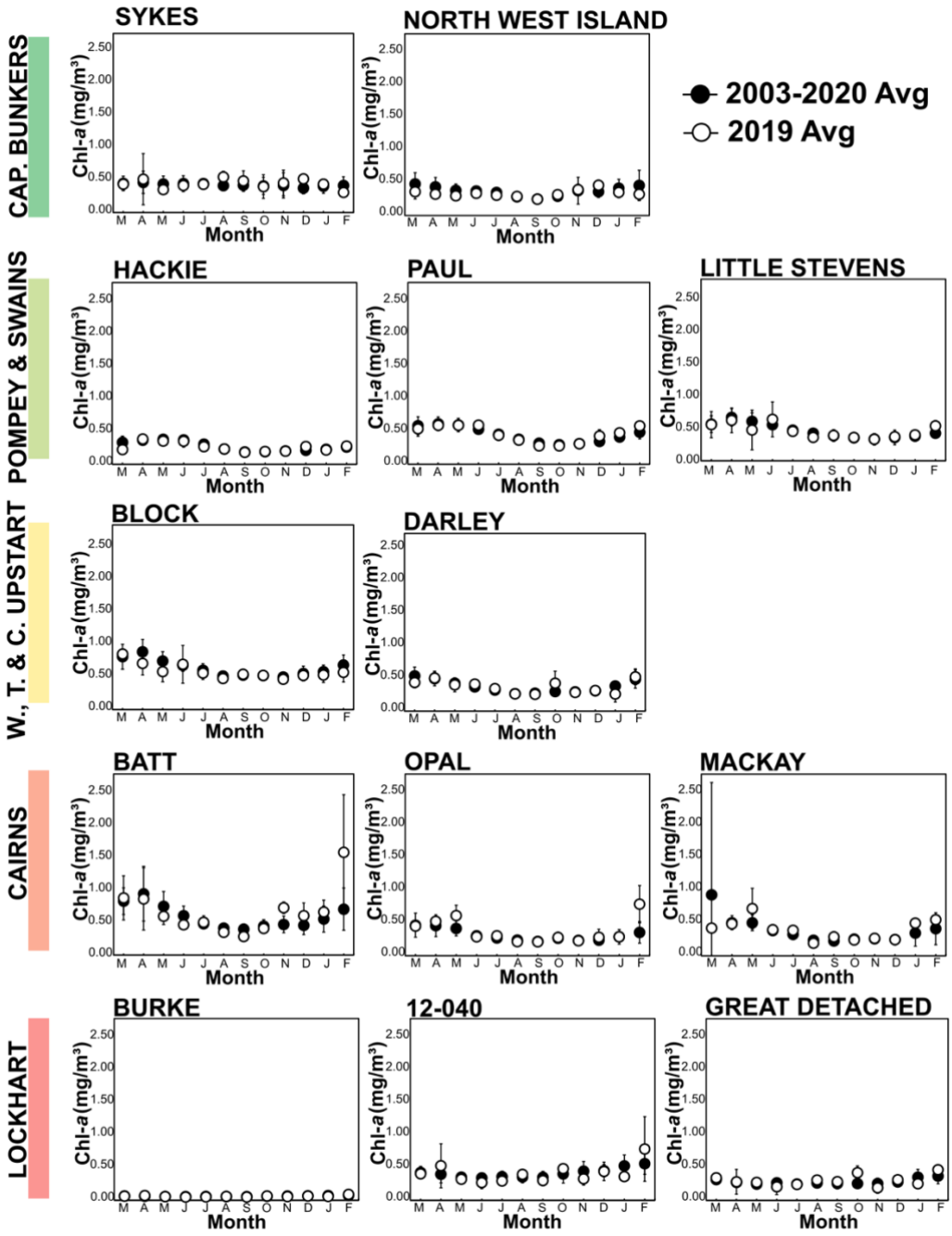

Figure S3.1 Combined 18-year (2003-2020) and 2019 average chlorophyll-*a* concentrations for each reef sampled in the current study. Each graph represents one reef, and bars on the left indicate Ecoregion from the GBR. Error bars represent the standard deviations. Data was sourced from the MODIS-Aqua database, obtained from the National Aeronautic and Space Administration (NASA) Giovanni website.

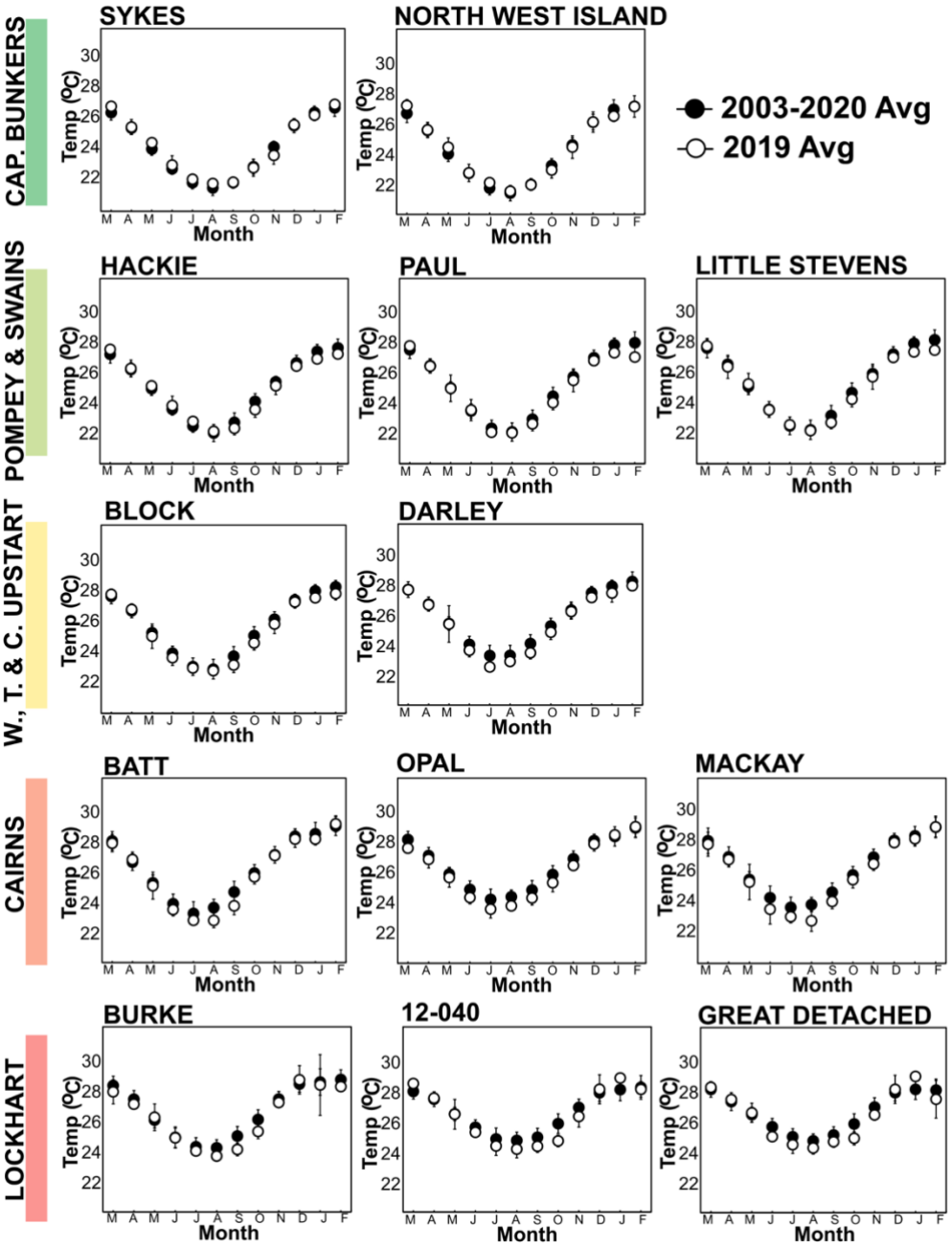

Figure S3.2 Combined 18-year (2003-2020) and 2019 average Sea Surface Temperatures for each reef sampled in the current study. Each graph represents one reef, and bars on the left indicate Ecoregion from the GBR. Error bars represent the standard deviations. Data was sourced from the MODIS-Aqua database, obtained from the National Aeronautic and Space Administration (NASA) Giovanni website.

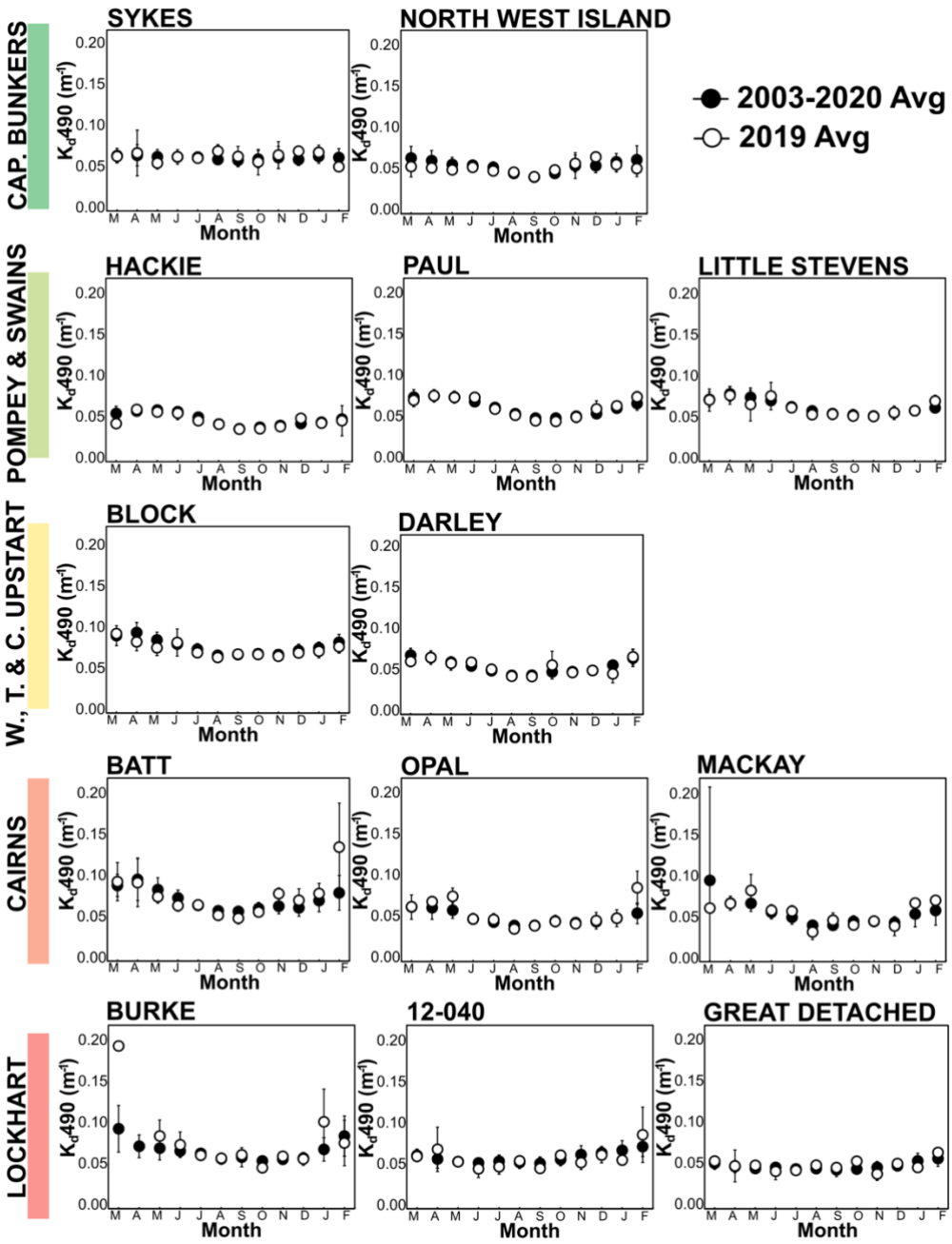

Figure S3.3 Combined 18-year (2003-2020) and 2019 average Diffuse Attenuation at 490nm for each reef sampled in the current study. Each graph represents one reef, and bars on the left indicate Ecoregion from the GBR. Error bars represent the standard deviations. Data was sourced from the MODIS-Aqua database, obtained from the National Aeronautic and Space Administration (NASA) Giovanni website.

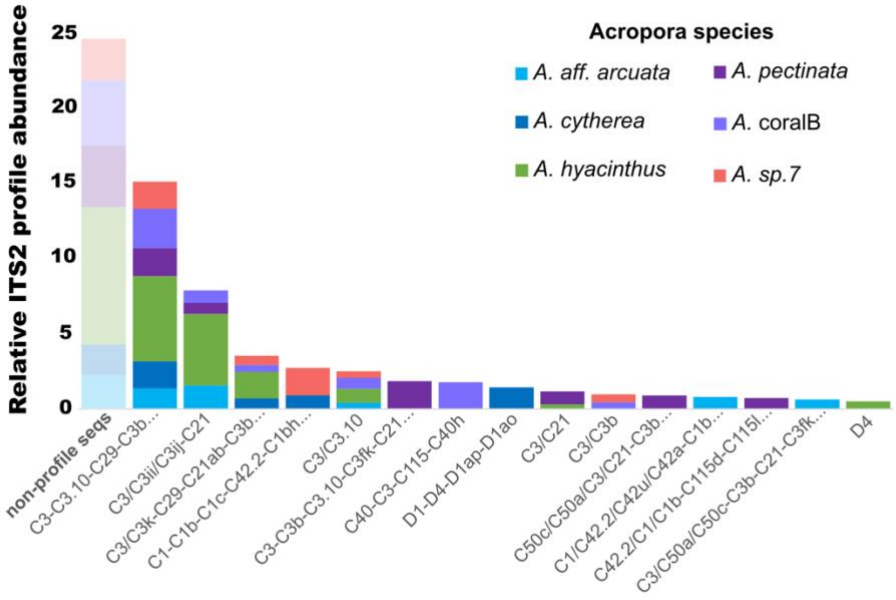

Figure S3.4 Relative abundances of the 15 most abundant ITS2 Type Profiles. Bars represent total relative abundance of each Type Profile, and colours represent portion of abundance to each of the six tabular *Acropora* species studied.

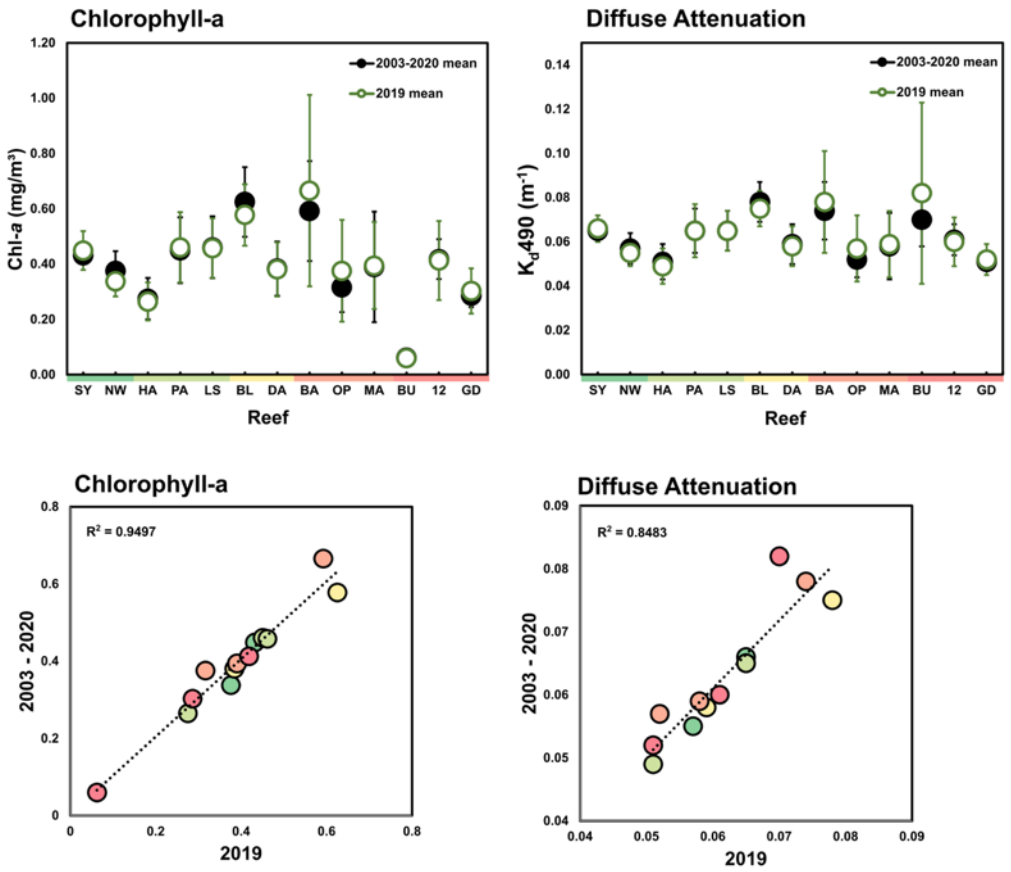

Figure S3.5 Graphs of 2019 and combined 18-year (2003-2020) average chlorophyll-a and Diffuse Attenuation for each reef sampled in the current study in order from the highest to lowest latitude along the GBR with **a)** coloured bar indicating Ecoregion where reefs are located being the Capricorn Bunkers: Sykes (SY) & North West Island (NW); Pompey & Swains: Hackie (HA), Paul (PA) & Little Stevens (LS); Townsville, Capy Upstart & The Whitsundays: Block (BL) & Darley (DA); Cairns: Batt (BA), Opal (OP) & Mackay (MA); & Lockhart; Burke (BU), 12-040 (12), & Great Detached (GD). Error bars represent the standard deviations & **b)** Regression plots show the correlation of 2019 and combined 18-year mean values, with data points coloured according to Ecoregion (as above). Data was sourced from the MODIS-Aqua database, obtained from the National Aeronautic and Space Administration (NASA) Giovanni website. See methods for calculations of each datapoint.

