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Understanding cryptic coral diversity: A 3D-reefscape genomics approach to assess dispersal dynamics and niche differentiation

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Abstract

Corals provide habitat for numerous marine species and ecosystem services for global human populations. However, they are vulnerable to local and global scale threats, especially climate change. Measuring demographic processes such as dispersal and ecological processes such as niche partitioning are important for predicting their responses to disturbances and environmental change. So far, correlations between coral genetics and the environment or spatial scale have largely been made over large habitat distinctions, such as depth, reef zone, and among islands or geographic regions. Reefs comprise structurally heterogenous landscapes and thus microhabitats may vary considerably, however, we have little understanding of how genotypes are distributed within reefs across fine spatial scales. Dynamics at fine spatial scales are particularly important in corals due to the frequent discovery of genetically divergent but morphologically indistinguishable coral taxa found sympatrically within reefs (*i.e.*, cryptic taxa, often with no obvious environmental distinctions) and evidence that dispersal distances may be small for some taxa. Technological advancements in both genomic sequencing and underwater imaging and computation can help to study fine-scale dispersal and determine whether cryptic taxa are ecologically partitioned. Reduced representation sequencing can be conducted on wild populations and gives access to genomic variation across hundreds of individuals. Structure-from-motion photogrammetry enables the characterisation of structural features of the reef and coral colonies within reefs; thus, it is possible to combine high resolution spatial mapping and micro-environment analyses with genotyped colonies using these two technologies.

Species of the Caribbean hard coral genus *Agaricia* (Order: Scleractinia) are arrayed over the entire depth range for photosynthetically dependent organisms, making them an ideal target for comparing mesophotic (>30 m depth) and shallow (<30 m) species, evaluating microhabitat differentiation, and assessing spatial structures across depths. My thesis uses this genus to explore questions related to spatial and environmental differentiation between and within taxa at scales from tens of kilometres to centimetres. The first data chapter (Chapter 2) of my thesis focuses on two mesophotic-occurring species: *Agaricia grahamae* and *A. lamarcki*. Despite presuming to be brooders with localised dispersal, no spatial population genetic structure was found over 10s of kms in either species. However, two sympatrically occurring cryptic taxa within each species were found. In *A. lamarcki* these taxa exhibited incomplete depth

partitioning between shallow and mesophotic depths, yet taxa within *A. grahamae* displayed no obvious environmental distinctions. Demographic histories of all taxa were characterised by gene flow among taxa. This chapter exemplifies the complexities found in corals, where (1) spatial genetic structures do not follow expectations, (2) morphologically similar, sympatric taxa exist both at the same depths and differentiated by shallow and mesophotic depths and (3) gene flow among taxa may be important for the evolution of corals. My second and third data chapters (Chapters 3 and 4) focus on fine-scale characterisation of genotypes across 3D-imaged reefscapes within three depth zones (5, 10, 20 m) and among four sites along the leeward side of Curaçao and spread over ~50 km. Chapter 3 describes the delineation of cryptic coral taxa and investigates dispersal within and between depths among all taxa. The cryptic taxa are defined by divergent genotypic clusters occurring sympatrically and some were found to be associated with particular depth profiles. Disparate spatial genetic structures were found among congeners, where taxa within *A. agaricites* and *A. humilis* presented isolation-by-distance and dispersal distances across metres and, in contrast, *A. lamarcki* taxa presented genetic homogeneity at distances >50 km. This chapter provides one of the few estimates of dispersal distances in corals, which is exceedingly low (across metres), highlights the widespread cryptic diversity within corals and finds substantial differences in dispersal, clonality and genetic diversity among congeners. In Chapter 4, I used photogrammetry to characterise the microhabitat around individually genotyped colonies of *Agaricia* by deriving novel geometric measures. Environmental niches for sympatric cryptic taxa were determined by describing microhabitats that coral colonies inhabit. Species and cryptic taxa exhibited subtle divergences in their physical microhabitat niches. This chapter tackles the question of how cryptic coral taxa co-occur in seemingly similar environments and demonstrates a novel photogrammetric approach to characterise the microhabitat.

My thesis applies new technologies and methods to help solve some of the mysteries of corals populations, namely, how far do larvae disperse? And what creates or preserves cryptic taxa? And in doing so, provides insight into coral ecology (interaction with microhabitat, spatial distribution, and dispersal) and evolution (cryptic diversification and hybridisation).

Declaration by author

This thesis *is composed of my original work, and contains* no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted *to qualify for the award of any* other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications included in this thesis

Chapter 2 - **Prata, K. E.**, Riginos, C., Gutenkunst, R. N., Latijnhouwers, K. R. W., Sánchez, J. A., Englebert, N., Hay, K. B., & Bongaerts, P. (2022) Deep connections: Divergence histories with gene flow in mesophotic *Agaricia* corals. *Molecular Ecology*, 00, 1-17, https://doi.org/10.1111/mec.16391

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Contributions by others to the thesis

	Contributions	Data chapters
Cynthia Riginos	Conception, design, funding, analysis and interpretation, and review	2, 3, 4
Pim Bongaerts	Conception, design, funding, analysis and interpretation, and review	2, 3, 4
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Statement of parts of the thesis submitted to qualify for the award of another degree

No works submitted towards another degree has been included in this thesis

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No animal or human subjects were involved in this research.

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Dedications

I dedicate this thesis to all animals without a voice, may your suffering at the hands of humankind be recognised and averted.

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Chapter 1: General introduction

Climate change has and is going to cause irreversible changes to our planet, including shifts in ecosystems and losses of species (Chen et al., 2011; IPCC, 2022; Román-Palacios & Wiens, 2020; Thomas, 2010). If governments across the world can eliminate the burning of fossil fuels and other thermal pollutants, then the recovery and adaptation of populations and species to new climates is more likely to occur (IPCC, 2022). Many models predicting the effects of climate change on species only consider current species distributions (Urban et al., 2016). Species distributions are used to explain the environments that species inhabit across large geographic scales, which may be an oversimplification of environmental tolerances (Lembrechts et al., 2019). For example, cryptic species may exist, or species may occupy specific habitats within these ranges and thus environmental niches of species would be overestimated if not taking this into account. Many climate models also do not consider species' dynamic responses such as demography, dispersal, evolution, and species interactions, which have been important for survival during past responses to climate changes (Urban et al., 2016). However, incorporating such processes into more sophisticated models relies on having the data available. Advances in genomic sequencing (*e.g.*, sequencing thousands of loci across the genome in many individuals of wild populations) enable identification of cryptic species and advances in remote sensing provide fine-scale and high resolution spatial and environmental data, which when analysed with genomic data can define more accurate environmental niches and uncover demographic and evolutionary processes.

Most importantly, affected species can respond by either dispersing into a refuge or adapting (Razgour et al., 2019; Travis et al., 2013). Dispersal is also important for spreading alleles amongst populations enabling genetic or evolutionary rescue (Hoffmann et al., 2021; Quigley et al., 2019). Using landscape and population genomics methods, we can estimate dispersal and compare environmental niches alongside other key species parameters (species identity, population size, extent of asexual reproduction etc.), and thereby better understand how closely-related species are differentially adapted to their environments across relevant spatial scales and predict how their populations will respond to disturbance and environmental change given their dispersal potential and other demographic information. This information can then be incorporated into such models and guide prioritisation of particular strategies that best

conserve species. Thus, the study of demographic and adaptive processes in wild and at-risk populations is critical for global decisions in conservation.

One of the ecosystems that are most threatened by climate change and other anthropogenic influences are coral reefs due to increases in the severity and frequency of marine heat waves, storms, ocean acidification, overfishing, pollution, and disease (Hoegh-Guldberg et al., 2007; Hughes et al., 2003; Pandolfi, 2003). Coral reefs support biodiverse ecosystems, harbouring species from most phyla and support worldwide economies through fishing, tourism, minerals, marine natural products (*i.e.*, unique chemical compounds used in medicines), erosion of coastlines and protection from storms (Bruckner, 2002; Chen et al., 2015; Hoegh-Guldberg et al., 2017). The foundations of these ecosystems are engineered by "hard corals", in the Phylum Cnidaria and the Order Scleractinia. Hard corals are vulnerable to thermal changes, particularly warming, and can experience coral bleaching, where they lose (or expel) their intracellular dinoflagellate symbiont (Family: Symbiodiniaceae) (van Oppen & Lough, 2018), which are responsible for most of their energy requirements from photosynthesis (Muscatine, 1990). Coral bleaching results in losses in fitness, disease, or death and has been a global concern for the decline and extinctions of coral populations for decades (Hoegh-Guldberg, 1999). Furthermore, as more carbon is absorbed by the ocean from atmospheric CO_2, the ocean becomes more acidic, this limits the ability for corals and shelled organisms to calcify (McNeil et al., 2004), which in the past has led to absences of calcifying organisms in the fossil record (Stanley, 2003).

Corals have especially complex life histories. They harbour partially asexual populations, are commonly hermaphroditic, are colonial, have indeterminate growth through asexual budding, have disparate dispersal capacities, and appear to inbreed, and hybridise frequently (Hughes et al., 1992). Additionally, the coral holobiont constitutes a mixed community of the cnidarian host and various symbiotic microbes including photosynthetic dinoflagellates which also dictate their ecology. These life history characteristics are atypical for most well-studied animals (*e.g.*, vertebrates) and population genomics provides a window to both measure the occurrence and extent of such traits and thus enable predictions of the responses of different species to disturbances and environmental change.

1.1 What is a species and why does it matter?

What exactly constitutes a species has been a matter of contention since the birth of evolutionary biology and even with the rise of genetics and genomics it remains philosophically contentious today (Hey, 2006; Richards, 2023). Specific definitions of species concepts vary but generally a species constitutes a unique group of organisms that should be genetically, phenotypically, and ecologically similar and it is the finest resolution of a named taxon (excepting subspecies). Probably the most influential definition is Mayr's biological species concept whereby species are defined as groups of individuals that are reproductively isolated from other groups (Mayr, 1942). Different species concepts can lead can to different conclusions about particular sets of closely related taxa (Hey, 2001). A major reason for this inconsistency in species definitions is because species lie on a continuum of divergence and within this continuum there is a "grey zone" of moderately diverged taxa with incomplete reproductive isolation barriers (Roux et al., 2016). Yet, it is important to decide on the conditions in which we called a group of individuals a species because these labels are required for conservation policy (*e.g.*, the IUCN Red list of threatened species), and species are the most frequent units used for biodiversity estimates (Coates et al., 2018). What we call a species also matters when measuring neutral demographic processes such as dispersal and selective processes such as adaptation to certain environments. For example, we *expect* that if populations all belong to the same species (and aren't markedly differentially adapted), when one population suffers from disturbance, migrants from unaffected populations should be able to supplement them (*e.g.*, demographic or genetic rescue, Carlson et al., 2014) and if environmental change would occur, individuals within a species that harbour adapted alleles should be able to share this variation amongst their populations (*e.g.*, evolutionary rescue, Carlson et al., 2014). Thus, whether groups of individuals within a species are linked and share a common evolutionary trajectory is important for the study of these processes. However, there are pervasive issues in recognising and identifying coral species; population genomics can be used to identify evolutionary distinct groups, and thus infer their population processes.

Coral species have traditionally been defined by their morphology (Wells, 1954). However, with the new era of genomic research, it is becoming increasingly clear that morphology is not always a great predictor of species delineations (Fukami, Budd, Paulay, et al., 2004; Huang et al., 2011) and that hybridisation between differentiated taxa may be common (Hobbs et al., 2022). To escape these sorts of issues (*i.e.*, morphological incongruence and hybridisation),

Mallet (1995) provides the "genotypic cluster" definition, where species are defined as distinguishable genetic groups of individuals that have few or no intermediates when in sympatry. The Phylogenetic Species Concept (*sensu* all versions) is similar to the genotypic cluster definition by defining groups by their genetic distinctiveness, but the phylogenetic concept does not consider geography in its definition, thus groups defined by the phylogenetic concept may be divergent due to geographic separation but harbour no reproductive barriers that would prevent these groups from homogenising if chance brought them back together. The genotypic cluster definition ignores morphology and does not rely on examining levels of genetic compatibility but rather infers it from the genetic distinctiveness of groups and thus focuses on the product (*e.g.*, genetic distinctiveness) rather than a particular process (*e.g.*, discovering an isolating barrier). Thus, the genotypic cluster definition is different to the Biological Species Concept (*sensu* Mayr), because groups could potentially reproduce, be viable and introgress, however it is assumed because of the genetic distinctiveness in sympatry that there exist reproductive barriers either endogenous or exogenous that are strong enough to prevent homogenisation of the groups, implying an evolutionary trajectory of divergence. Such consideration of evolutionary trajectories is employed in the Evolutionary Species Concept (*sensu* Wiley and Mayden). Of course, at different points in time or space these clusters may converge or diverge and thus further consideration may be required to formally name these as species under the Evolutionary Species Concept. Regardless, the genotypic cluster definition is useful for examining current demographic processes occurring within cohesive genetic groups. Using this definition may elucidate ecological processes that have either created or that maintain the genetic structure between groups in sympatry. Thus, this definition is useful in its simplicity and practicality to employ in coral population genetic studies (where cryptic taxa are apparent) as an initial hypothesis for studying demographic processes within groups and ecological processes that may isolate groups.

1.2 Ubiquitous cryptic taxa within species

Cryptic taxa are increasingly being uncovered across all phyla due to advances in genetic sequencing that offers high power to unveil this diversity (Bickford et al., 2007; Fišer et al., 2018; Pfenninger & Schwenk, 2007). Cryptic taxa are genetically divergent clusters that are found within named taxonomic species, thus, "cryptic" typically implies that taxa are morphologically unresolvable by human visual inspection (*e.g.*, macro-morphological

characters). If morphological differences are indeed absent at the micro-morphological scales, the lack of morphological distinguishability may be due to a few reasons: recent divergence (*i.e.*, not enough time has passed for differences to accumulate), morphological stasis (*i.e.*, maintain ancestral morphology due to similar selection pressures), morphological convergence (*i.e.*, independently converging on similar morphologies due to similar selection pressures) (Fišer et al., 2018). Cryptic taxa are important to study and acknowledge for several reasons. Firstly, elucidating the ecological mechanisms that produce this diversity is important for our understanding of the breadth of speciation processes and not exclusively on the divergence of groups with marked morphotypes. Secondly, due to their genetic independence, cryptic taxa require separate consideration when measuring neutral demographic processes. Finally, incorporating cryptic taxa into biodiversity assessments, species distributions and species coexistence patterns may provide novel insights into general patterns of biological organisation.

In population genetic studies of corals, cryptic taxa are widespread, (Bongaerts et al., 2010; Gélin et al., 2018; Johnston et al., 2017; Ladner & Palumbi, 2012; Matias et al., 2022; Prada & Hellberg, 2020; Richards et al., 2016; Rippe et al., 2021; Tisthammer & Richmond, 2018; Underwood et al., 2020; van Oppen et al., 2018; Warner et al., 2015). Cryptic taxa may either be morphologically indistinguishable or be represented by particular morphs that were once considered as different ecotypes (*e.g.*, particular groups that are adapted to different environments and harbour phenotypic differences yet interbreed freely). Conventionally, corals are split into species by studying differences in either their macro-skeletal morphologies or micro-skeletal morphologies (Veron, 1996). However, morphological determination of coral species can be obscured by high intraspecific morphological plasticity, where individual colonies can switch morphology over time naturally (Paz-García et al., 2015) or through transplant experiments (see Todd, 2008), even appearing to switch between morphospecies (*e.g.*, *Pocillopora damicornis* to *P. inflata*, Paz-García et al., 2015). Additionally, highly divergent groups can have a lack of morphological variability (Bongaerts, Cooke, et al., 2021). Thus, using morphology alone for identifying some species and relationships between species may be problematic (see Flot et al., 2011; Fukami, Budd, Paulay, et al., 2004).

Despite being morphologically cryptic, such coral taxa are often sympatric and may be ecologically partitioned. For example, a genomic study on *Pachyseris speciosa*, found three genetically divergent groups that were sympatrically distributed across multiple large-scale

regions (Bongaerts, Cooke, et al., 2021). These groups showed some evidence of depth partitioning, differing physiologies and different spawning times but using scanning electron microscope technology analysing micro-skeletal differences, the divergent clades could not be separated. Thus, despite being morphologically conserved, ecological differentiation was apparent. Furthermore, hierarchical patterns of genetic structure within taxonomic species are common. A genetic study aimed at exploring genetic diversity within the *Pocillopora eydouxi/meandrina* complex (Primary Species Hypothesis, PSH09) found that it was split into three divergent groups and within each of these three groups further structuring representing two or more genetic groups were found in sympatry at the reef scale (Gélin et al., 2017, 2018). Thus, thorough scrutiny of the data is required to determine the level of genetic structure that is relevant to demographic patterns.

Previous studies, using fewer (or less informative) markers may have missed discovering cryptic taxa, especially in less well-studied groups. Additionally, many coral species have not yet been examined due to the unavailability of traditional markers. Genomic investigations provide better clarity in resolving taxa when morphology obscures inferences (Johnston et al., 2017). Using explicit frameworks for defining cryptic taxa (as is done for species) is important to employ in population genetic studies of corals for consistency and comparison across studies. Cryptic taxa appear commonly in population genetic studies, it is important to first assess the presence of cryptic taxa before inferring demographic processes. Furthermore, assessing the ecological partitioning of taxa can help determine the possible causes of cryptic speciation and its broader implications.

1.3 Gene flow between species and taxa

Another phenomenon that should be considered in population genetic assessments of species, is that divergent groups may occasionally interchange genetic information. Gene flow amongst divergent groups may oscillate in time and at different stages of divergence. For example, the emergence of a physical geographic barrier may prevent gene flow amongst populations which diverge primarily due to genetic drift and then the release of this barrier may allow for gene flow to recommence (*i.e.*, secondary contact). Secondary contact can either reinforce divergence, provide introgressed alleles, lead to the merging of groups, or create new hybrid species (Todesco et al., 2016). Divergence without gene flow due to physical barriers as

opposed to divergence with gene flow has been considered a common form of speciation (Bird et al., 2012). There are many examples where populations remain divergent despite gene flow, presumably due to disruptive selection driving local adaptation to opposing environments (Bolnick & Fitzpatrick, 2007) but whether the initial speciation stages require a pause in gene flow are still in question (*e.g.*, can isolating barriers emerge despite high gene flow?). However, such questions may be difficult to answer, since continuously distributed populations may harbour divergent populations at the extremes but remain connected to neighbouring individuals across an allopatry-sympatry continuum representing a gene flow gradient (see Galindo & Grahame, 2008). Gene flow between divergent entities can be evolutionarily advantageous through producing novel combinations of loci (Marques et al., 2019), introgressing adaptive alleles into maladapted groups (Huerta-Sánchez et al., 2014) and generating intermediate or diverse phenotypes that occupy transitional or novel environments (Rieseberg et al., 2003). Through advances in genomic research, more cases of gene flow among semi-divergent groups are being discovered and that is has significant impacts on speciation and evolution (Feder et al., 2012). Thus, measuring the capacity and commonality of different species or divergent taxa to interbreed can help to understand the speciation process as well as potential adaptive sources when populations experience environmental changes.

There is considerable evidence for frequent interspecific hybridisation and introgression in corals. Hybridisation is suspected to be more common in synchronous mass spawning species (as compared to brooding species) where spatial or temporal barriers to hybridisation are usually lacking (Willis et al., 2006). Indeed, in crossing experiments, one third of 42 species pairs in the mass-spawning Indo-Pacific genera, *Acropora, Montipora* and *Platygyra* were able to form zygotes (Willis et al., 1997), and the evidence of pre-zygotic barriers were the variable success rates (*i.e.*, lower fertilisation success than conspecifics) and that hybridisation among species occurred less readily in the presence of conspecifics (Willis et al., 2006). In a spawning Atlantic genus *Orbicella* spp., evidence of pre- and post-zygotic barriers were found regarding spawning time and genetic compatibility, yet at another site these species hybridised well (Fukami, Budd, Levitan, et al., 2004; Levitan et al., 2004; Szmant et al., 1997) and thus the geography of hybridisation can also be varied. However, crossing experiments are only suggestive and genetic evidence in natural populations is required to substantiate the claim that hybridisation occurs readily in nature. For example, a recent review found 81 scleractinian species reported to hybridise using genetic evidence with most species being broadcasters and a few brooders (Hobbs et al., 2022), but many of the older studies had issues with the resolution

of markers. Regardless, recent genomic evidence supports hybridisation among species (Combosch & Vollmer, 2015; Quattrini et al., 2019) and that it could be evolutionary advantageous. For example, ancient introgression events amongst *Acropora* spp. corresponded with climatic changes and thus suggests hybridisation may have been responsible for the adaptive radiation of *Acropora* in the Indo-Pacific (Mao et al., 2018), at least within this group. There is building evidence that occasional hybridisation could occur naturally amongst coral species and thus could impact evolution (*e.g.*, the transfer of adaptive alleles).

Divergence with gene flow (*i.e.*, sympatric divergence) in corals seems likely given the abundance of semi-divergent cryptic taxa with both environmental associations, presence of a few intermediates and the lack of spatial barriers in the ocean (Knowlton, 1993). However, whether the initial stages occur in sympatry (or with gene flow) is difficult to prove (Bird et al., 2012; Momigliano et al., 2020). Demographic modelling has been applied to capture the likelihood different gene flow scenarios, often showing complex histories of both periods of gene flow and isolation (Matias et al., 2022; Prada & Hellberg, 2020; Rippe et al., 2021). Thus, gene flow amongst closely-related taxa in corals is likely. Importantly, population genetic studies should incorporate multiple species in the event that it does occur. Because investigating hybridisation in corals could lead to novel insights about local adaptation and speciation. Additionally, ignoring potential hybridisation may lead to including hybrid individuals within analyses that could bias inferences (*e.g.*, spatial structure or population genetic summary statistics). Examining gene flow amongst closely-related species and cryptic taxa in corals that are ecologically partitioned may provide further evidence to the growing body of literature that sympatric divergence occurs in nature and that hybridisation and introgression could be important sources of genetic diversity during environmental shifts or population declines.

1.4 Niche partitioning and the depth cline

Cryptic taxa (that may occasionally interbreed) are often sympatric. There are many processes that enable co-occurrence. Either these taxa formed in sympatry and local adaptation resulted in divergence and they now occupy different ecological niches, or they diverged in allopatry and are presently in secondary contact. If taxa diverged in allopatry and present intrinsic reproductive incompatibilities, during secondary contact competition may be driving

ecological divergence among taxa (*e.g.*, Gause's law). Regardless of how these taxa genetically diverged, it is expected that taxa will diverge in their ecological niche if interspecific competition becomes greater than intraspecific competition (Chesson, 2000). Thus, if the one taxon is of low abundance, then competition may not be high enough for competitive exclusion to occur (*e.g.*, density dependence). Interspecific competition can also decrease through disturbance (*e.g.*, physical damage from weather events, predators, or disease) and taxa may differ in their responses to disturbance (*e.g.*, temporal mass effects) (Shmida & Ellner, 1984). Furthermore, spatial mass effects may allow the co-occurrence of taxa in transitional habitat, where source populations of both taxa are nearby and provide a constant supply into shared adjacent habitats (*e.g.*, spatial mass effects) (Shmida & Ellner, 1984; Shmida & Wilson, 1985). Investigating niche partitioning of cryptic taxa could elucidate the particular ecological processes that enable their co-occurrence and may promote their divergence. Particularly, understanding these processes in ecosystems such as coral reefs, where high species diversity exists across fine-scales and cryptic taxa within corals continue to be discovered, could help inform ecosystem-level dynamics.

Depth is the main environmental axis that separates closely related species of marine taxa (including corals) with one half of all comparisons being separated by depth (Knowlton, 1993). Depth constitutes a steep environmental gradient where light is exponentially attenuated, temperature decreases, pressure increases, nutrients and chemical compositions change, and storm damage is less likely (Dollar, 1982; Lesser et al., 2009). The large environmental differences between depths create a unique and stark transition, as these environments are generally spatially proximate. Yet despite this spatial proximity, there are many coral examples where cryptic taxa are partitioned by depth (Bongaerts et al., 2011, 2017; Bongaerts, Riginos, et al., 2010; Carlon & Budd, 2002; Rippe et al., 2021; X. Serrano et al., 2014). For example, the soft coral, *Eucinea flexuosa* harboured two genetically divergent groups in different depths (*i.e.*, shallow, and deep habitat) despite no geographical differentiation of these groups across the Caribbean (Prada et al., 2008; Prada & Hellberg, 2013). Furthermore, reciprocal transplants between the two depth environments showed higher survival for colonies in their native depth profiles (Prada & Hellberg, 2013). Thus, these taxa appear to be adapted differentially to depth environments, although in some locations their distributions largely overlap. Divergence among these taxa appears to have initially occurred with gene flow and then a period of isolation before experiencing gene flow again (Prada & Hellberg, 2020). Thus, the depth

environment or environmental factors that scale with depth, create environmental niches for different taxa to specialise.