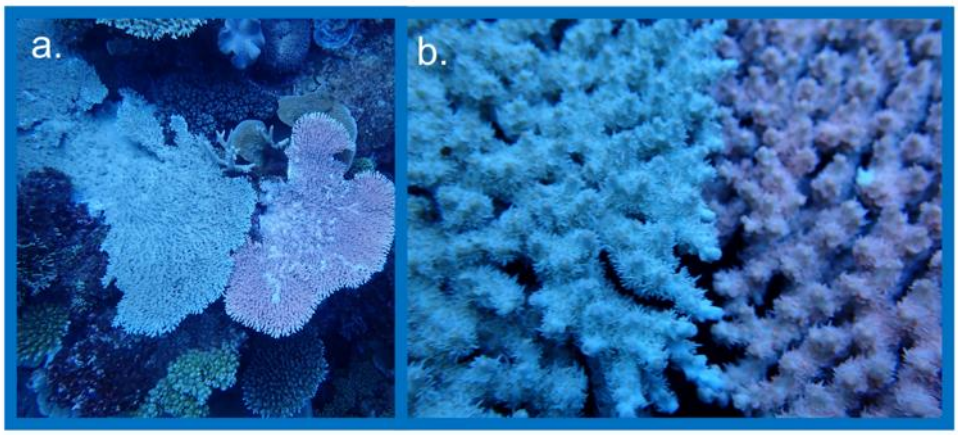

Figure S3.6 Figure depicts two colonies of *Acropora cytherea* (a. Whole colony imgaes and b. Close up of branch structure where colonies meet) that were found growing in close proximity at Block Reef on the GBR. The colonies as shown here are different colours and were found to host different ITS Type Profile communities (see results).

3.7.2 Supplementary Tables

Note: All supplementary tables can be found in **Appendix Chapter 3.**

Table S3.1 Sample collection metadata and Symportal ITS2 Sequence Profiles and relative abundances for each specimen.

Table S3.2 Raw results from Similarity Percentage (SIMPER) analysis.

Table S3.3 Environmental data showing averages and standard deviations for each month and year (2003 – 2020) for Sea Surface Temperature (SST), Chlorophyll-*a* concentration (Chl-*a*) and diffuse Attenuation (Kd490). Raw data was obtained from the National Aeronautic and Space Administration (NASA) Giovanni.

3.8 References

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Chapter 4

Morphology matters: Congruence of morphology and molecular phylogenies to resolve closely related coral taxa of tabular *Acropora.*

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4.1 Abstract

Effective research, conservation and management of key taxa relies on our understanding of biodiversity, and in turn accurate species identification and associated population connectivity. This is particularly true for reef-building corals that face continued anthropogenic pressures but where species delineation remains highly cryptic. Indeed, on the Great Barrier Reef (GBR), tabular *Acropora* have been identified as keystone taxa for reef resilience and recovery. However, historical ability to identify species within this 'tabular' coral group has remained elusive due to cryptic morphologies of closely related species. Here, in light of recent taxonomic revisions resolving 4 morphologically similar species of tabular *Acropora* on the GBR, we explore the ability of both molecular single nucleotide polymorphism (SNP) data and a set of novel morphological features to correctly delineate species. Specifically, we examine this ability within the framework of recently developed reef restoration efforts in the Whitsundays region that relies on species selection by non-experts. In sampling 5 sites spanning 12km (n = 85), we show clear presence of at least 4 tabular *Acropora* species occurring in sympatry throughout this region, and with high inter- but low intra-specific genetic differentiation amongst reefs. Importantly, some *in situ* morphological features, such as colour and corallite structure were congruent with molecular species delineations. These morphological features could in turn be used as tools for non-molecular based species identification in the field for research and management. Our data particularly highlights the challenges and opportunities to restoration practices in robustly resolving cryptic species diversity.

4.2 Introduction

Accurate understanding of coral biodiversity – and in turn the spatial genetic structure of key taxa – is critical for advancing research, conservation, and management of coral reefs in the face of ever deteriorating reef health (Sheets et al. 2018). Correctly identifying coral species and associated population dispersal is a fundamental pre-requisite towards this understanding (Thomas et al. 2018). Molecular studies are proving transformative in uncovering the presence of species complexes amongst key reef-building coral taxa (Schmidt-Roach et al. 2014; Bridge et al. 2023), often containing individuals that are considered morphologically cryptic (Ladner & Palumbi 2012; Matias et al. 2023) and in many cases living in sympatry. However, such progress can come at a cost to how well species can be delineated in real time for decision-making, including replication in sampling. For example, studies have unintentionally sampled cryptic taxa (Suggett et al. 2022; Voolstra et al. 2023; Howlett et al. in review) that, when unaccounted for, may hinder accurate population genetic inferences, and ultimately effective management (Sheets et al. 2018). Whilst recent taxonomic work has begun resolving species within cryptic coral taxonomic groups (e.g., Schmidt-Roach et al. 2014; Bridge et al. 2023; **Chapter 2**), capacity to correctly identify morphologically similar taxa in a consistent manner remains elusive without easily identifiable morphological traits.

Efforts to aid reef management through local restoration activities are accelerating worldwide (Bostrom-Einnarson et al. 2020), including on the Great Barrier Reef (GBR) (McLeod et al. 2019, Howlett et al. 2022). Restoration efforts to support recovery of reef systems often aim to produce self-sustaining ecosystems that are tolerant to future stressors (Voolstra et al. 2021, Shaver et al.

2022, Quigley et al. 2022). To achieve this goal, genetic diversity is often considered a key factor in ensuring resilience in restored ecosystems (Thomas et al. 2018; Sheets et al. 2018; Quigley et al. 2022; Voolstra et 2021, al. 2023) and in the protection of threatened species (Vardi et al., 2021). However, accurately identifying species can become particularly cumbersome when keystone taxa (or genotypes) are morphologically cryptic, requiring often costly, specialist and time-consuming genetic sequencing to resolve genetic populations, which can increase cost and reduce feasibility of restoration programs (Boström-Einarsson et al. 2020; Vardi et al. 2021). These problems are amplified where taxonomic non-specialist local communities must make practical conservation decisions (Hernández-Delgado et al. 2014). For example, field-based activities such as asexual fragmentation of donor colonies (Boström-Einarsson et al. 2020; Howlett et al. 2021) often require instantaneous identification and consequently there is a need for reliable field based diagnostic tools to ensure targeted biodiversity is captured (Howlett et al., in review). Whilst researchers are developing field based genomic tools for Scleractinia such as nanopore sequencing (Carradec et al. 2020) and standardized genotyping arrays (Kitchen et al. 2020), they often still require some morphological knowledge to identify lineages and inevitably introduce time and resource constraints to widespread application (Carradec et al. 2020; Kitchen et al. 2020). Ultimately, an inability to immediately identify taxa *in situ* and "on the fly" can lead to inaccurate sampling and reporting on true genetic and taxonomic diversity and thus impact effectiveness of restoration and scientific activities (Wiens 2004; Sheets et al. 2018; Howlett et al. in review).

On the GBR, tabular growth forms of *Acropora* are of particular interest in coral restoration activities (Howlett et al. 2021; Suggett et al. 2022) given their

role as keystone species for reef recovery via high structural complexity and growth rates, including early canopy formation that is an essential microhabitat for various reef organisms at different life stages (Oritz et al. 2021). Such forms are also often considered "aesthetically pleasing" contributing to their high tourism value (Oritz et al. 2021). However, tabular *Acropora* are notably impacted by recent bleaching (Hughes et al. 2017), thereby accelerating interest in these taxa for restoration on the GBR (Suggett et al. 2019; Howlett et al. in review). Amongst these tabular *Acropora*, certain species – namely *Acropora hyacinthus* (Dana 1846) – are often abundant throughout the GBR and attract a high volume of research and conservation focus (Ortiz et al. 2021; Quigley et al. 2022; Howlett et al. in review). Whilst considered for some time to form a 'cryptic species complex' (Ladner & Palumbi 2012) with a broad geographic range across the Indo-Pacific, recent taxonomic revisions have shown this putative '*A. hyacinthus* species complex' comprises at least 14 distinct species with many confined to distinct geographic regions, and at least five living in sympatry along the length of the GBR (**Chapter 2**). As with other taxa, species delineation within this '*A. hyacinthus* species complex' has been confounded by morphological similarities between taxa (Palumbi et al. 2012; Suggett et al. 2022). However, some morphological features, such as corallite structure, crowding, and colony colour can reliably delineate sympatric species in Japan in congruence with population genetic analysis (Ramirez-Portilla et al. 2021). Indeed, morphological analysis has identified some GBR species (e.g., *A. coralB*) as monophyletic clusters in a recent taxonomic revision of the '*A. hyacinthus* complex', but not others (e.g., *A. hyacinthus*, *A pectinata*, **Chapter 2**). Thus, inability to identify individual species based on morphology may reflect fundamental lack of knowledge for

phylogenetically informative morphological features of these distinct taxa as opposed to an inherent lack of discriminatory morphological features (Bridge et al. 2023). Consequently, analysis of novel morphological features that are unique to tabular morphologies of *Acropora* is warranted to resolve discriminatory traits for species identification independent of molecular analysis.

Reef restoration efforts have recently been established in the Whitsundays region of the GBR under Coral Nurture Program (CNP) to boost abundance and resilience of targeted corals and reefs through coral propagation and outplanting, with a focus of capturing functional and genetic diversity across taxa. However, population genetics and species diversity of distinct – yet morphologically similar – key tabular *Acropora* species is currently unknown in this region. We therefore compare genetic population structure, connectivity, and morphology of four closely related tabular *Acropora* species occurring in sympatry in the Whitsundays Islands group on the GBR through high-density sequencing of Single Nucleotide Polymorphism (SNP) genetic markers. We identify four distinct lineages – or species – that correspond with recent taxonomic revisions of this group (**Chapter 2**) and performed a morphological analysis to identify the discriminatory morphological traits that identify each of these taxa in this region. With this, we address the need for reliable morphological markers to identify cryptic taxa and explore the ability of several easily identifiable morphological features to accurately resolve populations identified through genomic analysis, providing a useful tool for biodiversity study and conservation efforts of these keystone taxa.

4.3 Methods

4.3.1 Sampling Location and Methodology

Sampling was performed in August 2022, in the Whitsundays region of the Great Barrier Reef (GBR), at 5 inshore fringing reef sites located around Hook, Black and Hayman Islands ~30 km off the coast from Airlie Beach, Queensland (Fig. 4.1). The Whitsundays region is a major tourism hub for the GBR, but reefs have been severely affected by weather anomalies, particularly severe cyclones (e.g. Tropical Cyclone Debbie in 2017) that has led to persistently reduced hard coral cover at several inshore exposed reef sites within the archipelago (AIMS Long Term Monitoring, 2021; McLeod et al. 2019). The frequent occurrence and predicted increase in intensity of cyclones in this region (Cheal et al. 2017) is particularly threatening to tabular *Acropora* that are highly susceptible to breakage and dislodgement in storms (Oritz et a. 2021). Following the success of restoration activities in Northern regions of the GBR that target "high value" tourism sites (Howlett et al. 2022), Whitsundays reef sites were recently selected for targeted restoration in partnership with local tourism operators. The 5 sites targeted for CNP restoration activities, Blue Pearl Bay (BPB), Luncheon Bay (LB), Stonehaven Bay (SB), Black Island (BI) and Wonderwall (WW) – and the sampling sites for this current study (Fig. 4.1B) – were selected to explore the diversity and connectivity of tabular *Acropora* populations in a restoration framework.

Sampling methods were designed to maximise collection of tabular *Acropora* 'morphotypes' resembling the '*hyacinthus*' morphologies (as identified in Rasmussen et al. in review) across the five sites to maximise biodiversity representation (Emerson et al. 2017). We employed a targeted sampling methodology to ensure comprehensive representation of all morphological and

genetic variations of *A. hyacinthus, A. pectinata, A coralB* and *A. sp.7* sampled from each site. Most sites (WW, BI, BPB, SH) were characterised by low abundance of tabular *Acropora* and thus we sampled each colony encountered. However, at LB tabular *Acropora* were relatively more abundant and therefore we focused on sampling morphological replicates of each species. Species representation and rate of colonies sampled at each site was therefore inconsistent across sites (Supplementary Material. Fig. S4.1).

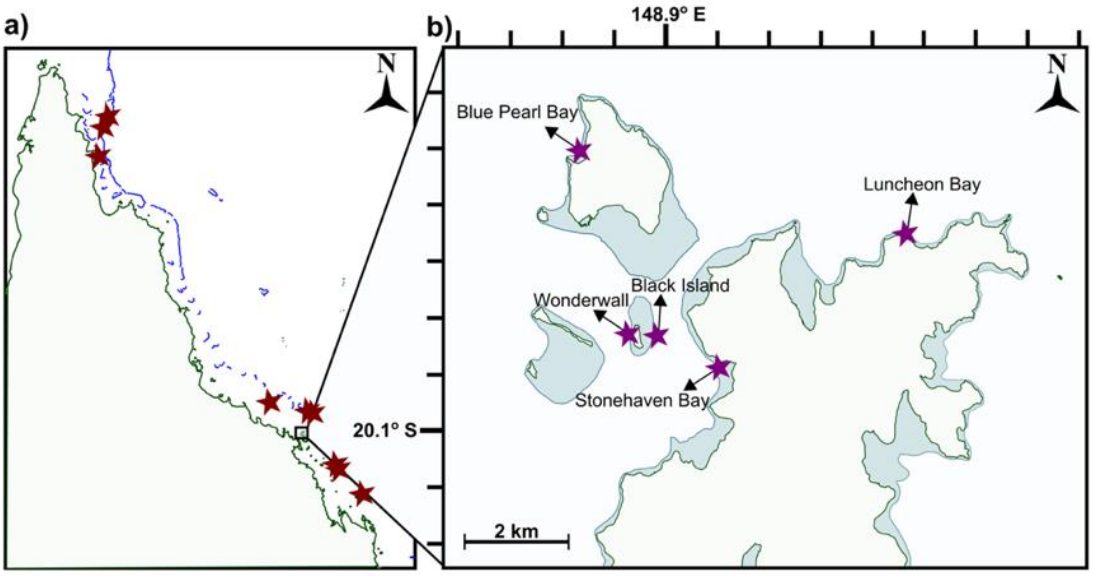

Figure 4.1 Sampling locations along the Great Barrier Reef (GBR) showing a) a map of the GBR sampling locations for **Chapter 2** specimens included in the present study (n= 9) with each red star indicating one specimen and b) inset map of sampling from the Whitsundays region with each purple star indicating a reef site sampled. Colonies sampled per site were BPB (n= 10), LB (n= 38), WW (n= 13), BI (n= 16) and SH (n= 8).

A total of 85 colonies were sampled via SCUBA by removing a 2-4 cm nubbin towards the colony edge that was immediately preserved in 99% molecular grade ethanol. *In situ* photographs were taken for all colonies with an Olympus TG-6 to aid species assessments and morphological analysis.

Photographs of each colony included a whole colony image, and macro images showing the vertical branchlets and radial corallites (Fig. 4.2). Additionally, 9 specimens from **Chapter 2** were included in the present study to act as anchor specimens for our genetic and morphological analysis for the species *A. hyacinthus*, *A. pectinata* & *A. coralB* bringing the sample count to n= 94 (Supplementary Material. Table S4.1). These specimens, also from the GBR (Fig. 4.1A), had already undergone extensive taxonomic assessments.