Light intensity is important to corals that rely on the symbiosis with *Symbiodiniaceae*, but light does not always correlate with depth. For instance, Vermeij & Bak (2002) studied light preferences of six *Madracis* spp. and found several strategies related to light capture. Species of *Madracis* were found to occupy specific microhabitats at certain depths in order to obtain particular light thresholds, *e.g.*, on horizontal surfaces, vertical surfaces, or in caves or crevices. Thus, taxa adapted to diverging light regimes may inhabit overlapping depth ranges through occupying different light microhabitats. There are many examples in corals where closely-related taxa harbour disparate but overlapping depth distributions (Johnston et al., 2022; Prada & Hellberg, 2013; Rippe et al., 2021), but also cryptic taxa where no obvious environmental associations are found (Gélin et al., 2018; Ladner & Palumbi, 2012; Matias et al., 2022). Although, many studies did not sample over depths, or record the depths in which they were collected. Assessing microhabitats may elucidate the particular environmental parameters (rather than depth alone) that may have caused genetic divergence of cryptic and sympatric coral taxa and enable their co-occurrence. This is important for understanding the ecology of certain groups given the climatic changes occurring on coral reefs.

1.5 The spatial movement of genes and individuals

Genetic and ecological investigation can illuminate ecologically partitioned cryptic taxa and examine the nature of their reproductive boundaries (as reviewed above). But what is most important for the short-term recovery of populations is their capacity to disperse propagules either by sexual or asexual reproduction (Hellberg, 2007). Populations may be aided by dispersing away from disturbances or into populations that have suffered a disturbance, and thus rescuing populations from local extinction. Dispersal also allows populations to colonise new habitat and expand ranges. If particular alleles or genotypes are favoured for particular environments, then dispersal can aid in the adaptive spread of beneficial locally adapted propagules across space. Many species harbour varied strategies regarding dispersal. Local dispersal can increase the local dominance of self-sustaining populations and promotes local adaptation. However, it may lead to small population sizes and demographic isolation, which carries the risk of negative repercussions from inbreeding (*e.g.*, lower adaptability to changing

environments and the surfacing of recessive deleterious mutations). Long range dispersal has the opposite consequences; large population sizes and demographic connectivity, with the ability to withstand many different environments but not specialise in any, however it carries the cost of dispersal, *i.e.*, will propagules find sufficient habitats or perish? (Ronce, 2007). Measuring different species dispersal capacities is an essential life history parameter for predicting responses of different organisms to disturbance and environmental change.

Evidence of divergent dispersal capacities can be found in corals. Often broadcasting species, which spawns gametes and fertilisation takes place in the water column, are likely to exhibit larger dispersal capacities due to longer pelagic larval durations (days to weeks). Brooding species, where only the sperm is released and fertilisation takes place within the maternal colony, are likely to disperse shorter distances due to the ability to settle within hours to days upon release from the maternal colony (Carlon, 1999). But directly observing the dispersal of minute larvae within the ocean is improbable and impractical (Riginos et al., 2016). Population genetic structure is commonly represented by F_{ST}, which describes the variance in allele frequencies among populations and evidence of significant differences can reveal spatial breaks to gene flow. Following theory, broadcasters often show no population genetic structure over large distances (100s to 1000s of kms, Baums et al., 2010; Nakajima et al., 2010; Serrano et al., 2014; van Oppen et al., 2015) and brooders may be highly structured over small to moderate distances (kms to 10s of kms, Casado-Amezúa et al., 2012; Goffredo et al., 2004). However, estimating gene flow or the number of migrants per generation (N_em) from F_{ST} is likely to be erroneous due to the large number of assumptions required to fit the island model (*e.g.*, no selection, all populations have equal population sizes and migration, migration is completely random and not spatially structured, populations are at equilibrium, see Whitlock & McCauley, 1999 for further discussion). Additionally, only one migrant per generation is required to maintain genetic homogeneity among populations assuming effective reproduction and no selection (Waples & Gaggiotti, 2006), which would not have very strong demographic effects. Population structure estimates could also be reflective of past spatial discontinuities and stepping-stone connectivity may lead to genetic homogeneity across large distances despite more limited dispersal (Hellberg, 2007). Finally, it is possible that multiple cryptic taxa were treated as a single entity in past studies, which can either inflate or deflate F_{ST} depending on circumstances (see Sheets et al., 2018). Thus, inferring ecologically relevant gene flow from F_{ST} may be unreliable.

Other methods for inferring effective dispersal from genetics have been applied in corals but there are still some limitations to inferences. For example, Assignment methods are used to estimate recent generational gene flow by assigning new recruits to parental populations (*e.g.*, Brazeau et al., 2005; Torda et al., 2013a, 2013b; Zvuloni et al., 2008). However, if not all source populations are targeted than conclusions could be erroneous. Spatial autocorrelation and isolation-by-distance analyses show that dispersal is likely to occur within metres for some brooders (Dubé et al., 2020; Gazulla et al., 2021; Gorospe & Karl, 2013; Ledoux et al., 2010; Underwood et al., 2006) and brooders and broadcasters can present similar patterns (Miller & Ayre, 2008; Underwood et al., 2009). Importantly, dispersal variance (σ^2) can be estimated from both of these methods using an independent individual density measure (Hardy & Vekemans, 1999; Rousset, 1997, 2000). Dispersal variance is relevant to the migration-drift balances that maintain genetic panmixia across a certain area and describes the dispersal kernel (*i.e.*, the probability distribution of dispersal distances) (Broquet & Petit, 2009). But it has rarely been estimated in corals (but see Gazulla et al., 2021; Gorospe & Karl, 2013; Ledoux et al., 2010). There is also large variation in realised dispersal for both modes. Hydrological and geographic conditions vary and either promote or hinder dispersal as well as create asymmetrical patterns (Hellberg, 2007; Riginos & Liggins, 2013). Applying a dichotomy of brooding vs. broadcasting may not be relevant for some species, and each species should be separately assessed and for particular geographic regions of interest. For example, a complex and highly abundant coral in the Indo-Pacific, *Pocillopora damicornis* has been shown to exhibit both broadcasting to produce sexual larvae and brooding of asexual larvae which appear to disperse far distances (Schmidt-Roach et al., 2013; Stoddart, 1983; Torda et al., 2013b; Ward, 1992). It is highly important to provide ecologically relevant estimates of dispersal that are region and species specific for predicting demographic recovery and adaptive spreads.

Both asexual and gamete dispersal are important factors that contribute to demographic processes in corals. Clonal dispersal from physical disturbances or asexual larvae may enable the spread and increase of resilient long-lived genotypes during times of reduced sexual reproduction (Lasker & Coffroth, 1999), but will also reduce genotypic diversity which could result in non-resilient populations susceptible to certain environments or disease (Zhu et al., 2000). Due to being sedentary, limited gamete dispersal and low densities of mates or other genotypes can result in Allee effects and self-fertilisation (Ayre & Miller, 2006; Gascoigne & Lipcius, 2004). Some species are gonochoric (*i.e.*, separately sexed colonies), others

hermaphroditic or switch between these modes (Baird et al., 2009). Hermaphroditism is more common in broadcasters and gonochorism is more common in brooders, but there are species with opposite combinations. Hermaphrodites could self-fertilise and thus inbreeding could occur in species despite high dispersal capabilities (Heyward & Babcock, 1986), although it is predicted that self-fertilisation and inbreeding would be more common in species with low dispersal (Carlon, 1999). Thus, along with genetic diversity, genotypic diversity should be assessed because it is important regarding the neighbourhood of potential mates that will contribute to the next generation.

1.6 Novel methodological approaches

Measuring environmental, biotic, or genetic patterns across spatial scales within the ocean is difficult. Particularly, due to the limits in the amount of time spent underwater collecting data while SCUBA diving and the lack of resolution in genetic markers regarding either interspecific diversity or intraspecific diversity for many marine taxa (and especially for corals) (*e.g.*, for mitochondrial makers Gijsbers et al., 2022; for microsatellites Sturm et al., 2020). Pairing new technological advancements in underwater remote sensing and high-throughput sequencing could potentially help to resolve these previously pervasive issues.

Structure-from-motion photogrammetry is a method of remote sensing, where repeated photographs are taken of the seafloor in order to create virtual reconstructions. Such reconstructions are comprised of points situated in three dimensions, with additional information on their direction (*i.e.*, point vectors) and colour (*i.e.*, RGB values). Using known distance markers, these points can be scaled. Thus, spatial distances amongst particular points of interest can be estimated with 3-Dimensional accuracy. Measures of structural complexity (*i.e.*, quantitative measures that describe physical structure) can be extracted from these models. Traditional measures of structural complexity used by coral reef ecologists include rugosity (*i.e.*, how convoluted a surface is), which uses a metal chain of known distance laid across the reef substrate and equates to the ratio of the length of the chain to the 2D length of distance it covered. Chain rugosity is used measure the health of coral reefs, due to the predicted correlation between more rugose substrate and more availability of habitats for coral reef organisms (Storlazzi et al., 2016). Using photogrammetry, these measures can be easily quantified as well as the implementation of improved measures that capture the third dimension

and provide higher accuracy and resolution, such as 3D surface rugosity (*i.e.*, the ratio of 3D area to 2D planar area) (Torres-Pulliza et al., 2020). Furthermore, many marine organisms are sessile (*e.g.*, corals, sponges, molluscs, algae) and thus locations of particular organisms can be mapped, and their features described (*e.g.*, surface area and morphology). So far, photogrammetry has only been used to describe community-level patterns, but the mapping of individuals allows the study of their distributions, microhabitats, and phenotypes. Photogrammetry thus enables individual-specific assessments that can advance our understanding of both population-level and ecosystem-level spatial and environmental patterns.

Reduced representation genomic sequencing which harnesses high-throughput sequencing technologies can be used on non-model species to assess genome-level diversity without the requirement of a genome (Andrews et al., 2016; Davey & Blaxter, 2010). This technology has become more accessible to researchers as the cost of sequencing has been steadily decreasing. A common method is Restriction-site Associated DNA sequencing (RADseq), which uses restriction enzymes to cut genomic DNA into fragments. These fragments are then size selected (~100 – 400 bp) for short read sequencing, so that sequencing effort is focused on particular sized fragments that are found at equivalent sites across many individuals. Restriction enzyme cut sites are found across the whole genome (*i.e.*, coding, and non-coding) and are conserved, thus different individuals within species and closely-related species are likely to share these loci. RADseq and other similar technologies often yield thousands of loci across closely-related species and thus enable both the study of population genomics and phylogenomics. For population genomics, single nucleotide polymorphisms (SNPs) are discovered, and SNP data are used to assess allele frequencies differences amongst individuals, populations, and species. RADseq enables powerful individual-based analysis such as, the assignment and admixture of individuals to different genetic groups (Manel et al., 2005), correlations of SNPs to spatial or environmental variables (Balkenhol, 2016), population genetic summary statistics (Parchman et al., 2018), allele frequency spectrum based demographic analyses (Gutenkunst et al., 2009), and kinship analyses (Flanagan & Jones, 2019). Thus, reduced representation genomic sequencing is a low cost and efficient method to study many individuals of closely-related species and is likely to provide robust insights into individual relationships, cryptic taxa, hybridisation, past gene flow scenarios and spatial or environment influences on genetic variation.

Combining Structure-from-motion photogrammetry with reduced representation genomic sequencing enables the pairing of spatial, genomic, and environmental data (see Bongaerts, Dubé, et al., 2021). Individual genotypes can be mapped on virtual reconstructions to perform spatially-explicit individual-based analyses. Within a certain area (across fine spatial scales), exhaustive sampling of all individuals can elucidate local processes, such as dispersal of both sexual and asexual propagules and provide estimates of individual densities. Photogrammetry provides characterisation of the physical environment surrounding individuals and thus can elucidate potential correlations between species and cryptic taxa with physical microhabitats.