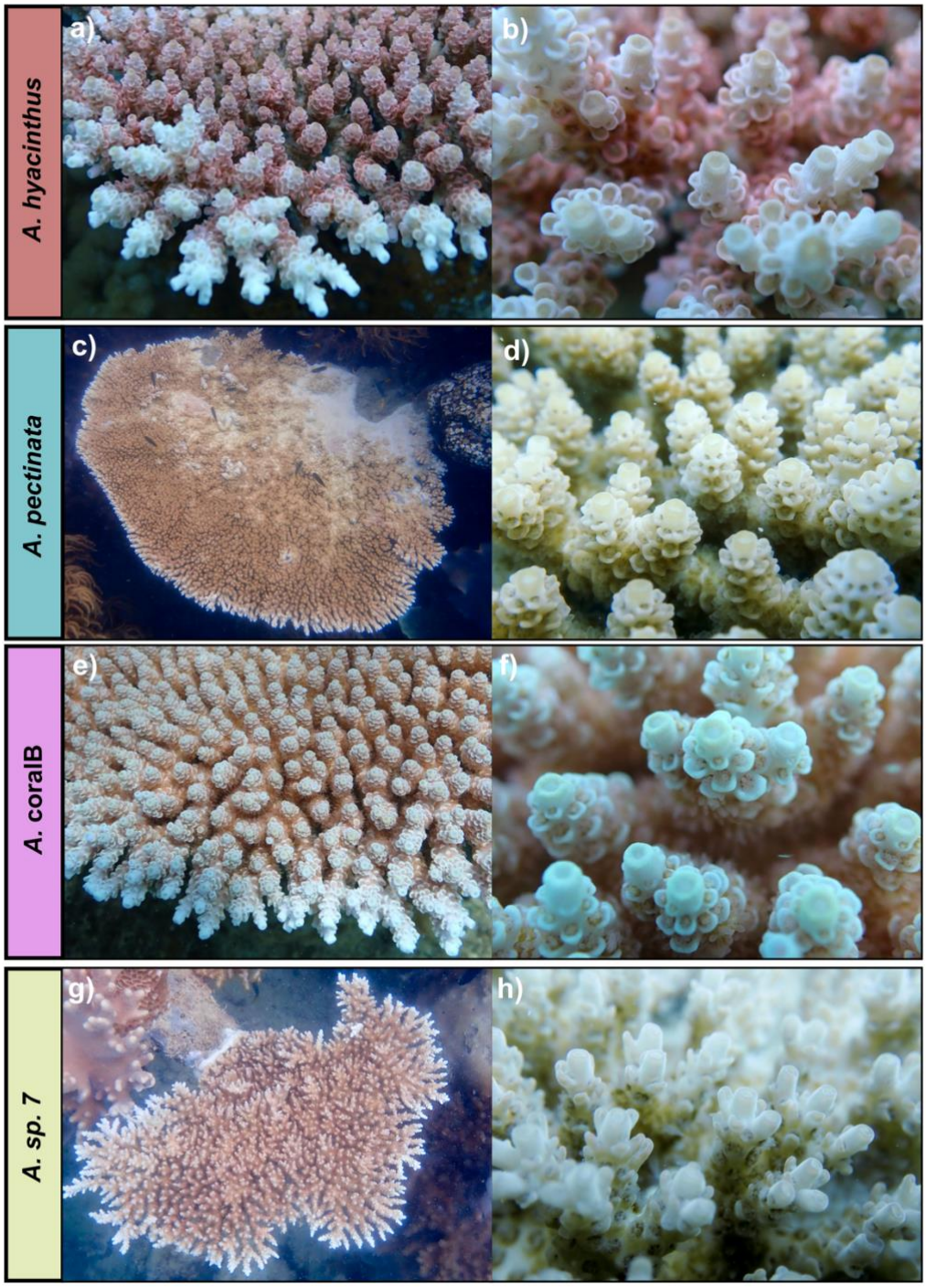

Figure 4.2 Photographs showing colony (first column) and corallite (second column) morphological features to be used for taxonomic identification and morphological analysis. Colonies shown are representatives of the four species targeted, being a,b) *A. hyacinthus,* c,d) *A. pectinata*, e,f) *A. coralB*, and g,h) *A. sp.7*.

4.3.2 DNA Extraction and Sequencing

DNA extraction and sequencing for generation of genome-wide single-nucleotide polymorphism (SNP) data was performed by Diversity Arrays Technology (DarT; University of Canberra, ACT). The DarT high density sequencing (2.5 mln reads) is a genome complexity reduction method of sequencing on Next Generation Sequencing (NGS) platforms (Jaccoud et al. 2001; Sansaloni et al. 2011). Genomic DNA was isolated according to DarTseq protocols using the NucleoMag kit (MACHEREY-NAGEL), and the extraction protocol can be found in the Supplementary Material (Protocol S4.1). SNPs were called using the DarT PL's calling algorithm (DarTsoft14) from approximately 357,420 unique sequences per sample. Samples were aligned to the reference genome for *Acropora millepora* (Acropora_amil_v2.1) and *Acropora hyacinthus* (Acropora_hyacinthus_chrsv1). Sequencing resolved 82,913 SNP loci across 92 individuals, with two specimens failing QC (BI_12 & LB_25, Supplementary Material. Table S4.1).

All resulting SNPs were filtered for quality control steps before population genetic analysis in R v.4.3.0 (R Core Team 2023) using the dartR package (v.2.9.7, Mijangos et al. 2022) unless otherwise stated. This package was specifically developed by Diversity Arrays Technologies (Gruber et al. 2018) to provide streamlined population genetic analysis on the SNP data recovered from their sequencing protocol. Upon initial interrogation, one sample was removed from the dataset that contained > 90% missing loci (SH_7), and four additional samples were removed after visual colony inspection (via *in situ* photographs) revealed they were unintentional replicates (Supplementary Material. Table S4.1). The remaining dataset was subject to the following filtering protocol; i) sequence tags with secondaries were filtered to retain just one SNP per tag at random, ii)

low coverage loci (< 5 read depth) were filtered to ensure only quality loci were retained, iii) SNPs with a repeatability < 0.99 were filtered, iv) loci were filtered at a threshold of 80% coverage, v) minor allele frequencies were filtered at a threshold of > 0.01, vi) individuals were filtered at a call rate of 80% coverage and vii) remaining monomorphic loci were filtered out. To ensure no contamination of Symbiodiniaceae in our data, we aligned sequences to available symbiont genomes (Aranda et al., 2016; Li et al., 2020; Shoguchi et al., 2013, 2018, 2021). All loci were free of symbiont contamination, and after filtering we retained 4,770 SNP loci and 81 individuals for downstream analysis.

4.3.3 Species Identification

To identify the populations – and therefore species – present we performed Discriminant Analysis of Principle Components (DAPC) and STRUCTURE analysis. We initially ran a DAPC using the find.clustes function (Jombart et al. 2010) in R v.4.3.0 (Rstudio Team 2023), where the optimal number of clusters (K_{DAPC}) was determined by the lowest Bayesian Information Criterion (BIC) score. In addition to this a STRUCTURE analysis was performed to provide additional population clustering and determine the proportion of individual ancestry to each population ($K_{STRUCTURE}$). STRUCTURE was run with the following parameters: Maximum number of clusters was set at K= 6 to account for the number of clusters determined by DAPC + 2, we set 5 runs for each assumed K with a 25,000 burn-in period and 100,000 MCMC iterations across each run. Results were analysed with pophelper (Francis 2017) in R Studio to identify the optimal mean log-likelihood (Mean L(K)) and delta (ΔK) $K_{STRUCTURE}$ to determine number of populations according to the Evanno method (Evanno

2005). Assignment to each population was averaged over the 5 runs and plotted to visualise individual ancestry. DAPC and STRUCTURE results were congruent, and we assigned individuals to populations according to KSTRUCTURE majority lineages. Anchor specimens (from **Chapter 2**) were used to assign species names to each population.

4.3.4 Population Genetics

Population genetics were next considered across the five reefs for each species (as well as with all the data combined) to examine both inter- and intraspecific genetic patterns. Initially, to identify closely related individuals that could present as clones, offspring, as well as siblings (*sensu* Speed & Balding 2015) in our dataset, a genomic relationship matrix (GRM) was computed using the gl.grm function in R. A low kinship value of 0.01 was set to identify any relationships above this threshold of genetic similarity. To quantify the genetic differentiation between species and between reefs F_{st} was calculated on the following scales, i) between species, ii) between sites (all species combined) and iii) between sites for species independently. F_{st} was computed with the function gl.fst.pop with 999 permutations to test for significance at 0.05. Sample size (n= 8) was insufficient for *A. coralB* populations to yield any F_{st} measurements.

AMOVA was performed using poppr v.2.9.4 (Kamvar et al. 2015) with 1,000 bootstraps to compare within and between population variation. Several AMOVAs were run; firstly, with all data combined to compare between species populations and between reef variation and then via separate analysis on each species to examine for any within population or between reef differences. Euclidean distance matrix and neighbour joining tree was constructed to visualise

the species assignments and relatedness amongst individuals and populations. 1000 bootstrap replicates were performed using Nei distances (function aboot) to identify branch support. Population structure and variation amongst individuals was further visualised by performing a principal component analysis (PCA) using glPCA function. Principle Components (PC) with an eigenvalue greater than 10, representing by the portion of the variation in the data that each PC represents, were considered.

STRUCTURE analysis on species populations was performed independently to further explore intraspecific variation. Parameters were again set to a 25,000 burn-in period followed by 100,000 MCMC with five runs per iteration of K, with the K values set to the number of sampling locations+1 for each species. Results were explored with pophelper (Francis 2017) to determine the optimal mean log-likelihood (Mean L(K)) and delta (ΔK) $K_{STRUCTURE}$ and plotted to visualise ancestry. Where $K_{STRUCTURE}$ resolved multiple intraspecific populations, individuals were assigned sub-populations when majority (> 50%) ancestry was of a unique population. One outlier specimen identified in the population genetic analysis (WW_11, Supplementary Material. Table S4.1) and confirmed by visual assessment (*in situ* photograph) was discarded from the dataset before individual species STRUCTURE and morphological analysis was performed.

4.3.5 Morphological Analysis

A morphological trait matrix was used to determine the set of visual traits that correctly identified species, and further if traits could delineate interspecific plasticity within species. A total of 10 morphological traits were recorded for each

sampled colony using *in situ* photographs taken from the time of sampling (Fig. 4.2). All traits were categorical in nature and were chosen according to traits identified in **Chapter 2** as potentially useful identifiable characteristics for the *Acropora* species in our current study. Traits that required physical measurements of colony features, such as branch length or corallite size, and hence precluded "rapid" species identification from photographs alone, were not included. We therefore recorded: 1) Branchlet Growth, 2) Colony Colour, 3) Incipient Axials, 4) Axial Colour, 5) Radial Shape, 6) Radial Angle, 7) Radial Uniformity, 8), Secondary Radials, 9) Radial Arrangement and 10) Rosette. Descriptions of all traits and categories are given in Supplementary Material S4.2 (and visual examples for each trait are shown in Fig. 4.2). All traits were recorded prior to genetic analysis to ensure no bias towards species assignments was present when generating the trait matrix.

Pearson's Chi-square (X^2) test for independence was first used to assess the goodness of fit of the morphological traits in explaining species clusters, enabling testing of the association between each morphological trait and the species assignments and identification of traits with a significant relationship to species identification. One trait variable – Radial Angle – had too few observations to meet the Chi-square test requirements and was discarded from further analysis. We performed Chi-square tests at a significance of 0.05 and 0.005 based on the standardized residuals (SR) and adjusted residuals (AR) that identify the variable and corresponding species most informative in explaining significant associations, as well as the strength and the direction of the relationship. Generally, residual values between -2 and +2 indicate the observed frequency is within the expected range and insignificant in explaining the

differences amongst groups, whereas values outside of this this range indicate a positive or negative association between the morphological trait and the species.

Two hierarchical clustering analyses were performed to visualise the clustering of individuals according to significant morphological variables in R v.4.3.0 (Rstudio Team 2023) using the package cluster v.2.1.4 (Maechler et al. 2022). Any traits that were found to be insignificant from Chi-squared tests were discarded. A dissimilarity matrix was then computed with the daisy function using the gower metric for measuring distances between categorical or mixed data types (Cook et al. 2022). Agglomerative Nesting (AGNES) Clustering was performed using the hclust function with Ward linkages. The agglomerative coefficient (AC) was computed to determine extent of clustering structure, with values closer to 1 with stronger clustering. AC was computed for 4 different linkage methods (complete, average, single and ward) to select the method with the highest clustering strength. In parallel, Divisive Analysis (DIANA) Clustering was also performed with the diana function, and the divisive coefficient (DC) measured to determine the amount of clustering structure found in a similar measure to AC. Branch tips were coloured according to species assignments, and further into sub-populations identified by STRUCTURE analysis to explore inter- and intraspecific patterns of morphological clustering.

4.4 Results

4.4.1 Species Identifications and Population Structure

Four population clusters (K_{DAPC}) were initially identified as optimum from DAPC determined from the lowest Bayesian Information Criterion (BIC), (Supplementary Material, Fig. S4.2). Further verification by exploring lineages

with STRUCTURE analysis ($K_{STRUCTURE}$) determined by both mean log-likelihood (Mean L(K)) and delta (ΔK) according to the Evanno method (Evanno 2005) resolved five optimum clusters ($K_{STRUCTURE}$= 5). However, one outlier individual (WW_11) in STRUCTURE, represented a single specimen in its lineage, where DAPC placed this individual within the *A. hyacinthus* cluster (Fig. 4.3); consequently, this individual was discarded from the dataset. Re-running clustering from these 80 individuals resolved the same four clusters from DAPC and STRUCTURE (Fig. 4.3). With the genetic anchor specimens resolving the following species populations: *A. hyacinthus* (n = 13), *A. pectinata* (n = 36), *A. coralB* (n = 7) and *A. sp.7* (n = 24).

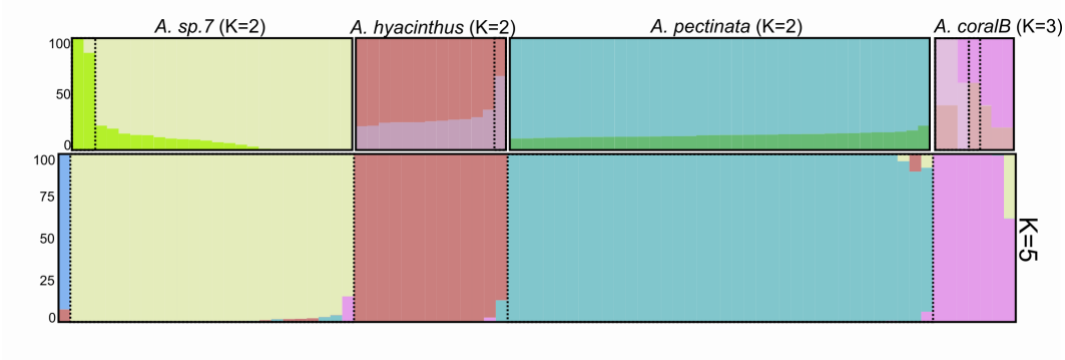

Figure 4.3 STRUCTURE plot showing proportion of individual ancestry to each species. Dotted lines indicate separation between clusters. The bottom plot indicates STRUCTURE analysis run on all data combined, with one outlier specimen (WW_11). The top plots show intraspecific ancestry when STRUCTURE was run separately on each species.

Only three individuals of *A. coralB* were identified as having some genetic similarity according to Identity by Decent (IBD) analysis (Endelman & Jannink 2012), with a range between 0.01 – 0.016 kinship values. These colonies included one from the present study (LB_15, Luncheon Bay) and two anchor specimens (19.GBR.57 & 19.GBR.122). Based on Speed & Balding (2015) kinship values

these individuals are potential "second cousin" relations. No other species clusters resolved any close relations.

Strong genetic differentiation was observed between species populations with high connectivity patterns indicated between reefs. When comparing between population differentiation, F_{st} measurements were highest between *A. pectinata* and *A. coralB* populations (0.336, $p < 0.001$ Table 4.1), and lowest between *A. hyacinthus* and *A. sp.7* ($F_{st} = 0.194$, $p = < 0.001$, Table 4.1). All species populations exhibited a high degree of genetic distance (Table 4.1). When considering all species as one and comparing between reefs, populations from the southern GBR exhibited significant differentiation to all other reefs and regions ($F_{st} \geq 0.061$, $p < 0.001$, Table 4.1). Moderate genetic distance was found between the Central GBR and both BI ($F_{st} = 0.058$, $p < 0.001$, Table 4.1) and WW ($F_{st} = 0.051$, $p < 0.001$, Table 4.1), which are both inshore sites in the Whitsundays (Fig. 4.1B). No significant genetic distances between reefs were recovered when exploring intraspecific genetic distance of the four species across Whitsundays sites (Supplementary Material. Table S4.3).

Table 4.1 Pairwise F_{st} scores (below diagonal) and significance (p-value above diagonal) for i) between species of tabular *Acropora* and ii) all specimens between reefs from the Whitsundays. Species are: A.pec (*A. pectinata)*, A.spB (*A. coralB)*, A.hya (*A. hyacinthus*) and *A. sp.7.* Reefs are: SH (Stonehaven), LB (Luncheon Bay), BPB (Blue Pearl Bay), BI, (Black Island), WW (Wonderwall), and South, Central and North indicating GBR regions for specimens from **Chapter 2**. Significant results are highlighted in **bold**. Significance of genetic distance is based on Wright (1978) with 0.05-0.15 = moderate, 0.15-0.25 = strong, and >0.25 = very strong.

i) SPECIES	A.pec	A.spB	A.sp.7	A.hya

A.pec		**0.000**	**0.000**	**0.000**
A.spB	**0.336**		**0.000**	**0.000**
A.sp.7	**0.252**	**0.266**		**0.000**
A.hya	**0.316**	**0.272**	**0.194**	

ii) REEF	SH	LB	SOUTH	BPB	BI	NORTH	WW	CENTRAL
SH		0.000	**0.000**	0.000	1.000	0.038	1.000	0.001
LB	0.013		**0.000**	1.000	0.000	0.985	0.000	0.502
SOUTH	**0.100**	**0.121**		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
BPB	0.022	-0.010	**0.126**		0.000	0.657	0.000	0.998
BI	-0.009	0.020	**0.169**	0.028		0.000	1.000	**0.000**
NORTH	0.013	-0.014	**0.061**	-0.003	0.044		0.000	0.000
WW	-0.015	0.020	**0.161**	0.023	-0.009	0.042		**0.000**
CENTRAL	0.028	0.000	0.048	-0.025	**0.058**	0.037	**0.051**	

Highest genetic variation was found between individuals (AMOVA, 50.38% variation, p = 0.01, Table 4.2) followed by between population variation (27.62%, p = 0.01, Table 4.2). For individual species no significant genetic differences were evident between reefs, although within reef and within samples genetic differences were resolved (AMOVA, $p < 0.03$, Table 4.2) with between sample differences explaining the greatest variation in all instances (within samples > 58% variation, Table 4.2).

Visual inspection of our neighbour-joining tree revealed all four species as strong genetic clusters with 100% bootstrap support for each clade (Supplementary Material, Fig. S4.3), although with little within-clade structure resolved. We found similar results with PCA clustering (Supplementary Material. Fig. S4.4), with 4 main clusters resolved, although low support from each principal component axis (Supplementary Material. Fig. S4.4).

Final STRUCUTRE analysis performed on species populations independently identified some intraspecific structure and genetic outliers amongst *A. hyacinthus*, *A. coralB* and *A. sp.7* populations. *A. hyacinthus* favoured a

$K_{STRUCTURE}$= 2 with just one individual demonstrating affinity to the second sub-population (A. hyacinthus-ii); however, all individuals exhibited some proportion of mixed ancestry (Fig. 4.3). *A. coralB* resolved 3 populations ($K_{STRUCTURE}$= 3, Fig. 4.3) and high admixture amongst all individuals. *A. sp.7* yielded 2 sub-populations ($K_{STRUCTURE}$= 2, Fig. 4.3) with 2 individuals of clear affinity to A. sp7-ii (Fig. 4.3).

Table 4.2 Analysis of Molecular Variance (AMOVA) results testing for significant differences between species, reefs and samples using 4,770 SNP loci. The first analysis was run on all individuals combined and tested for between species differences. The analysis performed on species populations independently tested for differences between reef populations. Df (degrees of freedom), SS (sum of squares), MS (mean of squares), F (Sigma = estimated variance), % var (percent of variation), *p*-value (significance), Phi (phi statistic). Significant results are highlighted in bold.

		Df	SS	MS	F	% var	*p*	Phi
All populations	between pop	3	6247.121	2082.374	50.01505	**27.61967**	**0.01**	**0.276197**
	between samples within pop	81	13550.27	167.2873	36.21746	**20.00026**	**0.01**	**0.276322**
	within samples	85	8062.454	94.8524	94.8524	**52.38007**	**0.01**	**0.476199**
	Total	169	27859.85	164.8512	181.0849	100		
		Df	SS	MS	F	% var	*p*	Phi
A. hyacinthus	between reefs	4	2295.024	573.756	30.525	8.803	0.140	0.088
	between samples within reef	9	3868.690	429.855	113.642	**32.775**	**0.010**	**0.359**
	within samples	14	2836.000	202.571	202.571	**58.422**	**0.010**	**0.416**
	Total	27	8999.714	333.323	346.738	100.000		
		Df	SS	MS	F	% var	*p*	Phi
A. coralB	between reefs	4	3237.375	809.344	-32.542	-5.347	0.840	0.370
	between samples within reef	3	2696.500	898.833	257.729	**42.350**	**0.030**	**0.402**
	within samples	8	3067.000	383.375	383.375	**62.997**	**0.010**	-0.053
	Total	15	9000.875	600.058	608.563	100.000		
		Df	SS	MS	F	% var	*p*	Phi
A. pectinata	between reefs	6	1624.452	270.742	-0.962	-0.438	0.770	0.262
	between samples within reef	29	8091.265	279.009	58.550	**26.675**	**0.010**	**0.266**
	within samples	36	5828.718	161.909	161.909	**73.764**	**0.010**	-0.004
	Total	71	15544.434	218.936	219.497	100.000		
		Df	SS	MS	F	% var	*p*	Phi
A. sp7	between reefs	4	1970.295	492.574	1.872	0.518	0.130	0.327
	between samples within reef	19	9041.811	475.885	116.197	**32.138**	**0.010**	**0.323**
	within samples	24	5843.797	243.492	243.492	**67.345**	**0.010**	0.005
	Total	47	16855.904	358.636	361.560	100.000		

4.4.2 Morphology

Initial analysis of the morphological trait matrix identified all traits as significant in explaining the species populations (X^2, (60, n = 80) = 267.57, $p < 0.001$, Table 4.3). However, when examining traits independently, four traits were not significant in association with the species populations (Secondary Radials, Radial Arrangement, Radial Consistency & Incipient Axials, $p > 0.05$, Table 4.3), and therefore independent from species clustering, and so discarded from the trait matrix for downstream analysis. The five remaining traits were found to have

associations with the species populations ($p < 0.002$, Table 4.3). Post-hoc testing of standardized and adjusted residuals found traits contributing most to delineating distinct species clusters – importantly, the same relationships were found by both standard and adjusted residuals (Supplementary Material. S4.4). For *A. coralB* just one variable, the Absence-Axial Tip, delineated this species from the other species (SR = 5.497, < 0.001 Table 4.4), whilst this trait could not delineate amongst any other species. The Indeterminate-Branchlet Growth was also only significant in delineating *A. hyacinthus* from the other species (SR = 2.555, p = 0.01, Table 4.4), and again had no effect in identification of the other three species. *A. hyacinthus* also exhibited a strong positive relationship with a Dark-Axial Tip (SR = 5.785, p = 0.000, Table 4.4) and either a Dusty Pink-Colour (SR = 2.368, p = 0.018, Table 4.4) or Dark Pink-Colour (SR = 2.925, p = 0.003, Table 4.4). A single variable was also found to have a strong positive relationship with *A. pectinata*, which was Nude-Colour (SR = 2.636, p = 0.008, Table 4.4); however, in this case, a significant negative relationship was found with three other variables indicating a lack of these traits in being discriminatory (Table 4.4). Three variables provided strong positive associations with *A. sp.7*, being Soft Pink-Colour (SR = 3.469, p = 0.001, Table 4.4), Absent-Rosette (SR = 4.383, p = 0.000, Table 4.4) and Cochlearform-Radial Shape (SR = 5.422, p = 0.000, Table 4.4).

Table 4.3 Chi-square results of morphological analysis. P-values with an asterisk (*) indicate significant results at a significance of 0.005. The results for the Incipient Axial trait were significant at a 0.05 significance threshold, although not at a 0.005 significance.

	df	X^2	p-value
All	60	267.571	**0.000***
Colour	9	53.143	**0.000***
Secondary Radial	3	2.623	0.453
Axial tip	6	86.834	**0.000***
Rosette	3	35.099	**0.000***
Radials arrangement	3	6.809	0.078
Radial consistency	3	6.011	0.111
Radials	3	54.194	**0.000***
Incipient Axials	3	7.947	0.047^
Branchlet Growth	3	14.911	**0.002***

Table 4.4 Chi-square standard residuals for morphological traits identified as significant. Traits are in the left-hand column with variables of each trait in the second column. Values are Standard Residuals calculated from Pearson's Chi-square analysis with values greater than ± 2 identified as significant variables in explaining the corresponding species population. Shading of significant results is according to the strength and direction of the relationship. Significance of each value is shown with an asterisk with * = $p < 0.05$, ** = $p < 0.005$ & *** = $p < 0.000$.

		A. hyacinthus	*A. pectinata*	*A. coralB*	*A. sp.7*
Colour	Nude	-0.875	**2.636***	-0.727	**-2.192***
	Soft pink	**-2.016***	**-2.162***	1.225	**3.469****
	Dusty pink	**2.368***	-0.843	0.359	-0.904
	Dark pink	**2.925****	-1.019	-0.887	-0.426
Axial tip	Dark_ring	**5.785*****	-1.713	-1.107	-1.561
	Soft_ring	**-2.253***	1.259	**-2.153***	1.279
	Absent	-1.453	-0.765	**5.497*****	-0.962
Rosette	Yes	0.863	1.061	0.634	**-2.277***
	Absent	-1.662	**-2.043***	-1.220	**4.384*****
Radials	Labellate	0.922	1.533	0.676	**-2.922****
	Cochlearform	-1.710	**-2.846****	-1.255	**5.422*****
Branchlet Growth	Uniform	-1.875	0.124	1.149	0.608
	Indeterminate	**2.555***	-0.169	-1.565	-0.828

Hierarchical clustering of the 5 informative discriminatory traits revealed a stronger cluster strength for AGNES (AC = 0.987, Fig. 4.4) clustering with ward linking, compared to DIANA (DC = 0.956, Supplementary Material. Fig. S4.5) clustering. We therefore focused all subsequent analysis on AGNES clustering. Visual inspection revealed clusters to converge on the main species clusters, with some mixing of individuals (Fig. 4.4). Interestingly, *A pectinata* formed two polyphyletic clades, possibly suggesting 2 distinct morphotypes. Also, *A. s.p.7* clustered as a sister lineage to the other species (Fig. 4.4), highlighting the distinct morphology of this species. A single specimen was identified each for *A. hyacinthus* and *A. sp.7* populations that resulted from the main cluster in both morphological clustering and STRUCTURE analysis (Fig. 4.4), thereby indicating some evidence of a unique morphology that correlated with some degree of genetic diversity.

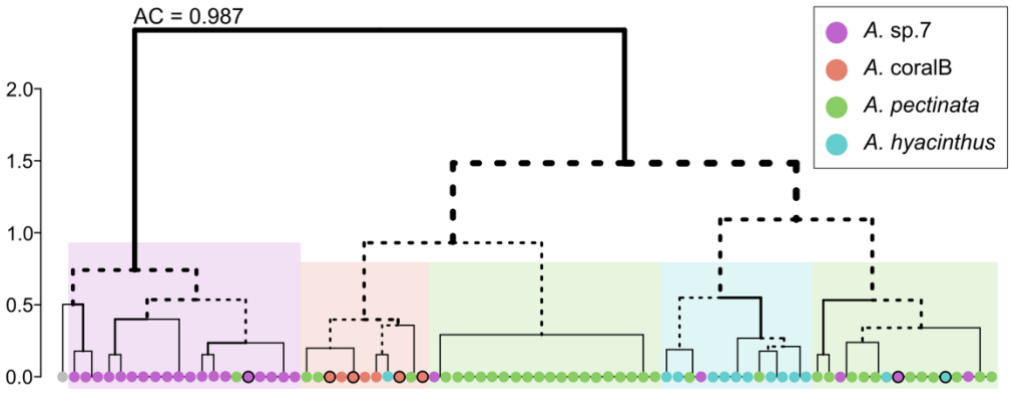

Figure 4.4 Agglomerative Clustering (AGNES) dendrograms of the five morphological traits identified as significant in Chi-squared analysis, with an Agglomerative Coefficient (AC) of 0.987 indicating the strength of the clustering. Colours indicate species, with Purple = *A. sp.7*, Red = *A. coralB*, Green = *A. pectinata* and Blue = *A. hyacinthus* and each branch tip represents a single colony, coloured by species. Branch tip circles with black outline indicate specimens that

formed genetic sub-populations in STRUCTURE analysis. Shading indicates clusters where majority of individuals form a single species.

4.5 Discussion

Capacity to correctly identify distinct evolutionary units, especially at the species level, is crucial for biodiversity research and conservation (Pante et al. 2015) but is often difficult for corals when morphologically similar and closely related taxa occur in sympatry (Thomas et al. 2018; Sheets et al. 2018; Rose et al. 2021). By exploring population genetics of tabular *Acropora* using a combined molecular and morphological framework across sites selected for restoration activities, we identified novel morphological features to resolve four closely related species. Specifically, we identified five traits – colony colour, radial corallite shape, presence of a rosette, branchlet growth and axial colour – to successfully delineate these taxa. We also found evidence of low intra-specific genetic differences (but high inter-specific differences) across reefs in the GBR Whitsundays region further highlighting the importance of identifying distinct species units when analysing population genetics. We discuss how these outcomes impact management activities, and notably the application of morphological tools through a restoration lens, whilst discussing future directions for the study of novel morphological features of *Acropora* – and broadly all coral – in species delineations.

Accounting for increased or maintained genetic diversity in restoration practices is key for boosting resilience of restored populations (Quigley et al. 2022). We observed low intra-specific genetic diversity and relatively high inter-specific genetic differences across all species and sites sampled in the

Whitsundays (Table 4.1), with no evidence of hybrid individuals. However, there was some intra-specific admixture in STRUCTURE analysis for *A. hyacinthus* (n= 1). *A. coralB* (n= 5) and *A. sp.7* (n= 2). Further, no clones or closely related individuals were observed within the Whitsundays. Collectively, these outcomes suggest high intra-specific genetic mixing and divergence of these species over time, and perhaps some past introgression (Mao et al. 2019). Such a trend is consistent with inaccuracies in connectivity patterns that have been reported previously when 'cryptic' taxa are not considered (Pante et al. 2015, Sheets et al. 2018). Whilst past reports on population structure for *A. hyacinthus* across the central to northern Pacific Ocean have consistently recovered multiple 'cryptic' lineages (Ladner & Palumbi 2012; Suzuki et al. 2016; Rose et al. 2021), the intra-specific population structure of individual 'lineages' remains largely unresolved, especially within the GBR. Where individual cryptic lineages of *A. hyacinthus* have been investigated in Japan and Micronesia, little population structure was observed (Cros et al. 2016; Nakabayashi et al. 2019), in line with our results of high intraspecific mixing of genetic material. Although it is possible the relatively small spatial scale of our current study (~12 km between the furthest reefs) may explain the low intra-specific genetic structure, this is in line with the scale of reef restoration practices where propagules are often sourced from the immediate reef surroundings (Howlett et al. 2021).

Morphological features of coral skeletons have remained the predominant basis of species delineations and taxonomies since coral species were first described (Kitahara et al. 2016). Such a basis is increasingly changing with the advent of molecular technologies (Bridge et al. 2023) that is in turn revealing the inaccuracies in traditional morphological species when compared with

phylogenetic lineages (Wiens 2004; Kitahara et al. 2016). Limited capacities of traditional morphological markers to identify species units is often alluded to when morphologically similar species are unable to be identified *in situ* (Ladner & Palumbi 2012; Suzuki et al. 2016). However, in exploring novel morphological features, our study demonstrates that it is possible to successfully delineate species into genetic lineages using morphological traits (Table 4.4), whilst also resolving uninformative features in species identification (Table 4.3). Morphological features such as corallite structure and colony colour have successfully delineated taxa amongst three tabular *Acropora* species in Japan (Ramírez-Portilla et al. 2021), an outcome we similarly demonstrate in delineating tabular *Acropora* species on the GBR. In line with previous observations (Ramírez-Portilla et al. 2021), we observed that colony colour is useful in delineating species in the field. Recent broad sampling along the GBR found evidence of morphological clustering amongst specimens of *A. hyacinthus* from the Palm Islands group in the central GBR, and for a south-eastern Australian population, indicating some ecological or geographic plasticity (**Chapter 2**).As such, at present, it remains unknown if our findings have broader applications to these taxa across the entire GBR, as opposed to being applicable only at the local scale for the Whitsundays region. Even so, we suggest that a restricted geographical application of this morphological-based taxonomic resolution caries important use for management.

Outcomes from our current study have consequences for the study and continued management and conservation of these tabular *Acropora* species. Firstly, at least 4 morphologically similar species of tabular *Acropora* – that previously would have all been considered *A. hyacinthus* – occur in sympatry in

this region of the Whitsundays. With limited knowledge of the true species abundances and distribution of these taxa an inability to identify each lineage can affect results of ecological studies as well as biological experimentation where results could be misinterpreted if more than one species are included in comparative experimentation. This raises questions for managers and practitioners around species representation, resilience and ultimately the biodiversity represented from such endeavors (Shaver et al. 2022). For example, caution has been raised for active restoration efforts in Japan for *A. tenuis* where population genetic analysis uncovered two sympatric cryptic populations – where any activity to manipulate wild abundances was considered irresponsible until practitioners were able to identify each lineage (Zayasu et al. 2021). Such caution may similarly apply for the GBR, where three populations of tabular *Acropora* were recently uncovered (when sampling was intended for *A. hyacinthus*) that represented three distinct species with high intra-specific gene flow (but low interspecific gene flow) amongst lineages (Suggett et al. 2022; Howlett et al. in review). Such examples demonstrate that current management activities likely under-account for biodiversity where identification of cryptic taxa is not resolved. Not only does this carry impacts for asexual propagation activity (Howlett et al. in review), but also may impact the effectiveness of sexual propagation crossing amongst parent colonies (Ramirez-Portilla et al. 2021).