1.7 My study system, *Agaricia*

The scleractinian coral genus *Agaricia* is a fantastic system to study fine-scale spatial processes and habitat differentiation due to *Agaricia's* (1) presumed brooding reproductive modes, (2) abundance across shallow to mesophotic depths, (3) environmental partitioning (*i.e.*, depth) across and within species, and (4) ecological importance within the Caribbean.

The Atlantic coral genus, *Agaricia*, comprises seven named species, *Agaricia agaricites*, *A. humilis*, *A. tenuifolia*, *A. fragilis*, *A. lamarcki*, *A. grahamae* and *A. undata*. Brooding reproductive modes are assumed for the genus (A. H. Baird et al., 2009) due evidence from larval studies that some species are brooders (*i.e.*, *A. agaricites* and *A. humilis*) (Van Moorsel, 1983). However, other species have not been directly assessed. An early genetic study using Amplified Fragment Length Polymorphism (AFLP) markers on populations of *A. agaricites* provided insight into local genetic structuring occurring between reefs <10 km apart and indicated self-recruitment due to recent recruits assigning to their local adult populations (Brazeau et al., 2005). However, a genomic study on populations of *A. lamarcki* found both cryptic genetic substructure and genetic homogeneity across 10s of km (Hammerman et al., 2018). Similarity, a genomic study on populations of *A. undata* found genetic homogeneity between reefs and islands separated by 10s of km, however genetic differentiation across regions that are 100s of km apart (Gonzalez-Zapata et al., 2018). Lastly, in a genomic study on *A. fragilis*, genetic homogeneity was found within an island, but spatial genetic structure was found between islands separated by 10s of km (Bongaerts et al., 2017). These studies have provided interesting insights into specific genetic structure patterns across different regions and appear to suggest different dispersal capacities amongst species. But no comparative study has

looked at multiple congeners across the same regions and spatial scales and thus differences could be attributed to local geographic conditions and specific population histories.

Species within *Agaricia* occupy divergent yet overlapping distributions across the entire depth gradient available to photosynthetic corals. The deeper occurring species (*i.e.*, *A. fragilis*, *A. lamarcki*, *A. grahamae* and *A. undata*) are abundant at mesophotic depths (> 30 m depth) and share similar morphologies (*i.e.*, unifacial plating or encrusting). The shallower species (*i.e.*, *A. agaricites*, *A. humilis* and *A. tenuifolia*), are abundant at shallow depths (< 30 m depth) and harbour either unifacial or bifacial plates (except *A. humilis*), encrusting, or domed (only *A. humilis*) morphologies. A study on both the host and symbiont genetics of *Agaricia* species within Curaçao (*A. humilis*, *A. agaricites*, *A. lamarcki* and *A. grahamae*) found that each species harboured a distinct but overlapping depth profile and unique symbiont profiles (Bongaerts et al., 2013). Within this study *A. lamarcki* harboured different symbiont profiles at shallow and mesophotic depths, sharing the same symbiont profile as *A. grahamae* at mesophotic depths. The nuclear marker (*atp6*) was able to resolve genetic differences amongst the shallow (*A. humilis*, *A. agaricites*) and deep (*A. lamarcki*, *A. grahamae*) species but not between the species with more similar depth distributions. Genomic-level divergence was found between two groups within *A. fragilis* separated by shallow and mesophotic depths (Bongaerts et al., 2017). Thus, the depth cline appears to structure both species and genetic groups within species of *Agaricia*.

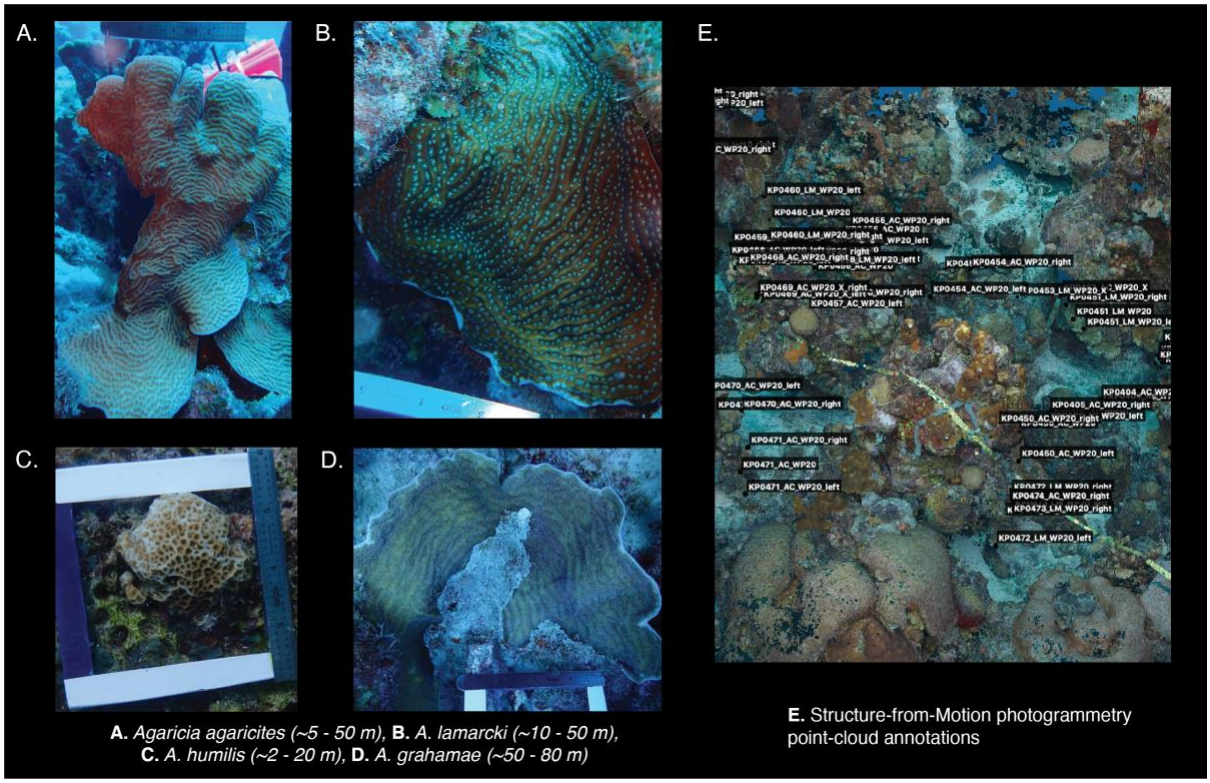


Figure 1.1. *Photographs of the focal species, (A) Agaricia agaricites, (B) A. lamarcki, (C) A. humilis and (D) A. grahamae (figure caption includes their dominant depth ranges). (E) A screenshot of the structure-from-motion photogrammetry point cloud including annotated colonies.*

In the Caribbean, many broadcasting 'framework' species have suffered population declines (*e.g.*, *Orbicella* spp. and *Acropora* spp.) which has led to relative increases in 'weedy' brooding species (*e.g.*, *Agaricia* spp. and *Madracis spp.*, among others) (Toth et al., 2019). Framework species are considered as such due to their dominance over the last 500,000 years, longevity, and asexual reproduction (in *Acropora* spp.) accruing significant reef cover. And 'weedy' species are considered as such due to relatively shorter lifespans and high recruitment rates (*sensu* Roff, 2020 and 'weedy' defined in Darling et al., 2012). There is a clear bias in the literature of studies focusing on the framework corals, however it is important to also understand these weedy groups. The family *Agaricidae* comprises many species that are dominant at mesophotic depths, and studies are suggesting that mesophotic coral reef ecosystems are important systems that enable refugia for shallower species whilst spouting unique diverse and productive ecosystems on their own (Bongaerts & Smith, 2019; Soares et al., 2020). Thus, investigating demography and niche partitioning within *Agaricia* species is important for understanding future species' responses to changes within the Caribbean in both shallow and mesophotic coral reef ecosystems.

1.8 Thesis aims and data chapters overviews

In my Thesis I aimed to use population genomics of *Agaricia* spp. corals and spatially explicit virtual 3D-constructs of the reef to investigate the causes and status of cryptic taxa and estimate dispersal within each taxon. Such inferences will add to our knowledge and understanding of the ecological processes that create and maintain species and how particular species can recover over ecological timescales.

Chapter 2 –I used high resolution genomic data (*i.e.*, nextRAD) and focused on spatial patterns and interspecific gene flow in the rarely considered mesophotic-specialist species, *A. grahamae* and depth-generalist species, *A. lamarcki*. Samples were collected from shallow and mesophotic depths within islands of Curaçao and Bonaire (southern Caribbean). This chapter comprised of a traditional population genetic study where samples were grouped per depth and location. I provide novel inference on spatial genetic patterns within a mesophotic-specialist species and one of the few emerging studies assessing gene flow scenarios across demographic histories of closely related species and cryptic taxa in corals. This chapter was published in *Molecular Ecology* (Prata et al., 2022).

Chapter 3 –I combined RADseq and photogrammetry to investigate fine spatial scale processes across three *Agaricia* spp. found at shallow depths, *A. humilis*, *A. agaricites* and *A. lamarcki*. A hierarchical sampling approach was implemented comprising of four locations across the leeward side of Curaçao and nested within each location, three depth plots at 5, 10 and 20 m were imaged using photogrammetry. Within each plot (25 x 2 m) all colonies of *Agaricia* spp. were exhaustively sampled for genotyping. I provide the most comprehensive, comparative study on dispersal amongst congeners, with one of the few estimates of dispersal variance in corals. Furthermore, I reveal cryptic taxa throughout *Agaricia* spp. and disparities in dispersal between congeners.

Chapter 4 – I used previous genetic assignments of cryptic taxa provided by Chapter 3 and derived quantitative measures from photogrammetry to assess microhabitat niche partitioning among cryptic taxa and species of *Agaricia*. I employ a unique approach for assessing coral niches and create novel quantitative measures to describe their microhabitats.

Chapter 2: Deep connections: Divergence histories with gene flow in mesophotic *Agaricia* corals

This chapter and its contents have been published in *Molecular Ecology*: Prata, K. E., Riginos, C., Gutenkunst, R. N., Latijnhouwers, K. R. W., Sánchez, J. A., Englebert, N., Hay, K. B., & Bongaerts, P. (2022) Deep connections: Divergence histories with gene flow in mesophotic *Agaricia* corals. *Molecular Ecology*, 00, 1-17, https://doi.org/10.1111/mec.16391

My contributions to this publication were substantial (Table 1.1). I utilised a dataset collected by my advisor and collaborators (KRWL, JAS, NE, KBH, JAS, PB). With the help of my advisors (CR, PB), I came up with the project concept and design. I performed all statistical analyses and interpreted the results with the guidance of my advisors and RNG. I wrote the original manuscript, CR and PB reviewed all drafts, RNG reviewed later drafts and the rest of the co-authors were involved in final edits and revisions which I led.

Table 1.1 *Author contributions to manuscript, "Deep connections: Divergence histories with gene flow in mesophotic Agaricia corals*

	***KP*[1]**	*CR*	*RNG*	*KRWL*	*JAS*	*NE*	*KBH*	*PB*
Project concept and design	**50**	10	0	0	0	0	0	40
Data collection	**0**	0	0	20	20	20	20	20
Statistical analyses	**80**	5	10	0	0	0	0	5
Interpretation	**60**	25	10	0	0	0	0	25
Original manuscript	**100**	0	0	0	0	0	0	0
Manuscript edits	**50**	17.5	5	2.5	2.5	2.5	2.5	17.5

[1]Letters donate author initials.

2.1 Abstract

Largely understudied, mesophotic coral ecosystems lie below shallow reefs (at >30 m depth) and comprise ecologically distinct communities. Brooding reproductive modes appear to predominate among mesophotic-specialist corals and may limit genetic connectivity among populations. Using reduced representation genomic sequencing, we assessed spatial population genetic structure at 50 m depth in an ecologically important mesophotic-specialist species *Agaricia grahamae*, among locations in the Southern Caribbean. We also tested for hybridisation with the closely related (but depth-generalist) species *Agaricia lamarcki*, within their sympatric depth zone (50 m). In contrast to our expectations, no spatial genetic structure was detected between the reefs of Curaçao and Bonaire (~40 km apart) within *A. grahamae*. However, cryptic taxa were discovered within both taxonomic species, with those in *A. lamarcki* (incompletely) partitioned by depth and those in *A. grahamae* occurring sympatrically (at the same depth). Hybrid analyses and demographic modelling identified contemporary and historical gene flow among cryptic taxa, both within and between *A. grahamae* and *A. lamarcki*. These results (1) indicate that spatial connectivity and subsequent replenishment may be possible between islands of moderate geographic distances for *A. grahamae*, an ecologically important mesophotic species, (2) that cryptic taxa occur in the mesophotic zone and environmental selection along shallow to mesophotic depth gradients may drive divergence in depth-generalists such as *A. lamarcki*, and (3) highlight that gene flow links taxa within this relativity diverse Caribbean genus.

2.2 Introduction

Mesophotic coral reef ecosystems lie below the well-studied shallow coral reefs (at ~30 - 150 m depth) (Lesser et al., 2009) and represent a substantial proportion of the world's potential coral reef habitat. These reefs have received considerable recent attention due their hypothesised role as ecological refuges (Bongaerts, Ridgway, et al., 2010; Bongaerts & Smith, 2019; Glynn, 1996; Semmler et al., 2017). Consequently, vertical genetic boundaries have been assessed within "depth-generalist" hard corals, namely those found in both shallow and mesophotic reefs. In such studies, vertical genetic structure has been commonly observed, although often varying among species and location (Bongaerts et al., 2017; Brazeau et al., 2013; Eckert et al., 2019; Serrano et al., 2014; Serrano et al., 2016; Studivan & Voss, 2018; van Oppen et al., 2011), refuting the concept of universal vertical replenishment (Bongaerts & Smith, 2019). Mesophotic coral communities, like their shallower counterparts, are also threatened by thermal anomalies and tropical storms (Bongaerts & Smith, 2019). Despite many surveys of connectivity in depth-generalists, patterns of horizontal connectivity in "mesophotic-specialist" species remain unexplored. If mesophotic-specialist species are horizontally isolated, then they would be more vulnerable to local disturbances than if populations are well-connected.

Scleractinian corals have traditionally been classified into two major modes of reproduction, which differentially affect spatial genetic structuring (Bongaerts et al., 2017; Carlon, 1999). In brooders, maternal colonies brood and release developed larvae with the ability to settle within hours (Carlon, 1999). This results in low dispersal potential and potential philopatry of the larvae (Warner et al., 2016). In contrast, larvae of broadcast spawners develop in the water column and are pelagic for longer (days to weeks) and thus have greater chances of dispersal (Carlon, 1999). Genetic surveys are often consistent with these expectations when examined over moderate to large distances (tens to thousands of km), where broadcast spawners generally exhibit low or negligible horizontal population structure (Baums et al., 2010; Huang et al., 2018; Nakajima et al., 2010; Serrano et al., 2014; Severance & Karl, 2006; Studivan & Voss, 2018; Tay et al., 2015; van Oppen et al., 2015), whereas brooding taxa typically have discernible population structure over similar distances (*e.g.*, Carlon & Budd, 2002; Casado-Amezúa et al., 2012; Goffredo et al., 2004; Gorospe & Karl, 2015; Stoddart, 1984; Underwood et al., 2006). However, these patterns are not universal, and it is still common for broadcasters to demonstrate fine-scale structure from local retention and brooders to demonstrate broad-

scale connectivity from a few widely dispersed propagules (Ayre & Hughes, 2000; Gorospe & Karl, 2013; Miller & Ayre, 2008; Riquet et al., 2021). At least in the Caribbean, most scleractinian coral species exclusive to mesophotic depths appear to be brooders (Bongaerts, Ridgway, et al., 2010). This suggests that these depth-specialist species could be highly structured and thus more vulnerable to local disturbances when compared to broadcast spawners.

Species boundaries in corals are poorly defined and are likely to be evolutionarily porous. Cryptic genetic groups are frequently described in corals (Arrigoni et al., 2019; Bongaerts, Cooke, et al., 2021; Gómez-Corrales & Prada, 2020; Ladner & Palumbi, 2012; Nakajima et al., 2017; Warner et al., 2015) and both homoplasy and phenotypic plasticity can lead to the misidentification of genetically distinct taxa (Forsman et al., 2009; Kitahara et al., 2016; Paz-García et al., 2015). Incomplete reproductive barriers between closely related taxonomic species are common (reviewed in Willis et al. (2006)), as are successful interspecific laboratory crosses (Willis, et al., 1997). Regular hybridisation between species or, reticulate evolution, has long been suspected to be an important aspect of coral evolution (Veron, 1995). Multilocus genetic or genomic approaches are moreover uncovering evidence for historical introgression (Mao et al., 2018) and frequent observations of contemporary admixture are consistent with intermixing of semi-differentiated taxa (*e.g.*, *Acropora* spp., Ladner & Palumbi, 2012; *Favia* spp., Carlon & Budd, 2002; *Madracis* spp., Frade et al., 2010; *Platygyra* spp., Miller & Benzie, 1997; *Pocillopora* spp., Combosch & Vollmer, 2015; *Porites* spp., Forsman et al., 2017; *Psammocora* spp., Stefani et al., 2008, *Seriatopora* spp., Bongaerts, Riginos, et al., 2010; *Stylophora* spp., Arrigoni et al., 2016; and octocorals, Prada & Hellberg, 2013; Quattrini et al., 2019). Recently, demographic modelling that is sensitive to detecting low levels of historical gene flow has shown evidence of this between taxa (*e.g.*, Cooke et al 2020; Ladner & Palumbi, 2012; Prada & Hellberg, 2021; Rippe et al., 2021). The examination of co-occurring closely related but genetically distinct groups can provide further insights into evolutionary dynamics of divergence with gene flow (Nosil, 2008; Bird et al., 2012). For example, morphologically cryptic and sympatric coral taxa may represent incipient species where specific habitats delineate taxa (*e.g.*, Carlon & Budd, 2002; Warner et al., 2015) or differences in reproductive timing (*e.g.*, Rosser, 2015). Determining the extent to which scleractinians can exchange alleles intra- or interspecifically can provide insights into possible rates of adaptive evolution such as in response to anthropogenic stresses.

Here, we focus on the genus *Agaricia* (Order: Scleractinia), which is one of the most speciose genera in the Caribbean and perhaps the most dominant group at mesophotic depths (Loya et al., 2019). *Agaricia* species have predominately plating morphologies and have been described as both hermaphroditic (Fadlallah, 1983) and gonochoric (Kerr et al., 2011). A brooding reproductive mode has been observed (through larval experiments) for three out of the seven species (*A. humilis*, *A. tenuifolia* and *A. agaricites*: Morse et al., 1988) and has therefore been assumed for the genus. *Agaricia* species are also presumed to have maternal inheritance of symbionts (Baird et al., 2009) resulting in host-endosymbiont specificity and with most species harbouring a distinct *Cladocopium* strain (Bongaerts et al., 2013). Within the Southern Caribbean, *Agaricia* species segregate by depth with some habitat overlap. Three species occur abundantly at mesophotic depths: depth-generalist, *Agaricia lamarcki* (most commonly found: ~15 – 50 m), and depth-specialists *A. grahamae* (~50 – 90 m) and *A. undata* (~60 – 90 m) (Bongaerts et al., 2013; Bongaerts, Frade, et al., 2015). Genetic structure has been assessed previously in *Agaricia* species, with horizontal spatial structure found over small to moderate distances in *A. agaricites* and *A. fragilis* (<40 km) (Bongaerts et al., 2017; Brazeau et al., 2005) but not in *A. lamarcki* and *A. undata* (considering similarly small to moderate distances: <40 km) (Hammerman et al., 2018; Gonzalez-Zapata et al., 2018). However, the spatial genetic structure of a dominant mesophotic-specialist, *A. grahamae*, has not yet been determined. This species shares the same *Cladocopium* strain with *A. lamarcki* at their sympatric depth zone (50 m), with *A. lamarcki* predominantly hosting a different strain at shallower depths (Bongaerts, Carmichael, et al., 2015; Bongaerts et al., 2013). Furthermore, mitochondrial markers (*atp6*, *nad5* and *cox1-1-rRNA*) have been unable to genetically differentiate *A. grahamae* and *A. lamarcki* (Bongaerts et al., 2013; Bongaerts, Frade, et al., 2015), although this is not surprising for Anthozoans with slow mutation rates of mtDNA, and shared haplotypes likely indicates their close-relatedness. Consequently, there is potential for hybridisation between *A. grahamae* and *A. lamarcki* as well as host divergence of shallow and mesophotic populations within *A. lamarcki.*

To evaluate horizontal genetic structure and interspecific gene flow of these ecologically important mesophotic species, we used a reduced representation genome sequencing approach (nextRAD) on specimens collected using a manned submersible and deep technical diving. We tested the following three hypotheses: (1) there is horizontal genetic structure between populations of mesophotic depth-specialist scleractinian species, *Agaricia grahamae* between Curaçao and Bonaire (~40 km); (2) gene flow occurs or has occurred between *A. grahamae*

and congener, *A. lamarcki* within the depth zone they share (50 m); and (3) depth-partitioning occurs within *A. lamarcki* between 15 and 50 m. After initial examination of the genetic data, we found two sympatrically occurring cryptic taxa each within both taxonomic species and thus *post hoc* decided to test the hypothesis that: (4) gene flow occurs between cryptic taxa or has occurred during their divergence history. To examine spatial genetic structure and identify hybrids, we used individual-based assignment models and multivariate analyses. For testing whether and when gene flow occurred during the divergence of taxa, we used the Diffusion Approximations for Demographic Inference (dadi) (Gutenkunst et al., 2009) to compare various demographic scenarios.

2.3 Methods

2.3.1 Sample collection

Specimens of *Agaricia* were collected at eight different locations on the leeward side of the islands of Curaçao and Bonaire in the Southern Caribbean, as part of the "XL Catlin Seaview Survey" carried out between March-April 2013 (Figure 2.1, S-T2.1). Samples were collected using technical SCUBA or the manned submersible ''Curasub'' operated by ''Substation Curaçao'', under permits from the Curaçao Government and the Bonaire Island Council. Specimens of the focal species *Agaricia grahamae* (Wells, 1973) were collected at a sampling depth of 50 m (±2 m), with two additional populations sampled at 60 m (±2 m) and 80 m (±5 m), whereas specimens of *Agaricia lamarcki* (Milne-Edwards & Haime, 1851) were collected at a subset of four locations within Curaçao at a sampling depth of 15 m (±2 m) or 50 m (±2 m). At one site, "CR60" (Figure 2.1, S-T2.1), additional samples were collected from the same colony (tissue connected) to assess for potential chimeras and from closely adjacent colonies (tissue not connected) to assess for potential clones due to fission. Morphological classification of the two species followed the taxonomic features specified by Wells (1973), Veron (2000) and Humann & DeLoach (2002). An additional 12 *A. grahamae* samples from San Andrés, Colombia (SA) were collected under permits by the National Environmental Licensing Authority (ANLA), and added as outgroup samples: three were collected from the upper mesophotic zone (60 - 65 m) and nine from the lower mesophotic zone (85 m). Small fragments of colonies were stored in salt-saturated buffer solution containing 20% DMSO and 0.5 M EDTA, and for a subset of specimens a skeletal voucher was collected.