By ensuring intra- and inter-specific diversity is represented, restoration practices aim to promote reefs that maintain genetic diversity - a key factor for future adaption and resilience (Drury & Lirman 2017). Here, our finding of low intra-specific genetic differentiation – suggesting high mixing of genetic material within species – is positive from a restoration lens where local sampling efforts of

individual species can yield the high genetic diversity that is targeted as best practice (Drury & Lirman 2017; Quigley et al. 2022). However, as with other reef biota (e.g., sponges, Griffiths et al. 2020), repeated sampling of the same donor colonies for propogation should be avoided to ensure genetic clones are not overrepresented. This also highlights the need for reliable species identifications to ensure species diversity – and not just broad morphological diversity - is represented. Additionally, whilst tabular *Acropora* in general have been described as keystone taxa for reef resilience and recovery on the GBR (Ortiz et al. 2021), studies in America Samoa have found differences in both growth rates and bleaching resilience amongst putative tabular *Acropora* species (Rose et al. 2021). Considering this, future restorations efforts may also focus on boosting target taxa to improve overall resilience to future stressor events. Such a notion clearly requires further study on the thermal tolerances of GBR taxa and hinges on the ability of practitioners to accurately identify these taxa.

Ultimately, with the easily identifiable morphological features identified in our present study we provide the opportunity for a low-cost and scalable identification tool to be used for both research and conservation. Going forward, field testing will determine the useability of these results e.g., how well can practitioners and researchers ultimately use these morphological markers to delineate species? We encourage more taxonomic revisions that include robust morphological analysis to ensure that when putatively 'cryptic' species are discovered and described, they are presented alongside morphological identification tools that can be widely used by both expert and non-expert audiences.

4.6 Acknowledgements

Thank you to all the crew of the Kiana, Brent Chatterton, Jack McAvaney and divemaster Maddie Gablehouse who worked with our team to coordinate fieldwork research efforts. We also want to thank all the researchers from the Climate Change Cluster (C3) from the University of Technology Sydney (UTS) for their assistance with field work and planning of this expedition. All field work was performed on the Great Barrier Reef under Great Barrier Reef Marine Park Authority (GBRMPA) permit G22/46543.1 (**EFC**). This research was supported by an Australian Government Research Training Program (RTP) Fee Offset Scholarship to **SHR**. Fieldwork through the Coral Nurture Program in the Whitsundays was supported by The Reef Islands Initiative is a Great Barrier Reef Foundation program, supported by funding from Lendlease, the Australian Government's Reef Trust, the Queensland Government and the Fitzgerald Family Foundation. Delivery of the Whitsunday Reef Islands Initiative is managed by Reef Catchments and is in partnership with the local Reef community including Traditional Owners, reef managers, scientific researchers, and tourism operators.

4.7 Supplementary Material

4.7.1 Supplementary Methods

Protocol S4.1

DNA extraction and sequencing for generation of genome-wide single-nucleotide polymorphism (SNP) data was performed by Diversity Arrays Technology (DArT; University of Canberra, ACT). The DArT high density sequencing (2.5 mln reads) is a genome complexity reduction method of sequencing on the Next Generation Sequencing (NGS) platforms (Jaccoud et al. 2001; Sansaloni et al. 2011). Samples were initially prepared by submerging a

tissue sample (10-15 mg) in molecular grade ethanol and shipped to Diversity Arrays Technology for subsequent DNA extraction and sequencing. Genomic DNA was isolated according to DArTseq protocols using the NucleoMag kit (MACHEREY-NAGEL). Briefly, samples were overlaid with 50 µL of a T1 Buffer and 6.25 µL of proteinase K and digested overnight at 60 °C. Centrifuged lysate was then transferred into a deep well plate and agitated with 6 µL beads and 90 µL MB2. Final extraction steps were then performed by a Tecan T100 robot according to DArT PL script. A total of 125 assays were performed with the PstI and HpaII restriction enzymes (Sansaloni et al. 2011) and "mixed fragments" were amplified in 30 rounds of PCR under the following conditions: 94°C for 1 minute, then 30 cycles of 94°C for 20 seconds, 58°C for 30 seconds and 72°C for 45 seconds, and finished with 72°C for 7 minutes. Post-PCR products were subject to 100 cycles of sequencing on an Illumina NovaSeq sequencer. Filtering on raw sequence reads was performed to remove barcode regions with < 30 Phred score (min 75% pass) and whole reads with < 10 Phred score (min 50% pass). SNPs were called using the DArT PL's calling algorithm (DArTsoft14) from approximately 357,420 unique sequences per sample. Samples were aligned to the reference genome for *Acropora millepora* (Acropora_amil_v2.1) and *Acropora hyacinthus* (Acropora_hyacinthus_chrsv1).

4.7.2 Supplementary Figures

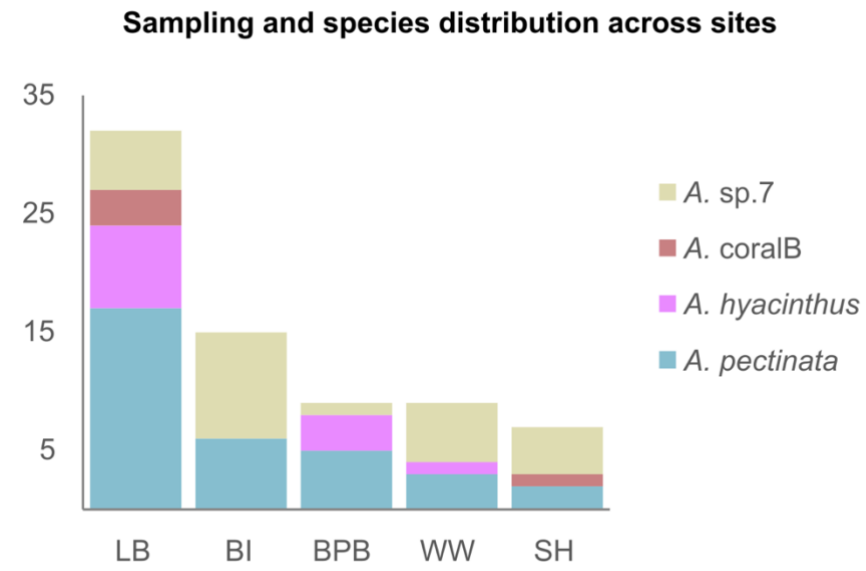

Figure S4.1 Distribution of species sampled across sites, with each bar indicating a sampling location in the Whitsundays region of the GBR, and each colour representing one of the four targeted species indicated in the key to the right. Sampling sites correspond to SH (Stonehaven), LB (Luncheon Bay), BPB (Blue Pearl Bay), BI, (Black Island), WW (Wonderwall).

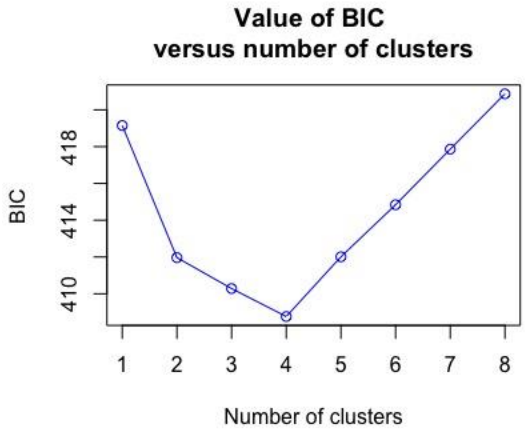

Figure S4.2 Plotted results from Discriminant Analysis of Principle Components (DAPC). Optimal number of clusters (K_{DAPC}) is chosen to be the lowest Bayesian Information Criterion (BIC) score ($K_{DAPC} = 4$).

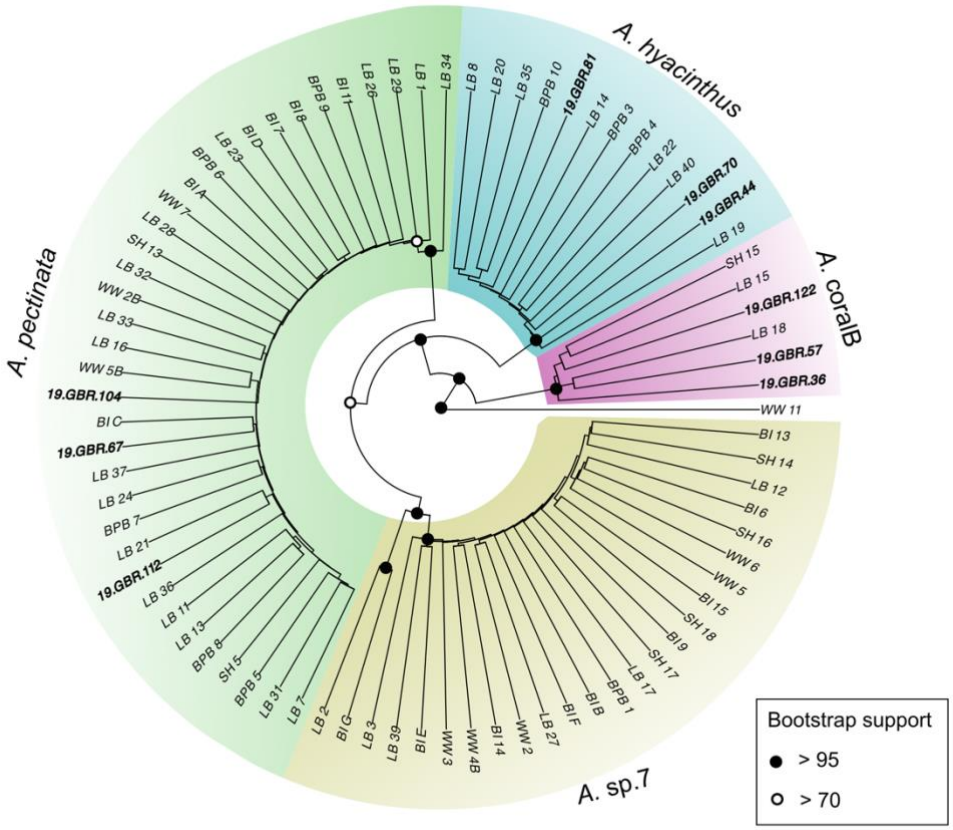

Figure S4.3 Neighbour Joining tree computed with SNP loci using Euclidean distances and 1000 bootstraps. Individuals highlighted in bold are anchor specimens from **Chapter 2** used to identify species. Node support is only shown when bootstrap values were > 70.

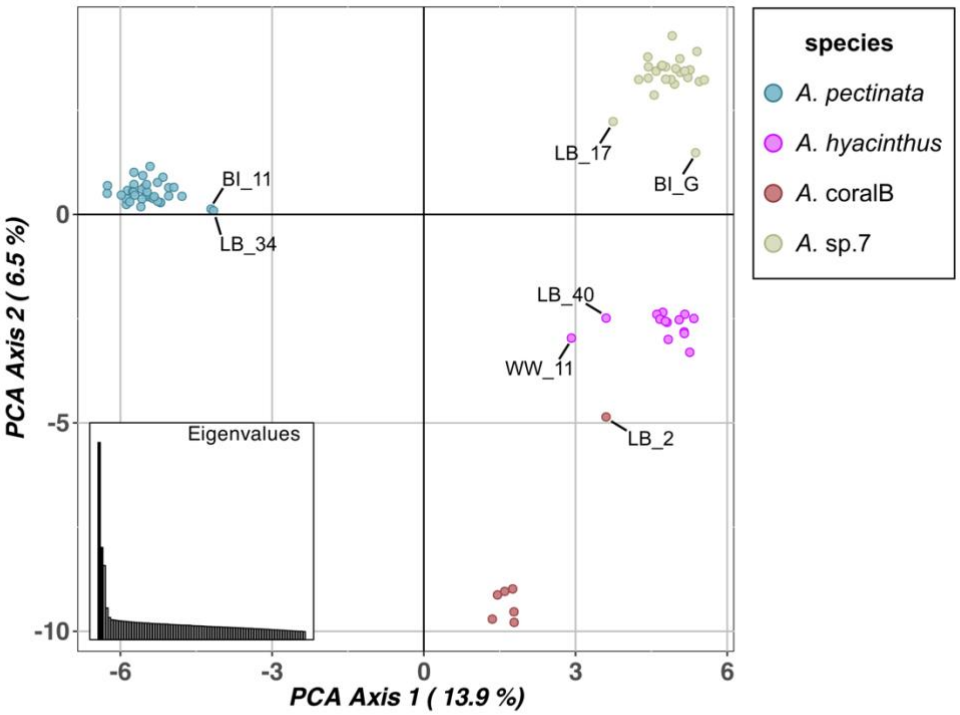

Figure S4.4 Principle Component Analysis (PCA) plot showing clustering of individuals according to the genetic species hypothesis populations. PCA aixs show support of principal component in explaining the data. Specimens with labels indicate outlier individuals that fell outside of the main cluster.

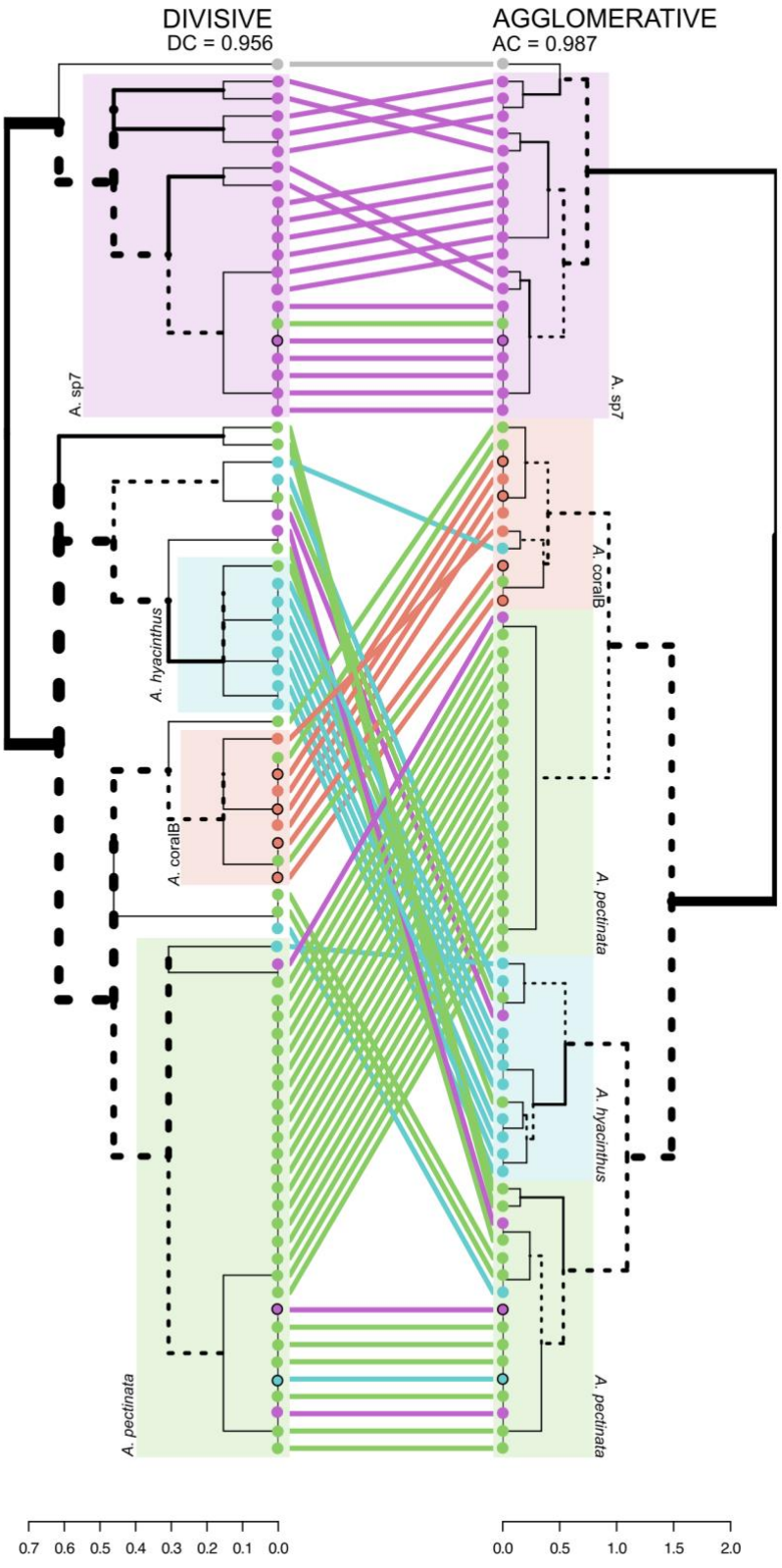
DIVISIVE
DC = 0.956
AGGLOMERATIVE
AC = 0.987
A. sp7
A. hyacinthus
A. coralB1
A. pectinata
A. sp7
A. coralB
A. pectinata
A. hyacinthus
A. pectinata
0.7 0.6 0.5 0.4 0.3 0.2 0.1 0.0
0.0 0.5 1.0 1.5 2.0

Figure S4.5 Comparison of AGNES & DIANA cluster dendrograms of the five morphological traits identified as significant in Chi-squared analysis. The alignment of these two clusters methods resolved an entanglement coefficient of 0.240 indicating a good alignment between the methods. Colours indicate species assignment, with Purple = *A. sp.7*, Red = *A. coralB*, Green = *A. pectinata* and Blue = *A. hyacinthus* and each branch tip represents a single colony. Branch tip circles with black outline indicate specimens that formed genetic sub-populations in STRUCTURE analysis. Shading indicates clusters where majority of individuals form a single species.