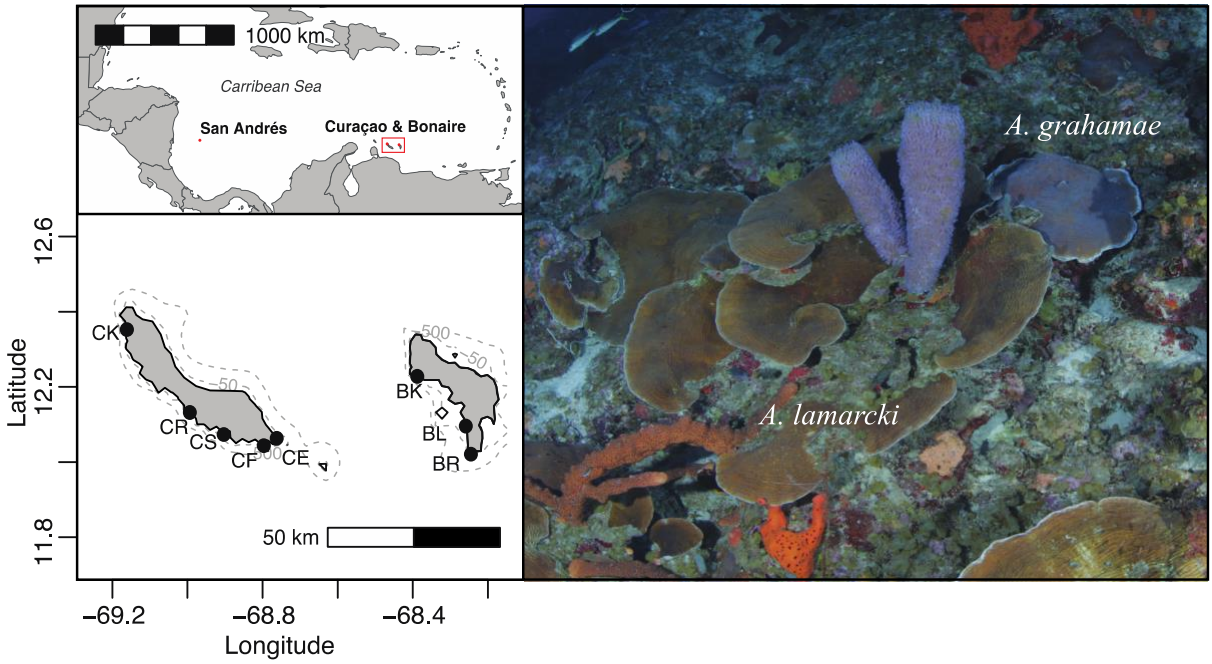


Figure 2.1. *Sampling locations for collected samples of Agaricia grahamae and A. lamarcki. Samples were collected from Curaçao and Bonaire, located in the Southern Caribbean, outgroup samples are from San Andrés (top left). Samples of A. grahamae were collected from five sites in Curaçao and three sites in Bonaire and A. lamarcki were collected in four sites in Curaçao: CK, CR, CS and CE (bottom left). Photograph of the two study species (right).*

2.3.2 DNA isolation, library preparation and sequencing

Isolation of genomic DNA from the coral host was carried out as reported in Bongaerts et al. (2017), using centrifugation steps to reduce endosymbiont contamination. Symbiodiniaceae were then isolated from two *A. grahamae* specimens (to sequence separately as a subtraction reference), using fluorescence-activated cell sorting (BD FACSAria Cell Sorter) at the Queensland Brain Institute. Quality and yield of gDNA were assessed using gel electrophoresis and a Qubit fluorometer, with a subset of samples (*A. grahamae* n = 176; *A. lamarcki* n = 51; Symbiodiniaceae n = 2) selected for downstream sequencing. For *A. lamarcki*, the Symbiodiniaceae ITS2 profile was determined for several of these samples in a previous study (n = 6; Bongaerts et al., 2015), and we screened the profiles of an additional 41 samples using the same ITS2-DGGE method against reference samples from that study. Library preparation was carried out using the nextRAD method (SNPsaurus, LLC), using a 9bp selective sequence ("GTGTAGAGG") to amplify loci consistently between samples. Genomic DNA was fragmented and ligated with adapter sequences using Nextera reagent (Illumina, Inc), and sequenced across a total of six HiSeq 2500 (Illumina, Inc) lanes using 100bp single-end chemistry and following the manufacturer's recommended protocol. Samples that failed in the

initial run (three lanes), were purified using AMPure XP beads to remove potential inhibitors and sequenced again on the additional HiSeq lanes.

2.3.3 Sequence clustering and variant calling

TRIMGALORE v.0.4.5 (https://github.com/FelixKrueger/TrimGalore) was used to remove adapters and low-quality ends (Phred below 20) and discarding reads that were less than 30bp. Read clustering was conducted using the IPYRAD pipeline v.0.7.22 (Eaton & Overcast, 2017) using default settings, excepting: minimum depth statistical/majority = 10, filter for adapters = 1, maximum uncalled bases = 5, maximum heterozygotes = 8, and maximum number of SNPs per locus = 20. Initial filtering, symbiont contamination removal and defining clonal lineages followed Bongaerts et al. (2017) (available through: https://github.com/pimbongaerts/radseq), unless otherwise indicated. We used BLASTN to identify and remove any matches to three Symbiodiniaceae databases (RAD isolates from Bongaerts et al. (2017), *Breviolum minutum* (ITS2 type B1) genome (Shoguchi et al., 2013) and *Cladocopium* (ITS2 type C1) genome (Liu et al., 2018). Other potential microbial contamination was removed through a BLASTN search against the NCBI non-redundant nucleotide database, extracting positive matches (max. e-value of 10^{-4}) that were classified as non-Cnidarian taxa (using the NCBI Taxonomy Database). RAD loci were truncated to 100bp prior to downstream analysis. Two sequencing duplicates (but from the same library preparation) of each species were included in the dataset to assess genotyping error and as a comparison to identify natural clones. The occurrence of any genetically identical individuals (clones) were then evaluated through assessing the distribution of allelic similarities between all pairs of individuals. Pairs of individuals that had 96% similar reads and above were deemed as clonal groups and one representative of each pair was retained. This threshold was chosen due to a combination of the genetic similarity of sequencing replicates (at similarities of 99%), a break in the distribution of pairwise allele similarities, and the maximum similarity (95%) that was observed for individuals occurring at different sites. Two datasets were retained: one removing putative clonal individuals and a second with all individuals.

The VCF file containing SNP data was filtered using VCFTOOLS v.0.1.16 (Danecek et al., 2011) to have a minor allele count of three due to many singletons and doubletons likely being sequencing or PCR errors and thus have consequences on downstream population genetic analyses (Andrews et al., 2016; Linck & Battey, 2017). To remove sites that were not

represented across most individuals, we removed sites that had >50% missing data across individuals and used a minimum depth of five per site. Certain individuals with low coverage across sites (<50% of sites genotyped) were removed. The removal of individuals with high missing data was conducted before more stringent filtering as these individuals will bias which sites are retained. Lastly, as different missing data filtering thresholds can change observed genetic patterns substantially, we applied four different thresholds for sites: (1) 50% maximum missing data of sites across individuals, (2) 20%, (3) 10% and (4) 5% and these datasets were compared for congruence across analyses. Results from the 20% missing data dataset are presented here unless reported otherwise due to congruence of results across the four thresholds.

2.3.4 Population Structure

To assess population structure of both species and initial evidence for hybridisation and introgression between species, methods that do not use *a priori* population assumptions were used. Models employed include: model-based multilocus population assignment methods based on maximum likelihood, (ADMIXTURE, v.1.3.0, Alexander et al., 2009) and Bayesian, (STRUCTURE, v.2.3.4, Pritchard et al., 2000) optimisation criteria as well as Principal Component Analysis (PCA) and likelihood-based genetic clustering (using functions *glPCA()*, *snap.clust()* and *find.clusters()* in the package, ADEGENET v.2.1.3 Jombart, 2008 in R v.3.6.3. The consensus of all four methods was used to identify the number of genetic clusters (K) within the datasets and to assign individuals to these clusters. The datasets including clones and without clones were compared for differences. Both "all SNPs" and putatively "neutral" datasets were compared for differences in genetic clustering patterns. The "neutral" datasets were created by removing outlier SNPs found using PCADAPT R package v.4.3.3 (Luu et al., 2017). The PCADAPT method identifies SNPs that exhibit significantly large correlations with certain PC axes relative to the genomic background, based on Mahalanobis distance and corrections using a genomic inflation factor. The calculation of *q*-values was used to determine which SNPs to retain with a false discovery rate <10% calculated by the QVALUE package v.2.18.0 (Storey et al., 2019) in R.

For ADMIXTURE and STRUCTURE analyses, the datasets were randomly trimmed to one SNP per contig to reduce correlations caused by physical linkage. Replicate datasets (10 replicates) with one random SNP per contig were created for comparison. In ADMIXTURE we ran each

dataset with a cross-validation of 100 for K = 1 - 7. In STRUCTURE we ran each dataset with a Burn-in of 100,000 and 50,000 MCMC repeats for K = 1 - 7. Cross-validation error between runs, log-likelihood ratios, and Evanno's Best K (Evanno et al., 2005) were evaluated to find the most likely number of clusters for each dataset in both analyses. For the Principal Component Analysis, the number of PC axes were deemed appropriate by assessing a drop between eigenvalues (where the slope becomes less steep as the cut off) as well as qualitatively assessing any structure on each axis iteratively until structure dissipates. For likelihood-based genetic clustering using the *snap.clust()* function, we chose the number of genetic groups (K) with lowest value for the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) between K = 1 – 10 for each dataset. We found two cryptic and sympatric genetic groups within each species (referred to as AG1 and AG2 within *A. grahamae* and AL1 and AL2 within *A. lamarcki* hereafter), these were treated as separate groups in subsequent analyses.

We calculated deviations from the Hardy-Weinberg Equilibrium for genetic groups within each taxonomic species using the *summary()* function in ADEGENET, then used Bartlett's test for homoscedasticity and a t-test for differences in means between the expected and observed heterozygosity in the package STATS v.3.6.3. We calculated population genetic statistics for each species treating each genetic group (e.g., AG1 and AG2) as populations, in the HIERFSTAT package v.0.0.4 (Goudet, 2005) in R for F_{IS} and Weir and Cockerham's F_{ST}. We tested for significance using Goudet's G-statistic with 1,000 permutations. A chi^2 test was used to detect correlations between the frequency of the genetic group in each depth profile for *A. lamarcki* using STATS.

2.3.5 Hybridisation and Introgression

Potential hybrids and individuals with various levels of mixed ancestry were found within in the assignment methods results. We investigated putative hybridisation using NEWHYBRIDS v.1.1 (Anderson & Thompson, 2002). NEWHYBRIDS incorporates the predictable patterns of inheritance seen in mating events rather than using only allele frequencies. We assessed whether individuals had genotypes consistent with any of eight hybrid classes: (1 and 2) pure parental, P1 or P2, (3) first- F1 or (4) second-generation, F2 hybrids (F1 hybrid offspring), (5 and 6) first generation backcross from F1 into each parent group, b-1 or b-2, or (7 and 8) second-generation backcross into each parent group, b-1-1 or b-2-2. The analysis was run for

≥10,000 steps and ≥10,000 MCMC with both Jeffery's and Uniform distributions testing for the eight possible genotype scenarios and each run was repeated five times. The number of steps the analyses was run for was determined by convergence of the parameters. We show results from the 5% missing datasets with neutral and unlinked SNPs due to inability to estimate hybrid classes with higher missing data thresholds.

2.3.6 Spatial Genetic Structure

We assessed the spatial genetic structure of the genetic groups within both *A. grahamae* and *A. lamarcki* to look for potential spatial and or environmental barriers to gene flow. We applied Redundancy Analysis (RDA) to assess the relative exploratory power of geographic distance and depth environment to SNP genotype data. RDA performs a multiple linear regression between matrices (Legendre & Legendre, 2012) and is a commonly used technique for assessing the relative contribution of multiple predictors. It is effective for uncovering predictors of population genetic structure (e.g., Forester et al., 2018; Legendre & Fortin, 2010). We utilised the partial RDA model for each taxonomic species: SNPs ~ latitude + longitude + depth environment (categorical) + condition (genetic group). Because cryptic genetic groups within each species were found, conditioning was used to evaluate the relative contribution of depth and geographic location to SNP variance considering the variance explained by the genetic groups. Missing genetic data was imputed with the most common SNP following Forester et al. (2018). For *A. grahamae*, we calculated the over-water distance matrix between locations using the function *lc.dist()* in the package MARMAP v.1.0.4 (Pante & Simon-Bouhet, 2013) in R. Here, we calculated the least-cost path between our locations which avoids land masses. These distances were then transformed into PCoA coordinate scores for input as exploratory factors into the RDA model. For *A. lamarcki*, which was only sampled in sites in Curaçao, we used the raw latitudes and longitudes as input into the RDA model because the over-water distance was similar. We used the *rda()* function in the package VEGAN v.2.5 (Oksanen et al., 2018) in R. We began with the full models and used permutation tests (1,000 permutations) to permute the genotypes randomly and assess the global model, RDA axes and marginal significance using PERMANOVA (*anova.cca()* function) (Legendre et al., 2011).