4.7.3 Supplementary Tables

Table S4.1 Sample collection metadata and loci captured for samples in the current study. Sample ID's beginning with 19.GBR* and species anchor specimens from **Chapter 2** (see methods). Items that failed QC were discarded from the analysis.

Sample_ID	GBR section	Location	Site	Permit	loci	Failed_QC
SH_5	GBR North-Central	Hook Island	Stonehaven donor site #1	G22/4654 3.1	53122	
SH_7	GBR North-Central	Hook Island	Stonehaven donor site #1	G22/4654 3.1	5925	Y, > 90% missing data
SH_13	GBR North-Central	Hook Island	Stonehaven donor site #1	G22/4654 3.1	53168	
SH_14	GBR North-Central	Hook Island	Stonehaven donor site #1	G22/4654 3.1	48453	
SH_15	GBR North-Central	Hook Island	Stonehaven donor site #1	G22/4654 3.1	46061	
SH_16	GBR North-Central	Hook Island	Stonehaven donor site #1	G22/4654 3.1	44411	
SH_17	GBR North-Central	Hook Island	Stonehaven donor site #1	G22/4654 3.1	46396	
SH_18	GBR North-Central	Hook Island	Stonehaven donor site #1	G22/4654 3.1	51092	

BPB_1	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	54273	
BPB_2	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	50777	Y, replicate (BPB_4)
BPB_3	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	52162	
BPB_4	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	52587	
BPB_5	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	54498	
BPB_6	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	55812	
BPB_7	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	55667	
BPB_8	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	55709	
BPB_9	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	54139	
BPB_10	GBR North-Central	Hayman Island	Blue Pearl Bay Nursery site #1 (Bommie #2)	G22/4654 3.1	52175	
WW_2	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	54526	
WW_3	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	53896	
WW_5	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	54860	

WW_6	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	52279	
WW_7	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	56256	
WW_8	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	54060	Y, replicate (WW_4B)
WW_9	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	52434	Y, replicate (WW_2B)
WW_11	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	46303	outlier_(morphology & STRUCTURE)
WW_1_A &B	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	40362	Y, > 20% missing data
WW_2_A &B	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	55226	
WW_3_A &B	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	50091	Y, replicate (WW_7)
WW_4_A &B	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	55075	
WW_5_A &B	GBR North-Central	Black Island	Wonderwall donor site #2	G22/4654 3.1	51529	
BI_6	GBR North-Central	Black Island	Black Island_1	G22/4654 3.1	48493	
BI_7	GBR North-Central	Black Island	Black Island_1	G22/4654 3.1	54390	
BI_8	GBR North-Central	Black Island	Black Island_1	G22/4654 3.1	54311	
BI_9	GBR North-Central	Black Island	Black Island_1	G22/4654 3.1	49995	
BI_11	GBR North-Central	Black Island	Black Island_1	G22/4654 3.1	50697	
BI_12	GBR North-Central	Black Island	Black Island_1	G22/4654 3.1	n/a	Y, failed sequencing QC
BI_13	GBR North-Central	Black Island	Black Island_1	G22/4654 3.1	52999	
BI_14	GBR North-Central	Black Island	Black Island_1	G22/4654 3.1	54140	
BI_15	GBR North-Central	Black Island	Black Island_1	G22/4654 3.1	53188	
BI_A	GBR North-Central	Black Island	Black Island_2	G22/4654 3.1	53107	
BI_B	GBR North-Central	Black Island	Black Island_2	G22/4654 3.1	52835	
BI_C	GBR North-Central	Black Island	Black Island_2	G22/4654 3.1	55042	
BI_D	GBR North-Central	Black Island	Black Island_2	G22/4654 3.1	54630	
BI_E	GBR North-Central	Black Island	Black Island_2	G22/4654 3.1	54880	

BI_F	GBR North-Central	Black Island	Black Island_2	G22/4654 3.1	54281	
BI_G	GBR North-Central	Black Island	Black Island_2	G22/4654 3.1	57710	
LB_1	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	50662	
LB_2	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	59010	
LB_3	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	49559	
LB_4	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	28904	Y, > 20% missing data
LB_5	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	30748	Y, > 20% missing data
LB_7	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	52225	
LB_8	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	46055	
LB_9	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	38221	Y, > 20% missing data
LB_11	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	52381	
LB_12	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	54505	
LB_13	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	52981	
LB_14	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	50591	
LB_15	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	48793	
LB_16	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	51747	
LB_17	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	42566	
LB_18	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	49016	
LB_19	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	47782	
LB_20	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	49300	

LB_21	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	51556	
LB_22	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	47717	
LB_23	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	50528	
LB_24	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	51555	
LB_25	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	n/a	Y, failed sequencing QC
LB_26	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	50082	
LB_27	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	50837	
LB_28	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	52875	
LB_29	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	54706	
LB_30	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	39857	Y, > 20% missing data
LB_31	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	43343	
LB_32	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	43403	
LB_33	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	53553	
LB_34	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	48378	
LB_35	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	49566	
LB_36	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	53444	
LB_37	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	53329	
LB_38	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	30271	Y, > 20% missing data
LB_39	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	42404	

LB_40	GBR North-Central	Hook Island	Luncheon Bay donor site #3	G22/4654 3.1	49572
19.GBR.67	GBR Central	Block Reef	n/a	G19/3936 4.1	51771
19.GBR.104	GBR Far-North	Burke Reef	n/a	G19/3936 4.1	56343
19.GBR.112	GBR Far-North	12-040 Reef	n/a	G19/3936 4.1	54082
19.GBR.57	GBR South-East	Little Stevens Reef	n/a	G19/3936 4.1	49290
19.GBR.36	GBR South-East	Paul Reef	n/a	G19/3936 4.1	48800
19.GBR.122	GBR Far-North	Great Detached Reef	n/a	G19/4282 9.1	48308
19.GBR.44	GBR South-East	Paul Reef	n/a	G19/3936 4.1	49308
19.GBR.70	GBR Central	Block Reef	n/a	G19/3936 4.1	46037
19.GBR.81	GBR Central	Darley Reef	n/a	G19/3936 4.1	49762

Table S4.2 Morphological Glossary for 10 traits explored in the current study.

1) Branchlet Growth	Description:	A distinguishing feature of tabulate Acropora species is the presence of short vertical secondary branchlets protruding from the dominant horizontal branches. The consistency of spacing and uniformity in growth direction of these branchlets can vary amongst colonies.
	Categories:	Monotropic = All branchlets appear to grow in the same direction in a uniform manner and length. Pleiotropic = Branchlets appear to grow in seemingly random directions, with variations in branch length and angle.
2) Colony Colour	Description:	Colour of coral colony distinguished from *in situ* photographs (see Figure 2). Colour recorded from whole colony. No colour cards were used, so broad colour categories were considered.
	Categories:	Nude = Overall colony colour displays a nude tone, ranging from almost white to light brown tone. Pink = Colonies were categorised into one of three pink shades being soft pink, dusty pink or dark pink.
3) Incipient Axials	Description:	Acropora are defined by having dominant corallites at their branch tips (or axis) known as axial corallites. In some taxa, poorly defined, newly developed, or false axial corallites can be identified as incipient axials. For tabulate Acropora some taxa appear to have numerous incipient axials budding off the branchlets, whilst in other taxa each branchlet is independent and will bud no further axial branchlets.
	Categories:	Few or None = Majority of branches appear to have no incipient axial growth. Many = Majority of branches appear to have the presence of incipient axials.

4) Axial Colour	Description:	In resolving the taxonomy of tabulate Acropora, (**Chapter 2**) I identified a unique feature that seemed common amongst many tabulate Acropora. This feature was the presence of a dark coloured ring at the aperture of the axial corallite. See Figure 2. for examples of this.
	Categories:	Absent = No visual colour change present at Axial aperture. Soft = Soft coloured ring present. Dark = Dark coloured ring present.
5) Radial Shape	Description:	Radial corallites for Acropora are those that grow along the branches and are often a unique morphology compared to the axial corallite. Two main grown forms were present in the current study. See Figure 2. for visual examples of feature.
	Categories:	Flaring Labellate = This grown form is distinguished by a flaring lip at the opening of the corallite. Cochleariform = Corallites may present a similar flaring lip to labellate forms, although with a reduced internal wall connecting the corallite to the axial branch.
6) Secondary Radials	Description:	Some corals can display two different radial shapes on the one branchlet, often with one appearing to be the more dominant radial type.
	Categories:	Present = Second common radial shape present Absent = Only one radial shape present
7) Radial Angle	Description:	The direction of growth of the radial corallite can vary from appressed to the branchlet wall to laterally forming a 90-degree angle.
	Categories:	Lateral = The flaring lip of the radial corallite is at an almost 90-degree angle to the branchlet Vertical = The flaring lip of the radial corallite curves upward to grow almost vertically to the main branchlet.
8) Radial Consistency	Description:	The size and shape of radial corallites can vary within a colony, with some taxa displaying a range of corallite sizes and morphologies.
	Categories:	Uniform = Corallites were consistently the same size and shape across the branchlets Indeterminate = Majority of corallites appeared to be different sizes and/or shapes across branchlets
9) Radial Arrangement	Description:	The arrangement of radial budding along a branchlet can vary amongst taxa, ranging from a unform budding pattern to an irregular budding pattern.
	Categories:	Structured = Radial budding along branchlets is regular and structured, with 'rows' of radials forming along the branchlet. Irregular = Radial budding is irregular, with indeterminate patterns along branchlet.
10) Rosette	Description:	A distinguishing feature of many corals in the 'hyacinthus' group is the presence of a rosette-like feature formed by the growth pattern of the radial corallites around the axial when viewed from above. See Figure 2. for visual example.
	Categories:	Present = Rosette feature present. Absent = Rosette feature absent.

Table S4.3 Results for the Pairwise Fst scores (below diagonal) and significance (p-value above diagonal) for each species between reefs. Shaded regions indicate

reefs where species was not sampled. NaN values indicate insufficient sample size to yield results from analysis.

		SH	BI	WW	BPB	LB	SOUTH	CENTRAL	NORTH
A. pectinata	SH		0	0.867	0.018	0.043		0.961	0
	BI	0		0	0	0		0	0
	WW	-0.024	0		**0.662**	**0.399**		0.382	0
	BPB	0.012	0	**0.007**		**0.049**		0.684	0
	LB	0.012	0	**0.007**	**0.006**			0.961	0
	SOUTH								
	CENTRAL	-0.021	0	0.008	-0.013	-0.011			0
	NORTH	0	0	0	0	0		0	

		SH	BI	WW	BPB	LB	SOUTH	CENTRAL	NORTH
A. hyacinthus	SH								
	BI								
	WW				**0.001**	**0**	**0.006**	**0**	
	BPB			**0.001**		-0.003	0	0.611	
	LB			**0.369**	NaN		0.494	0.604	
	SOUTH			**0**	0	0		0	
	CENTRAL			**0**	0	0	0		
	NORTH								

		SH	BI	WW	BPB	LB	SOUTH	CENTRAL	NORTH
A. coralB	SH					0.911	0	0	0
	BI								
	WW								
	BPB								
	LB	-0.022					0.304	0.997	0.98
	SOUTH	NaN				0.008		0	0
	CENTRAL	NaN				-0.043	NaN		0
	NORTH	NaN				-0.032	NaN	NaN	

		SH	BI	WW	BPB	LB	SOUTH	CENTRAL	NORTH
A. sp7	SH		0.78	0.051	**0.047**	0.731			
	BI	-0.003		0.656	0.073	0.49			
	WW	0.009	-0.001		0.114	0.782			

	BPB	**0.034**	0.023	0.02		0.755			
	LB	-0.004	0	-0.004	-0.012				
	SOUTH								
	CENTRAL								
	NORTH								

Table S4.4 Chi-square adjusted residuals for morphological traits identified as significant. Traits are in the first column with variables of each trait in the second column. Values are Standard Residuals calculated from Pearson's Chi-square analysis with values greater than ± 2 identified as significant variables in explaining the corresponding species population. Shading of significant results is according to the strength and direction of the relationship. Significance of each value is shown with an asterisk with * = $p < 0.05$, ** = $p < 0.005$ & *** = $p <$ 0.000.

		A. hyacinthus	*A. pectinata*	*A. coralB*	*A. sp7*
Colour	Nude	-0.9827	**3.6519**	-0.7816	**-2.6916**
	Soft pink	**-2.2417**	**-2.9666**	1.3058	**4.2201**
	Dusty pink	**2.6021**	-1.1434	0.3775	-1.0862
	Dark pink	**3.2165**	-1.3822	-0.9348	-0.5124
Axial tip	Dark_ring	**6.3833**	**-2.3328**	-1.1701	-1.8847
	Soft_ring	**-2.5581**	1.7642	**-2.3422**	1.5883
	Absent	-1.6027	-1.0408	**5.8069**	-1.1605
Rosette	Yes	0.9876	1.4979	0.6943	**-2.8493**
	Absent	-1.8380	**-2.7876**	-1.2921	**5.3026**
Radials	Labellate	1.0533	**2.1630**	0.7405	**-3.6527**
	Cochlearform	-1.8926	**-3.8865**	-1.3305	**6.5633**
Branchlet Growth	Uniform	**-2.1269**	0.1736	1.2483	0.7540
	Indeterminate	**2.8478**	-0.2325	-1.6714	-1.0096

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Chapter 5

GENERAL DISCUSSION

5.1 Summary

Accurate understanding of biodiversity provides the foundation for biological science and conservation of the natural world (Sandall et al. 2023). To achieve this understanding, we require robust taxonomies to discover and describe species on which we can build and share knowledge (de Queiroz 2007; Pante et al. 2014). Such a goal is especially important when there are taxonomic uncertainties concerning keystone species, notably Scleractinian hard corals that form the structure of coral reefs (Kitahara et al. 2016; Cowman et al. 2020). Through my research presented in the preceding chapters, I have shown how a robust taxonomic revision of a key coral species complex, being the *Acropora hyacinthus* (Dana, 1846) complex (Ladner & Palumbi 2012), has revealed a higher diversity of species than previously documented (**Chapter 2**). I subsequently explored how resolving coral species diversity based on this new knowledge has implications for understanding of coral-algal symbiosis association specificity (**Chapter 3**), and in turn resilience and health of these taxa. I further investigated the importance of taxonomic resolution for conservation efforts, leading the way for the application of field based morphological species identification tools that could aid both reef conservation and research where morphologically similar species are living in sympatry (**Chapter 4**). Here, I have synthesised these findings, highlighted the importance of taxonomy, whilst also providing insights into the knowledge gained by other fields of biology and conservation with resolved species boundaries.

Advancements in molecular technologies have vastly improved collective knowledge on the evolutionary history of life on earth, leading to revisions of taxonomies across all taxonomic levels. However, several factors including incomplete lineage sorting, uninformative molecular markers and slow rates of mitochondrial evolution have hindered progress in resolving systematics of corals and other Anthozoa (Kitahara et al. 2016; Quattrini et al. 2018). To overcome this hurdle for corals, recent developments of Anthozoa specific bait sets for target enrichment of the ultraconserved element (UCE) and exon region of the genome have proved promising for higher taxonomic resolution of corals, both across shallow (species) and deep (order) evolutionary levels; such an approach was previously inaccessible without whole genome sequencing (Quattrini et al. 2018; Cowman et al. 2020). Here, I applied target enrichment of UCE to a species complex (*A. hyacinthus* complex, Ladner & Palumbi 2012) that was previously discovered, but not yet taxonomically resolved (**Chapter 2**). Species boundaries were investigated through several phylogenetic, species delimitation and morphological methods; an integrated approach that allowed for several lines of evidence to support species hypothesis and final taxonomic revision (Pante et al. 2014) (**Chapter 2**). Here, I was able to resolve species boundaries, leading to the discovery of four new species of coral and resurrection of five previously synonymised nominal species (Figure 5.1).

With improved taxonomic resolution of the *A. hyacinthus* complex (**Chapter 2**) I discovered that at least four morphologically similar and sympatric species of *Acropora*, all with morphological affinity to *A. hyacinthus*, occurred on the Great Barrier Reef (GBR) (**Chapter 2**). Further, past investigations have revealed that two morphotypes of *Acropora cytherea* (Dana, 1846), a species of

tabular *Acropora* that is closely related to *A. hyacinthus* also occur in the GBR (Ladner & Palumbi 2012). By sampling of these six closely related tabular *Acropora* species along the latitudinal gradient of the GBR, I investigated both the intra- and interspecific coral-algal associations of these corals and revealed correlations with latitude, possibly driven by cooler temperatures in the southern region of the GBR (**Chapter 3**, Fig. 5.1). I also found these closely related tabular *Acropora* species were generalists with their symbiont associations, commonly associating with type profiles of the C3 (genus *Cladocopium* LaJeunesse & H.J.Jeong 2018) radiation, which is common amongst *Acropora* (LaJeunesse et al. 2004; Tonk et al. 2014; Epstein et al. 2019; Butler et al. 2023). I did, however, identify background levels of rare ITS2 type profiles amongst all species. Whilst uncommon, these rare type profiles may prove to be useful in exploring abilities of coral colonies to switch or shuffle their dominant symbiont types when faced with stress events such as heating anomalies (Thomas et al. 2019). With known variations amongst bleaching susceptibility and recovery of tabulate *Acropora* after stress episodes (Hoogenboom et al. 2017; Brodnicke et al. 2019; Cornwell et al. 2021; Quigley et al. 2022b), exploration of these algal associations and health of individual species of coral is essential in assessing risk and planning mitigation and recovery practices. For example, in America Samoa a study identifying four species of tabular *Acropora*, belonging to the *A. hyacinthus* complex, found that each species contained different symbiont types, and that some species displayed higher bleaching tolerances compared to others (Rose et al. 2021). Further investigations into bleaching tolerances amongst sympatric species of tabular *Acropora* on the GBR may therefore reveal similar patterns and aid in management and restoration activity.

Finally, the presence of four morphologically similar species living in sympatry raises questions about the ability for both researchers and conservationists to effectively identify species and record diversity. Biodiversity is considered a key factor for successful reef restoration (Quigley et al. 2022a); however, the ability to identify biodiversity hinges on the ability of practitioners to correctly identify species. In the taxonomic revision from **Chapter 2** I performed an initial morphological analysis that revealed some unique morphological traits that proved informative for clustering distinct species of tabular *Acropora*. Interestingly, I found commonly investigated morphological features for *Acropora* such as the skeletal coenosteum formation and Axial corallite form and size (Wallace 1999) were generally uninformative in delineating between species of tabular *Acropora*; in contrast several descriptive traits, such as corallite shape and vertical branchlet structure, were more informative in distinguishing between species (**Chapter 2**). I built upon this knowledge and explored the application of morphology to identify species and populations of four tabular *Acropora* species that co-occur in five reef restoration sites in the Whitsundays region of the GBR (**Chapter 4**).

With recent mass coral bleaching and predicted future ocean warming (Hughes et al. 2017, 2018), there has been an increase in reef restoration efforts globally, including on the GBR (McLeod et al. 2019, Howlett et al. 2022). Such acceleration of restoration interest has placed an urgent need on reef restoration practitioners to understand the species and genetic diversity of the corals (Vardi et al. 2021; Quigley et al. 2022a). Whilst molecular technologies can aid in assessments of population genetics, accurate species identifications, especially amongst morphological similar and closely related taxa, is necessary for the

interpretation of results and application of restoration measures (Sheets et al. 2018; Suggett et al. 2022; Howlett et al. in review). Here, through molecular analysis, I found each of the four species of tabular *Acropora* to display high intraspecific genetic mixing, although low interspecific genetic mixing, indicating connectivity across sites. Interpretation of these results relies on the correct identification of species – whereas confusing these four species as a single species (e.g. based on previously accepted taxonomy (Wallace 2012)), would have yielded results of four populations – not species – and would have grossly overestimated population genetic patterns and species abundance (Sheets et al. 2018). To ensure correct species identification are made, both non-taxonomist researchers and conservationists require easy to use and low-cost tools to confidently identify morphologically similar species. I have therefore provided a baseline in which five informative morphological features – colony colour, radial corallite shape, presence of a rosette, branchlet growth and Axial colour – were identified that were found to accurately classify species in congruence with molecular species identifications (**Chapter 4)** (Fig. 5.1). Importantly, not all morphological features were reliable in identifying each species; for example, colour was unable to correctly identify *A. coralB* from the other three species. Also, an absence of a rosette (formed by radial corallites around the Axial) was an informative trait to distinguish the unresolved *A. sp.7* from the other three species, which all displayed a rosette. Of course, how well these traits can be independently applied to resolve taxonomy confidently still waits to be tested. Regardless, these findings highlight the need for investigations into novel morphological features and provide evidence that universal morphological features are not informative for species delineations of all coral species.

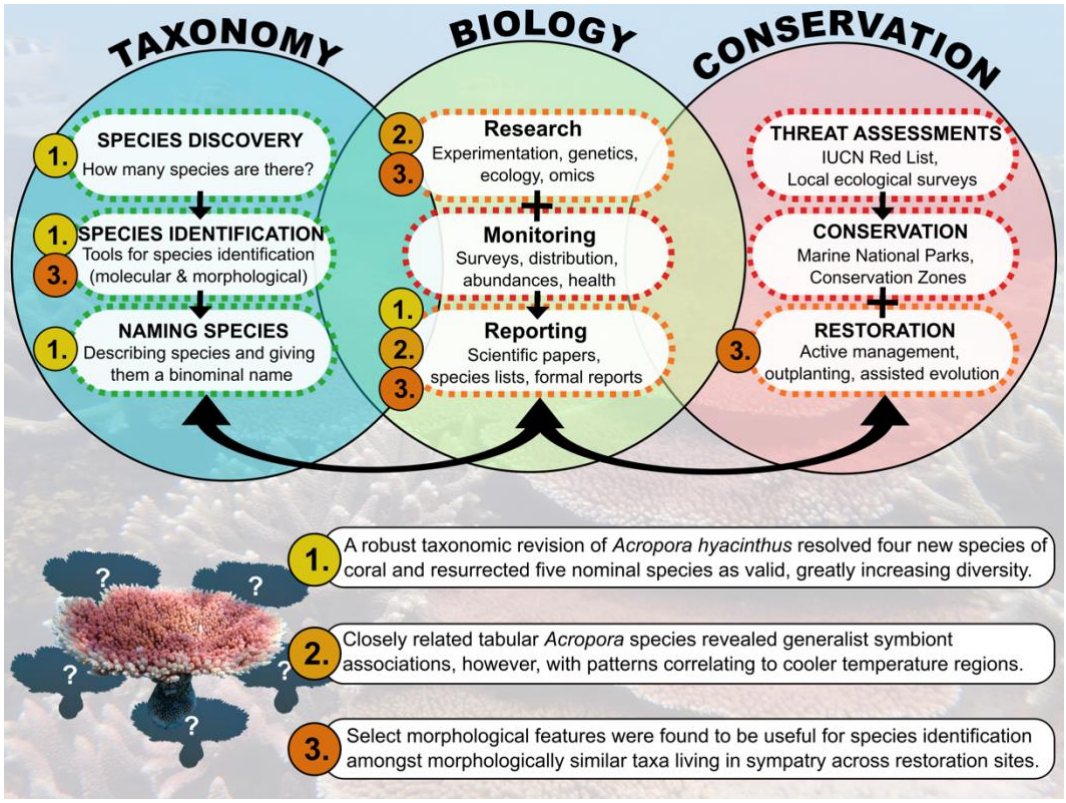

Figure 5.1 With the knowledge gained thought this thesis I have addressed the three aims from **Chapter 1** resulting in increased taxonomic diversity of a coral species complex. Here, activities for each scientific discipline are outlined in i) Green to indicate areas where I have filled in knowledge gaps, ii) Orange for activities that I have provided groundwork for on which further study can be built upon, and iii) Red for areas that were not directly addressed, although necessitate further investigation and are unpacked below. The main outcomes from each aim (1 = **Chapter 2**, 2 = **Chapter 3**, 3 = **Chapter 4**) is shown below, and the activities which they targeted are labelled in the main figure.

5.2 Coral biodiversity is greater than what is currently accepted.

Throughout my research it has become apparent that science is lacking vital information on the true species diversity of corals, including the often-abundant genus *Acropora*, which contributes immensely to the structural complexity of many coral reefs. In this thesis I have resolved species diversity of a keystone group known to include important reef builders that provide shelter to

reef organisms and that play a critical role in reef recovery as “pioneer” species (Ortiz et al. 2021); however, this revision covers just a fraction of the diversity existing in coral reefs. As discussed in **Chapter 1**, a broad *Acropora* phylogeny revealed over 50% of specimens included could not be reliably identified as any of the > 400 nominal species of *Acropora*, which includes both accepted and synonymised species (Cowman et al. 2020; Hoeksema & Cairns 2023).

The recent work by Cowman et al. (2020) found *Acropora* to form six major Clades (I – VI), with the tabular *Acropora* complex researched in this thesis forming just a fraction of Clade VI (Cowman et al. 2020; **Chapter 2**). In revising the taxonomy of this complex, I was unable to resolve species boundaries for at least five lineages (**Chapter 2**). Of these, one species appears to be particularly common on the GBR (*A. sp.7*) and although this species is clearly distinct in this region (**Chapters 2** and **4**) I was unable to resolve phylogenetic or geographic boundaries to formally describe this taxon. Indeed, another recent taxonomic revision of the species *Acropora tenuis* (Dana, 1846) (Bridge et al. 2023), which focused on one of three subclades within Clade I (*sensu stricto* Cowman et al. 2020) *Acropora* phylogeny (Bridge et al. 2023) discovered two novel species and resurrected five nominal species but was unable to resolve species boundaries for five lineages.

Between the Bridge et al (2023) *A. tenuis* revision and the one I performed in **Chapter 2** covering a portion of Clade I and Clade VI of *Acropora* (Cowman et al. 2020), there is evidence of at least ten additional species of *Acropora* that require further investigations and formal species descriptions. Importantly, across these two revisions of *Acropora* the accepted species diversity has increased by 16 species in the past year, not including these additional 10 species yet to be

described. Adding to this, evidence is mounting that several other species of *Acropora* form complexes with more diversity than that currently documented (*Acropora samoensis*, Rosser et al. 2015; *Acropora* spp*.,* Richards et al. 2016; *Acropora pruinosa*, Pipithkul et al. 2021). Furthermore, during my studies I attended a taxonomic workshop focused on resolving *Acropora* diversity in the Red Sea to reveal hidden species diversity in this region. An outcome of this was the initial indication of a plethora of undiscovered, or valid nominal species that have been erroneously synonymised, hailing from this unique location that is not reflected in coral taxonomy (Wallace et al. 2012), warranting further investigation. This is especially concerning, considering several species that were thought to occur from the Pacific Ocean across to the Red Sea (*A. hyacinthus*, *A. anthocercis*, *A. spicifera*, *A. tenuis*) have all been found to have much smaller ranges, mostly restricted to the Pacific Ocean (**Chapter 2**; Bridge et al. 2023), thereby suggesting species in the unique region of the Red Sea and Western Indian Ocean have been incorrectly identified and the true species diversity is unknown. Collectively, it is clear that despite recent progress, including the revision performed here (**Chapter 2**), much work is required to revise taxonomies for *Acropora*, and possibly broader for other coral genera.

5.3 For effective taxonomy collaboration is necessary, and agreement across scientific disciplines is required

Performing a taxonomic revision, especially for globally distributed organisms, is no simple task (Fig. 5.2). An example of the issues preventing resolution of the taxonomy for corals can be found in Wepfer et al. (2020), which provided insight into the incompatibility with the accepted taxonomy of the coral

genus *Galaxia* and their molecular phylogenetics via broad scale geographical sampling. Wepfer et al. (2020) was however criticised for failing to collect voucher specimens, topotype material and investigate the taxonomic history of the genus *Galaxia*, which would have provided the authors means to revise the taxonomy formally (Bonito et al. 2021). With this, Bonito et al. (2021) acknowledged that "good taxonomy can be difficult and can be time consuming". In response, Wepfer et al. (2021) acknowledged that whilst some criticism was warranted, they also encountered logistical issues that prevented the collection of voucher material. Such logistical issues can be common when dealing with local permitting and export constraints such as those put in place by the Convention on International Trade in Endangered Species (CITES), in which concern has been raised about the accuracy of species or genus identification of corals on export permits without taxonomic expertise (Green & Hendry 1999). Additionally, Wepfer et al. (2021) noted that many researchers do not have access to high-cost sequencing technologies that have been shown to resolve species boundaries corals, which limits progress where funding is lacking.

Whilst the example of (of Wepfer et al. 2021) is just one of many, it highlights just a few of the impediments faced by taxonomy. Taxonomic resource accessibility is improving to aid in research and species revisions. Online resources such as the Biodiversity Heritage Library make original taxonomic works much more accessible than it would have been in the past via digitisation of old records (Gwin & Rinaldo 2009) (Fig. 5.2). Across museums, networks of taxonomists and museum curators are making historical collections digital, allowing easier access to vital specimens such as holotype material for species identifications and taxonomic works (Popov et al. 2021) (Fig. 5.2). The vast

network of people and resources discussed here provides a small insight into the scale of taxonomic works such as the revision in this thesis (**Chapter 2,** Fig. 5.2). Although as discussed earlier, I have also highlighted the importance in performing such taxonomic revisions, necessitating further investment and collaboration for these foundational projects.

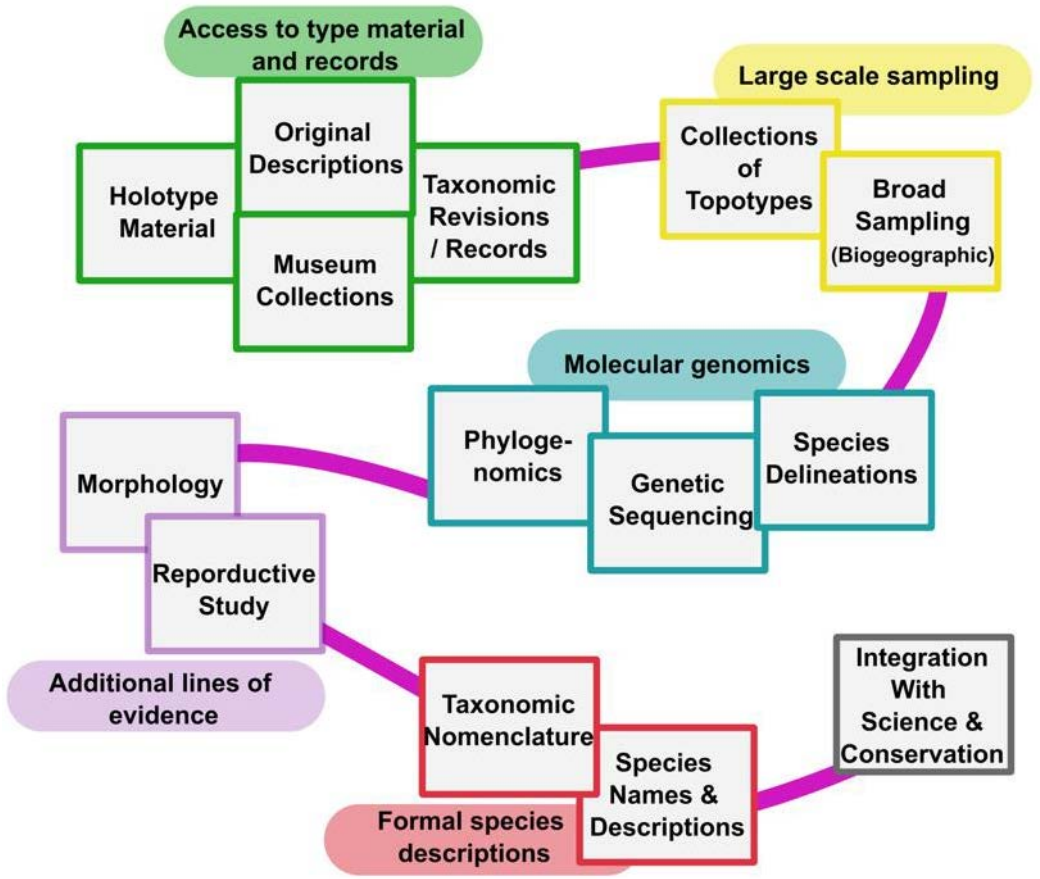

Figure 5.2 A thorough taxonomic revision requires a collaborative effort across institutes and scientific disciplines. Here, a framework is provided showing each step of the taxonomic process, noting the institutions and specific requirements of each stage.

An often-overlooked issue that affects progress in taxonomy that became apparent in my studies is the inadequate use of taxonomic citations for scientific works that deal with species (Wägele et al. 2011; Meier 2016). Specifically, acknowledgement of original authority when a species is referenced in a scientific manuscript. For example, *A. hyacinthus* was first described by James Dwight Dana in 1846 as *Madrepora hyacinthus*, and the genus name was then updated to *Acropora* by Verrill in 1902. Taxonomic nomenclature deems 'Dana, 1846' to be the original taxonomic source for this species, thus a taxonomic citation would acknowledge this original source upon mentioning this species in a manuscript, which would appear as *Acropora hyacinthus* (Dana, 1846). Despite this, a Google Scholar search for the term "*Acropora hyacinthus* (Dana, 1846)" reported just 174 results of this exact phrase occurring in a publication, even though, as mentioned in **Chapter 1**, there are > 3,000 reports mentioning the species "*Acropora hyacinthus*" according to a Google Scholar search for this species.

Lack of taxonomic citations for the origin of species names is concerning as it leads to low citations for taxonomic publications, even if the findings – being the new species discoveries – are widely applied (Agnarsson & Kuntner 2007; Wägele et al. 2011; Meier 2016). This is further driven by the fact that many taxonomic works are too long for most high impact journals, leading to publication of these important works in "lower impact" taxonomic journals, creating a low impact loop (Zhang 2006; Agnarsson & Kuntner 2007). Ultimately, this has been shown to have potential to negatively impact success for taxonomists in competitive funding opportunities (Agnarsson & Kuntner 2007; Wägele et al. 2011). Indeed, the Journal Zootaxa was created for the reason of providing a journal for rapid and cost-effective publications of descriptive

taxonomic works to accelerate species discovery (Zhang 2006). However, low citations outside of other taxonomic works has ultimately led to a low impact factor for this Journal, driving this citation issue in taxonomy (Gornaeu et al. 2022). To resolve this issue, a change is needed in publishing conventions to require correct and common use of taxonomic citations in text and authorities listed in references. At the minimum, the first mention of a species in scientific reports should cite the authority which should in turn be reflected in the reference list (Wägele et al. 2011). The important factor here is the requirement for publishers to ensure full authorities are listed in references and cited in text, as whilst some authors may provide in text citations for scientific names (e.g. *Acropora hyacinthus* (Dana, 1846)), the citation issue which drives the impact factor of taxonomic works is dependent on the literature listed in references and not just in text citations (Wägele et al. 2011). For corals, this is not a difficult task, as online repositories such as the World Register of Marine Species (WoRMS Editorial Board, 2023) provide all the detail required to check the authority and original source for species descriptions of corals and other marine taxa with minimal effort. This in turn would increase citations and impact factor for journals and position taxonomy with the profile it warrants as the baseline for many studies.