2.3.7 Demographic Inference

To explore the possibility of gene flow occurring during the divergence of our taxa, we used the Diffusion Approximation of Demographic Inference *dadi* v.2.1.1 (Gutenkunst, et al., 2009) in PYTHON v.3.6. We treated the two clusters found previously within each species as separate populations (within *A. grahamae*: AG1 and AG2 and within *A. lamarcki*: AL1 and AL2). The Joint Allele Frequency Spectrum (JAFS) was used to examine the likelihood of various demographic scenarios by modelling forward-in-time changes to the JAFS using solutions to the Chapman-Kolmogrov Forward equation (*i.e.*, the Diffusion Approximation). The JAFS is a matrix of bins comprising SNP counts for each combination of haplotype frequencies between the populations. We tested five different divergence scenarios for our 2-population comparisons: (1) divergence with no migration (no mig), (2) divergence with continuous symmetrical migration (sym mig), (3) divergence with continuous asymmetrical migration (asym mig), (4) divergence with ancient symmetrical migration followed by isolation (anc mig) and (5) divergence in isolation followed by symmetrical migration (sec cont) (see S1 for schematics of models). For each model, we incorporated inbreeding due to finding a statistically significant positive F_{IS} within each taxon (Blischak, Barker, & Gutenkunst 2020). Parameters for each of these models were fit for the JAFS of each pairwise population comparison, within (*i.e.*, AG1 and AG2) and between the known species (*i.e.*, AG1 and AL1), thus we assessed divergence patterns within six paired groups. The folded JAFS was used due to the ancestral state of each allele being unknown without an available outgroup as a reference. The JAFS were constructed by representative individuals from each population, which had >0.95 admixture assignment using neutral, unlinked (one SNP per contig) and 20% missing loci datasets. The *subsample()* function in *dadi* was used to randomly select a subset of haplotypes for analysis and maximise the number of SNPs due to missing data issues and to avoid difficulties with modelling inbreeding when projecting SNP frequencies to smaller sizes. In each paired population comparison, singletons and doubletons were masked because these entries were unreliable due high error rates in sequencing. Model parameters were optimised by simulating a model JAFS and calculating the likelihood of each model fit to our dataset JAFS using the Nelder-Mead simplex as the optimising algorithm. We assured convergence by running optimisations until independent runs with the same parameter scores (≤1% difference) and the lowest AIC occurred ≥2-3 times. We then compared ΔAIC and log-likelihoods (using the likelihood ratio test when nested) using the most likely replicate of each demographic scenario. The residuals between the simulated JAFS and the dataset JAFS were qualitatively

assessed for random distributions across the JAFS and forming a normal distribution at zero. To assess Goodness-of-fit we used non-parametric bootstrapping to see if the likelihood of parameters from the analysis dataset fit the distribution of likelihoods from the bootstraps. We applied the Godambe Information Matrix (GIM) (Coffman et al., 2016) using bootstraps to calculate the confidence intervals of the parameter estimates.

2.4 Results

2.4.1 Sequence Clustering and Variant Calling

Reduced representation sequencing was performed on 227 individuals to obtain 686,608 SNPs and 130,890 loci. We identified 41 individuals with ≥96% genetic similarity (putative clones), representing 20 clonal groups and one individual of each clonal group was kept for subsequent analyses (ST-1, 2, S2). Filtering measures and genetic assignment of species groups obtained four main datasets with a maximum 20% missing data per locus threshold (see S-T2.2 for specific filtering results): 1. All individuals dataset including both species (161 individuals, 4,306 neutral SNPs), 2a. *A. grahamae* with San Andrés outgroup (118 individuals, 3,104 SNPs), 2b. *A. grahamae* with no outgroup, outlier individuals and putative hybrid (106 individuals, 2,725 neutral SNPs) and 3. *A. lamarcki* (41 individuals 2,515 neutral SNPs). The congruent results from clustering analyses agreed with our morphological taxonomic classification of the two species: *A. grahamae* and *A. lamarcki* and further analyses were performed on these groups separately. Congruent results also found two distinct genetic clusters within both species that were found sympatrically across sites and depths within each species (named: AG1, AG2, AL1 and AL2).

2.4.2 Population Structure

Morphologically identified *A. grahamae* and *A. lamarcki* formed separate genetic clusters and one individual that was morphologically classified as *A. grahamae* had 0.5 mixed ancestry at K = 2 in ADMIXTURE and had intermediate PC1 scores between the two species clusters (Figure 2.2). This individual was treated as a provisional F1 hybrid. At K = 3, two cryptic clusters within *A. grahamae* (AG1 and AG2) occurred sympatrically at sites within Curaçao, Bonaire and outgroup San Andrés, Colombia. At K = 4, *A. lamarcki* also spilt into two clusters (AL1 and AL2). Across all methods, K = 4 was deemed most likely for this dataset.

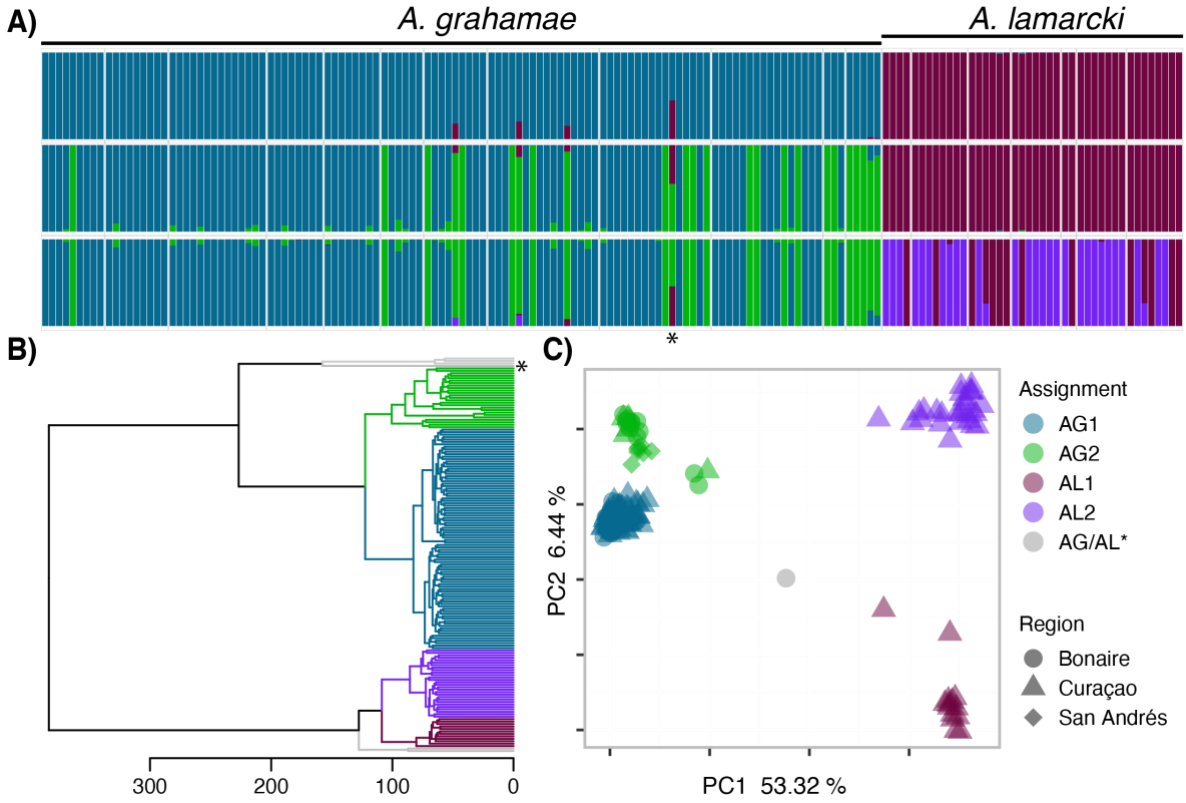


Figure 2.2. *ADMIXTURE, NJ-Tree and PCA results show genetic distinction between species and further substructure within each species. A) Ancestry proportions with ADMIXTURE analysis of 161 samples using 1,465 unlinked and neutral SNPs (119 Agaricia grahamae and 42 A. lamarcki) for K = 2 – 4. Each bar on the x-axis represents a sample and the y-axis is the proportion of ancestry. Genetic clusters are represented by colours. B) Neighbour-joining tree of the same samples with 4,306 neutral SNPs using genetic distance. Grey individuals represent outliers. C) PCA of the same samples using 4,306 neutral SNPs displaying PC1 and 2. * indicates the putative hybrid between nominal species.*

The same two clusters were found in the subset *A. grahamae* dataset at K = 2 (Figure 2.3). At K = 3, three individuals formed a separated cluster and appear as outliers separated from both *A. grahamae* groups on PC1. AG2 was more commonly found in Bonaire compared to Curaçao sites (10 vs. 4) and were more closely related to most individuals collected from San Andrés (SA) (7/8 assigned to AG2 at K = 2). The AG2 individuals from San Andrés formed a separate cluster at K = 4. Across all population structure methods, K = 3 was deemed most likely. We removed the *A. grahamae*/*A. lamarcki* putative hybrid, three outlier individuals and outgroup samples from San Andrés from the *A. grahamae* dataset to eliminate confounding elements for Hardy-Weinberg Equilibrium, spatial and hybrid analyses (i.e., dataset 2b).

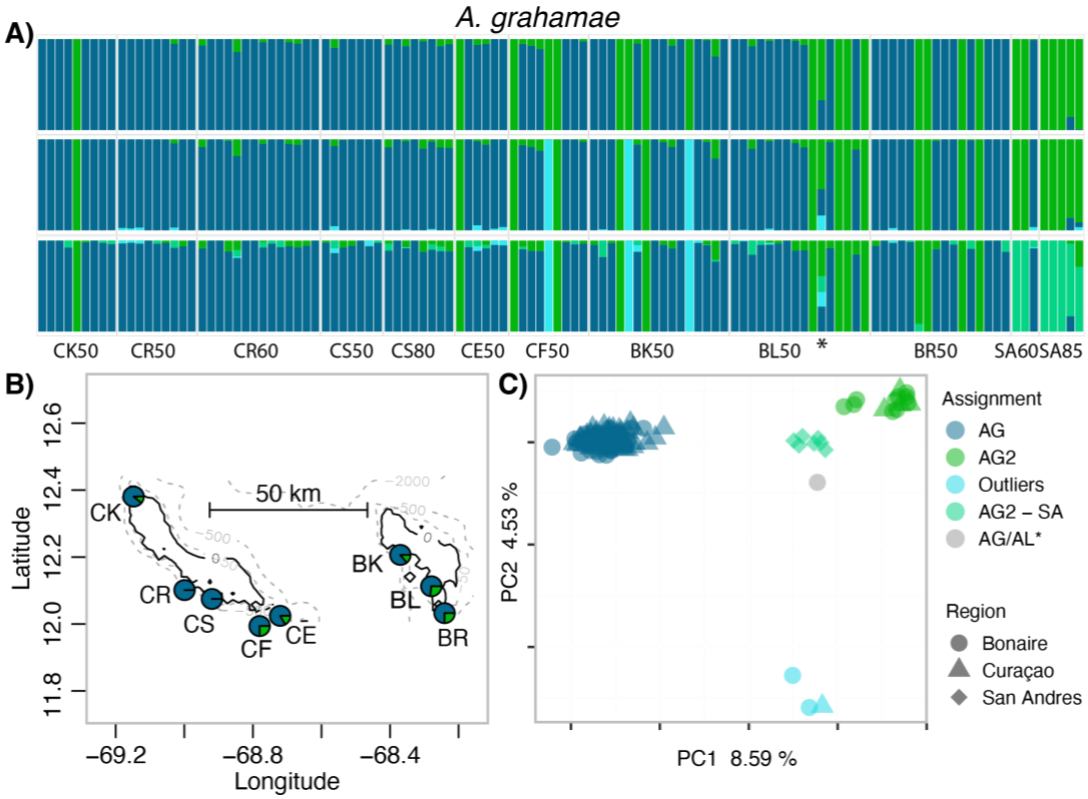


Figure 2.3. *ADMIXTURE and PCA results show genetic structure within A. grahamae, these genetic groups occur sympatrically at most sites within Curaçao and Bonaire. A) Ancestry proportions with ADMIXTURE analysis of 118 samples using 813 neutral and unlinked SNPs (K = 2 - 4) for the 10% missing dataset. B) Map of Curaçao and Bonaire indicating the proportion of each cluster at each site. C) PCA results using 3,104 SNPs. Site codes consist of one letter for region, one letter for site and numbers for depth sampled at, SA corresponds to outgroup, San Andrés. * indicates the previously found putative hybrid between A. grahamae and A. lamarcki*

A. lamarcki also had the same two clusters found in the "all individuals" dataset that occurred sympatrically at all sites and within both depths. AL1 was more commonly found at 50 m (12/19) and AL2 at 15 m (22/24) (Figure 2.4, χ^2 = 10.96, df = 1, p<0.01). Across all methods for the *A. lamarcki* dataset, K = 2 was deemed most likely. After having subset each of AG1, AG2, AL1 and AL2, into separate datasets, we found no further genetic structure (increasing K did not reveal any more clusters or improve likelihood greatly) across all population structure analyses.