On the impediments to taxonomy, there has been recent concern raised around the legacy and continued use of "eponyms" – a species named after a specific person – that are considered offensive or problematic for various reasons (Wolsan 2007; Guedes et al. 2023). As taxonomic nomenclature is designed to be stable over time, the code states that original species names take priority for valid species. Whilst some eponyms may today be considered offensive, the

International Code of Zoological Nomenclature (ICZN) does have the authority to reject a taxonomic name if it is deemed unsuitable or invalid, which includes names that may be considered offensive. Further, retracting and renaming all species names containing eponyms is a significant task that would take considerable time and effort and would further confuse the scientific and taxonomic literature, causing issues for conservation and surveying of species across time (Garbino 2023). While noted that some historical names may cause offence, the continued use of eponyms allows for a switch to the story and instead provide celebration and recognition of worthy figures in scientific names (Garbino 2023; Jost et al. 2023). For instance, in **Chapter 2** I discovered a new species of coral which was given the name *Acropora coralA*. This species was discovered from South-Eastern Australia and was named in acknowledgement of Dr Vicki Harriott who spent much of her life dedicated to research of Australia's South-Eastern subtropical reefs. Here, my colleagues and I decided to name this species after a deserving woman. In acknowledging this, we are unable to predict however if in the future certain attributes that may be accepted by today's society are considered offensive to future generations (Guedes et al. 2023). There is also the question of what deems a person worthy of having a species named after them, and should any name be available or is there a criterion that must first be met? (Poulin et al. 2022). Indeed, some have suggested that the use of eponyms should continue, however, with suggestions on increasing diversity of those honoured such as celebrating female scientists, as we have done here (Poulin et al. 2022; Jost et al. 2023) Considering this, whilst I support the use of eponyms we must acknowledge that there is some degree of risk in naming a species after a person, even if they are revered by today's society.

5.4 Science and conservation require easy access to species identification guides.

For scientific research outside of the field of taxonomy, species are identified based on available guides or the most recent or accessible taxonomic resources. For corals, and also for other organisms (Berrilli et al. 2023) - where identification of species is uncertain amongst closely related taxa – many researchers have given the term 'cryptic' to groups of morphologically similar species that reveal some degree of genetic differentiation (for example; *A. hyacinthus & A. cytherea*, Ladner & Palumbi 2012; *A. hyacinthus,* Nakabayashi et al. 2019; *Oribcella faveolate*, Gómez-Corrales & Prada 2020; *A. tenuis*, Matias et al. 2023). What these studies often fail to do is corroborate that these genetically distinct lineages are in fact cryptic – which would assume they are morphologically indistinguishable – through morphological investigation. An occurrence that may relate to the lack of taxonomist involvement in such molecular studies. Indeed, when morphology has been performed on assumed cryptic species of tabular *Acropora* authors have revealed taxa than can in fact be identified morphologically (**Chapter 2** and **4**; Ramírez-Portilla et al. 2021). Although molecular studies can derive interesting results from population genetic analysis without identification of individual species or lineages (Sheets et al. 2018), there is the inability to then share knowledge pertaining to a distinct species if it has not been correctly identified, or formally described if it is an undiscovered species. This leads to difficulties with integration of data across studies and platforms (Sandall et al. 2023). As a solution, robust taxonomies that include thorough morphological analysis and provide easy to use tools for species

identification are key, providing species with universal names, distributions, and morphologies. However, as discussed earlier the effective update of taxonomy, including the correct citations of scientific works, is required by researchers to support such taxonomic endeavours.

5.5 Why taxonomy is important for conservation of coral reefs

For coral reef conservation, the overarching goal is to protect vulnerable ecosystems and species, and aid in coral recovery thus boosting coral reef resilience for the future (Hein et al. 2021), often for the protection of biodiversity (Quigley et al. 2022a). For biodiversity to be protected and targeted by conservation efforts, there is a need to first understand the biological diversity of organisms, especially at the species level. An inability to identify diversity in conservation may lead to the extinction of threatened or endemic species, due to overestimations of abundances when multiple species are believed to be one (Pimm et al. 2014; Sandall et al. 2023). For example, in the Horn of Africa primate conservation efforts are heavily influenced by species lists, which are often not up to date with current taxonomic resolution and thus unintentionally direct conservation away from highly threatened species (Gippoliti 2022). For corals, the IUCN Red List of Threatened Species is a considered source for assessment of species-level extinction threat; however, for species such as *A. hyacinthus* the last assessment was performed in 2008 (Aeby et al. 2008), before it was known that this species forms a complex with smaller geographic ranges of each lineage (Ladner & Palumbi 2012). Efforts may therefore not be directed to threatened species due to outdated threat assessments and species lists (Mace 2004) (Fig. 5.3).

Reef conservation increasingly involves many reactive approaches, such as restoration or assisted evolution to boost recovery and resilience of ecosystems (Hein et al. 2021; Suggett & van Oppen 2022) (Fig. 5.3). Restoration projects that involve activities including coral propagation and gardening are directly impacted by the information of species diversity present (see Suggett et al. 2022; Howlett et al. in review). For example, recent reef restoration efforts on the GBR which were aimed at exploring genotypic diversity of *A. hyacinthus* discovered that sampling efforts contained three distinct species of coral (Howlett et al. in review). If taxonomic expertise was not provided in this case to identify species, then it may have been assumed that there were three populations of *A. hyacinthus* in this region and management decisions would have been based on these results. Instead, as with results from **Chapter 4**, Howlett et al. (in review) found low intraspecific genetic diversity of species across this region, indicating a high degree of mixing of genetic material within species. In another conservation lens, the application of larval propagation to boost coverage and recovery of species is a technique being tested in reef restoration (Boström-Einarsson et al. 2020, Suggett & van Oppen et a. 2022). This method, however, relies on accurate identification of species to efficiently breed hosts and produce viable offspring, especially if targeting certain species. Ultimately, a resolved taxonomy and clear species boundaries is the best option for ensuring efficiency and success of such endeavours.

On a broader scale, biodiversity credits are driving a multibillion-dollar market where offsetting or protecting biodiversity loss is placed in policy and management globally (Niner et al. 2017; Stephens 2023). The focus here is that biodiversity is protected from extinction due to mostly anthropogenetic activity

(Niner et al. 2017). For these biodiversity offsets to be successful, there is a need for an understanding of biodiversity so focus can be targeted on vulnerable ecosystems, species, or populations (Mace 2004). Ultimately, lack of knowledge of how many species there are on Earth leads to an inability to effectively protect biodiversity that is hidden (Appeltans et al. 2012). For the GBR, an area that is considered incredibly biodiverse, past biodiversity offsets have not been effective in protecting diversity (Bos et al. 2014). In driving the need for future biodiversity management and offsets, I have found (**Chapter 2**) that the GBR – and other regions globally – host both more species of coral than previous estimates, but also with smaller ranges with some species shown to be regionally endemic, such as *A. anthocercis* (**Chapter 2**).This increase in known biodiversity, and reassessment of species ranges, highlights the heightened value but also threat to coral reefs, which are already vulnerable to predicted future environments (Hughes et al. 2017, 2018).

When resolved taxonomies lead to an increase in the diversity of life there is a need for revisions of species biogeography and recorded abundances, which can have flow on effects into conservation measures (Bickford et al. 2007). In revising the taxonomy of the '*Acropora hyacinthus* complex' (**Chapter 2**) the discovery of novel species and resurrection of previously synonymised nominal species led to an increased diversity for tabular *Acropora* (Fig. 5.1). Importantly, none of the accepted nominal species resolved within this Chapter retained the broad geographic ranges documented in previous taxonomic revisions, which considered species such as *A. hyacinthus, A. anthocercis* (Brook, 1893) and *A. spicifera* (Dana, 1846) to be global species found in coral reefs from the Red Sea and Western Indian Ocean, all the way to the Central Pacific Islands and north to

Japan (Wallace 1999). Instead, I revealed *A. hyacinthus* to be biogeography restricted to the Central Pacific Ocean and Eastern Australia, *A. anthocercis* was found to be a Great Barrier Reef endemic, and *A. spicifera* to have a Coral Triangle and Western Australian biogeographic distribution. This has concerning implications for the study and conservation of these species. Anything identified as any one of these species outside of these revised ranges is likely not the formal species now identified, which impacts integration of data through time (Pante et al. 2015). Even more concerning, the true extinction threat to these species is likely much higher than previous estimates (Bickford et al. 2007). For instance, the International Union for the Conservation of Nature (IUCN) Red List records *A. hyacinthus* as a near-threatened species with a declining population, however, the metrics considered for this assessment included the broad global biogeography documented from previous taxonomic works (Wallace 1999; Aeby et al. 2008). With the known importance of tabular *Acropora* species – which includes *A. hyacinthus* – in reef recovery and resilience, there is now an urgent need for revised threat assessments that truly capture the distribution, abundance and threat level faced by these tabular *Acropora* species (Ortiz et al. 2021), so conservation and management can make informed and effective decisions in the protection of key taxa. Ultimately, this taxonomic revision now drives the need for revised threat assessments, such as those used for the IUCN Red List of Threatened Species, for all nominal and novel species resolved in **Chapter 2** (Fig 5.1).

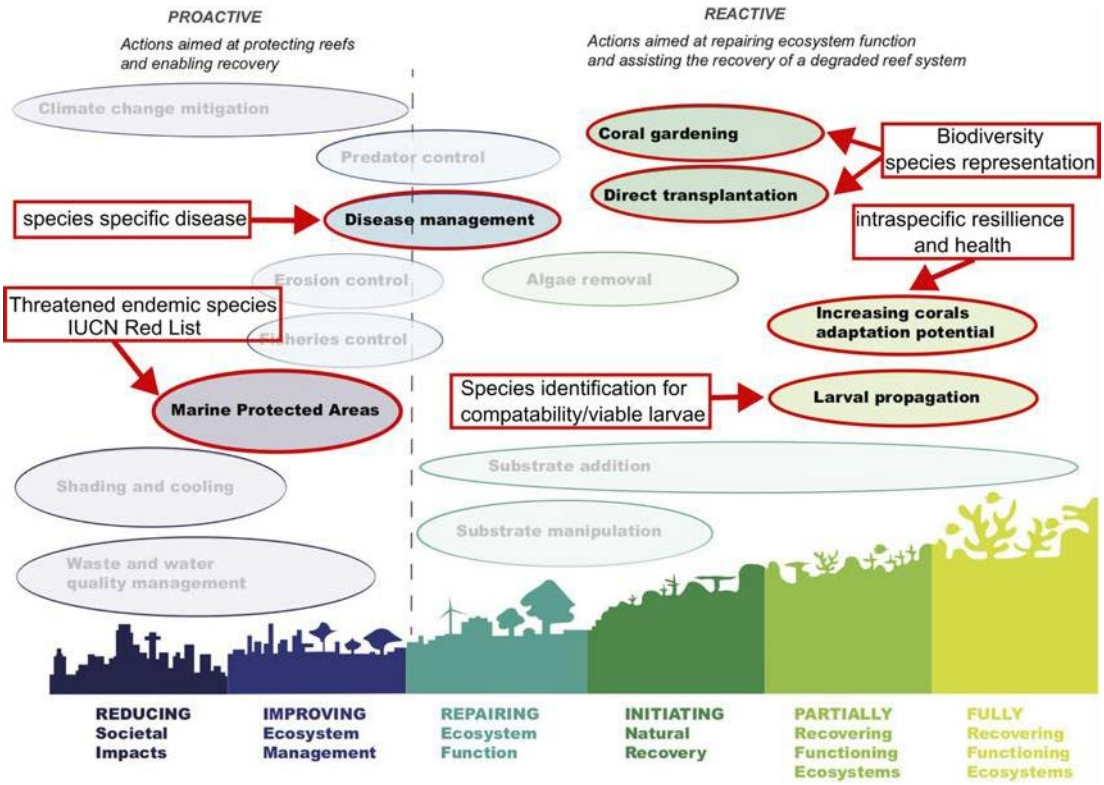

Figure 5.3 Integration of science with traditional conservation (proactive) and restoration (reactive) management types for coral reefs. This figure is adapted from Hein et al. (2021) which displays the range intervention types and where they fall on the conservation continuum. Here, measures which are directly affected by species-level taxonomic resolution of corals are circled in red and species-specific impacts shown for each measure indicated. Measures which are not directly affected by species-level diversity on of corals are shaded in grey.

5.6 Future Directions

In conducting the research and evaluating the major outcomes of this thesis there are clear knowledge gaps that should be a focus for future research efforts. Possibly the clearest outcome is the need for more taxonomic revisions throughout the genus *Acropora*. Multiple lines of evidence have now indicated that the taxonomic diversity of *Acropora* is greater than what has been reflected in traditional taxonomic works (**Chapter 2**; Cowman et al. 2012; Bridge et al. 2023). With the success of recent novel molecular techniques, such as the development

of targeted baits for Anthozoa in the sequencing of UCEs we now have an opportunity to confidently resolve molecular species boundaries for difficult groups of Scleractinia that were difficult with past technologies (Quattrini et al. 2018). However, with over 400 nominal species of *Acropora* (Hoeksema & Cairns 2023), performing revisions covering all existing nominal species and accounting for the discovery of new species this is a task that will require collaborative efforts. We have provided a starting point here for future revisions of *Acropora*, especially those residing in Clade VI of the *Acropora* phylogeny (Cowman et al. 2020), and perhaps a logical next step would be to target known species complexes that reside with Clade VI, such as the *Acropora cytherea* (Dana, 1846) species complex (Ladner & Palumbi 2012; Cowman et al. 2020).

The usefulness of undertaking taxonomic revisions ultimately hinges on the accessibility of new taxonomic works and the ability for non-taxonomists, including researchers and conservation practitioners to confidently identify resolved species and diversity. As shown in **Chapter 4**, and in line with previous reports for tabular *Acropora* (Ramírez-Portilla et al. 2021) we have found that previously considered 'cryptic' species can be identified morphologically when novel informative morphological features are explored. This provides a direction for the exploration of morphology across all species of *Acropora* and can have direct applications in research and conservation where species-level diversity is concerned, without the need for time consuming and costly molecular interventions (Boström-Einarsson et al. 2020; Vardi et al. 2021). With the informative traits resolved for species identification in **Chapter 4**, the next stage would be to test this identification method out in a field setting with non-taxonomists to test for feasibility. Evolving from this, future taxonomic works for

coral have a baseline for performing morphological analysis alongside molecular phylogenies to produce non-expert species identification tools to allow for easy integration of new (and existing) species into research and conservation measures.

Finally, as discussed above there needs to be more collaborations between taxonomy and conservation. Much of coral conservation and restoration is concerned with protection of vulnerable ecosystems and protecting biodiversity (Hein et al. 2021; Quigley et al. 2022a). Whilst taxonomy is concerned with discovering and describing biodiversity, so it can be studied and ultimately protected if vulnerable (Dayrat 2005). Improved integration across these platforms may provide new avenues for taxonomic investigations, and ultimately success of conservation and restoration efforts focused on biodiversity and protection of threatened species. For example, restoration efforts that may want to propagate species of coral to restore a damaged reef may be interested in the species diversity of a site to ensure restoration efforts capture and protect the existing diversity. The ability to successfully perform this task requires initial taxonomic knowledge of the targeted species and requires an ability of practitioners to identify the diversity in the field. Further, as taxonomic resolution is improved, we are discovering many species to have smaller ranges than previously accepted, leading to higher rates of endemism (Pimm et al. 2014). This knowledge is crucial for conservation efforts that may want to focus on protection and resilience of threatened local taxa. For this to happen, collaborations across disciplines are necessary, and the application of taxonomic tools for non-experts – such as morphological species identification tools – is an area that necessitates further study.

5.7 Concluding Remarks

With impending threats to coral reefs under continually changing climates it has never been more important to discover, record and protect biodiversity. For coral reefs, the hard corals that form the architectural structure of reefs are the keystone species for the survival of reefs as they exist today, however, much of the diversity of these crucial coral species is unknown. In this thesis I have revealed a much greater diversity of a species complex within the genus *Acropora* and have explored the impacts and applications of this resolved taxonomic diversity on other areas of biodiversity research and conservation. I have for the first time investigated coral-algal associations of newly discovered species of coral on the Great Barrier Reef and shown how resolved species diversity can impact reef restoration efforts where diversity was previously hidden. These foundations provide a direction for future taxonomic revisions and explore the utility of these taxonomies across research and conservation efforts.

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