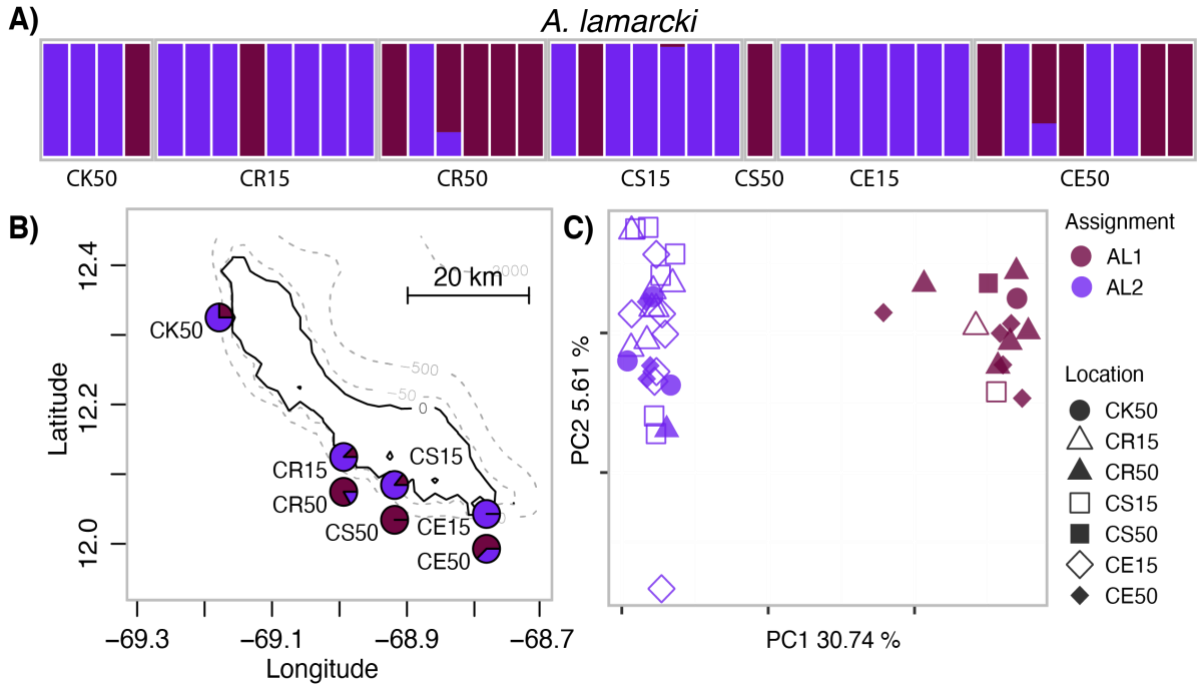


Figure 2.4. *ADMIXTURE and PCA results show genetic substructure within Agaricia lamarcki. Samples were collected from two depth profiles at 3 sites within Curaçao and one depth in at one site. A) Ancestry proportions with ADMIXTURE analysis of 41 samples using 1,328 unlinked and neutral SNPs for K = 2. Each bar on the x-axis represents a sample and the y-axis is the proportion of ancestry. Genetic clusters are represented by colours. B) Map of the study sites within Curaçao, pie charts represent the proportion of each genetic cluster found at each site. C) PCA of the same samples using 2,515 neutral SNPs.*

Hardy-Weinberg Equilibrium estimates were calculated for the cryptic taxa and F-statistics were calculated for each species with cryptic taxa treated as populations. Cryptic taxa within each taxonomic species were not in HWE (S-T2.3) with an excess of homozygosity. Inbreeding within populations for each species was high (AG: F_{IS} = 0.18 and AL: F_{IS} = 0.19) accompanied by substantial population differentiation between cryptic taxa (AG: F_{ST} = 0.17, p<0.01, AL: F_{ST} = 0.18, p<0.01).

Regarding the Symbiodiniaceae associated with *A. lamarcki*, corals predominately associated with the two ITS2 profiles (C3/C3d/C3.N6/C3.N7 and C3/C11/C11.N4/C3.N5) reported in Bongaerts et al. (2013, 2015; although sometimes with an extra unidentified band) and were partitioned between 15 m and 50 m depth. However, there was no association observed with the AL1 and AL2 lineages (S3), with both profiles occurring in both lineages.

We found groups of colonies with the same genotype ("genets") at most sites, with 17 genets observed for *A. grahamae* and three for *A. lamarcki* populations (S-T2.1, S-2.2), and each genet was always restricted to a single site (and depth). Most genets consisted of two colonies

("ramets"), although one genet for *A. grahamae* and one for *A. lamarcki* was represented by three ramets. At CR60 we took multiple samples for five *A. grahamae* colonies as well as four clusters of closely adjacent colonies, and these always represented the same genotype.

2.4.3 Hybridisation

2.4.3.1 Between *A. grahamae* and *A. lamarcki*

From the ADMIXTURE results for the dataset including both species, 115 individuals assigned to *A. grahamae* with >0.98 assignment and 42 individuals assigned to *A. lamarcki* with >0.99 assignment at K = 2. Of the admixed individuals, three were the outlier individuals identified previously within the *A. grahamae* dataset and one putative hybrid (also identified in the PCA results) had 0.5 admixture (Figure 2.2). The three outlier individuals and outgroup samples from San Andrés were removed to create another dataset for input into NEWHYBRIDS (n = 149, 5% missing, SNPs = 555). In NEWHYBRIDS, all individuals assigned as pure species with 0.99 probability (P1 or P2) apart from the putative F1 hybrid which assigned as a F1 hybrid with a 0.99 probability.

2.4.3.2 Between cryptic groups within *A. grahamae*

We also assessed the patterns of admixture between the two *A. grahamae* lineages (AG1 and AG2). ADMIXTURE results presented 55 individuals assigned to AG1 >0.99 assignment and 14 individuals for AG2 at K = 2. The remaining 37 individuals predominately assigned to AG1 (0.8 – 0.98). We used dataset 2b (without outgroup, AL/AG hybrid and outliers) with more stringent filtering for input into NEWHYBRIDS (n = 106, 5% missing, SNPs = 514). NEWHYBRIDS assigned 86 individuals with predominately P1 assignment (0.62 – 0.99) (pure parental AG1) and 12 individuals with predominately P2 assignment. The remaining individuals: 6 individuals (predominately assigned to AG1) and 2 individuals (predominately assigned to AG2) had higher assignments to hybrid categories (S-T2.4). Most notably, the highest probabilities for these admixed individuals were second generation back-crosses into each parental. Due to the limitations of the NEWHYBRIDS analysis, we were not able to estimate genotype classes of more advanced backcrosses which these individuals may more accurately represent.

2.4.3.3 Between cryptic groups within *A. lamarcki*

Within the *A. lamarcki* dataset, ADMIXTURE found 12 individuals assigned to AL1 >0.99 and 26 assigned to AL2 >0.99 and three individuals were admixed. Two of these admixed individuals were assigned to AL1 with ~0.7 and 0.78 assignment and one with 0.98 to AL2. A more stringent filtering dataset was used for input to NEWHYBRIDS (n = 41, 5% missing, SNPs = 622). In NEWHYBRIDS, two individuals presented higher assignment to hybrid classes than pure parentals: one *A. lamarcki* individual collected from CE50 had a 0.99 probability of being a F2 (offspring of two F1s), and another from CR50 had mixed assignments, 0.34 for pure parental AL1, 0.41 for a first generation back-cross into AL1 and 0.25 for second generation back-cross into AL1. The second individual, potentially representing a further back cross into AL1.

2.4.4 Spatial Genetic Structure

No strong correlations between genotype or depth, latitude, and longitude for either species were uncovered with redundancy analyses. For *A. grahamae*, although the global model was significant (R^2 = 0.04, R^2_{adj} = 0.002, F = 1.05, p = 0.005) it only explained 4% of the variance. None of the canonical axes were significant in explaining variation in SNPs (*i.e.*, RDA1 – 4: P≥0.1) and PC1 explained more variation than RDA1 (0.015>0.011). This was further substantiated through removal of each term, apart from Condition(Clusters), improving the AIC of the model. For *A. lamarcki*, the full model was globally non-significant (R^2 = 0.07, R^2_{adj} = 0.0002, F = 1.00, p = 0.483) as was each reduced model. Thus, neither horizontal (by space) nor vertical (by depth) locations were substantial predictors of genetic variation within cryptic taxa in both taxonomic species.

2.4.5 Demographic Inference

The demographic modelling results from *dadi* consistently found that models including migration (gene flow) had higher likelihoods than those with no migration (Tables 2.1, 2.2, Figure 2.5 and S-2.4). In the "no migration" models, divergence time approached the lower bound parameter limit. Thus, gene flow likely occurred during the divergence of the two species (*Agaricia grahamae* and *Agaricia lamarcki*) as well as during the divergence of the two cryptic taxa found within each species (AG1, AG2, AL1 and AL2). The haplotype frequency patterns within the JAFS were more comparable to the divergence with migration

scenario than the no migration scenario, particularly in the shared low frequency bins (Figure 2.5), as shared alleles in such bins are expected to be elevated from migration. Divergence time (T) in the isolation models was always less than migration models due to attempts to optimise these frequency bins (Table 2.1, Figure 2.5). For all pairwise comparisons the symmetrical and asymmetrical continuous migration models showed similar results, with the asymmetrical migration models having only minimal differences in migration rates. Thus, only the continuous symmetrical migration models are reported here. In the ancient migration model optimisations between all population-pairs, T2 (the second epoch where populations diverged in isolation) approached the lower the parameter limit (~0) and thus equating this model (ancient migration) to the continuous symmetrical migration scenario. The continuous symmetrical migration and secondary contact models had equal likelihood (<2 AIC) for within taxonomic species comparisons (AG1 vs. AG2 and AL1 vs. AL2, Table 2.1). For both AG groups vs. AL1, secondary contact had higher likelihood >2 AIC than continuous migration model (Table 2.2). But for AG groups vs. AL2, the secondary contact model had equal likelihood with continuous migration. Migration rates estimated from *dadi* were larger between more closely related pairs than the more distant pairs, suggesting that more divergent taxa are likely to experience less gene flow (Tables 2.1 and 2.2). Parameter uncertainties were often larger than 0.5 of the parameters and thus are not shown. These were also not converted into real time units because of the unreliability of parameter estimates due to a low number of SNPs used as well as having unreliable mutation rate and generation time estimates. The main purpose of this analysis was to detect whether gene flow occurred over the divergence history between all taxa, and thus conversion into real time units was not necessary for the aims of this study.

Table 2.1. *Demographic modelling performed between genetic groups within Agaricia grahamae (AG1 and AG2) and Agaricia lamarcki (AL1 and AL2) support histories of divergence with gene flow. Maximum likelihood estimates for each demographic scenario and each parameter is scaled by θ.*

Model	LogL	AIC	χ^2	θ[a]	N_1[b]	N_2[b]	F_1[c]	F_2[c]	m[d]	T1[e]	T2[e]
Between *A. grahamae* genetic groups											
No mig	-558.54	1127.09	1181.93	239.50	2.12	150	0.00	0.00	-	0.41	-
Sym mig	-499.09	**1010.18***	591.89	141.06	2.98	1.40	0.00	0.40	1.05	1.19	-
Anc mig	-499.15	1012.30	593.28	140.80	2.98	1.40	0.00	0.40	1.05	1.19	0.00
Sec cont	-497.26	**1008.52***	536.81	148.19	2.91	1.43	0.00	0.46	1.27	0.65	0.38
Between *A. lamarcki* genetic groups											
No mig	-551.26	1112.52	467.64	264.47	7.58	1.98	0.03	0.00	-	0.43	-
Sym mig	-516.33	**1044.66***	385.34	200.97	26.9	1.57	0.46	0.30	0.49	1.01	-
Anc mig	-516.27	1046.54	385.56	200.26	26.9	1.56	0.46	0.24	0.51	1.01	0.01
Sec cont	-515.94	**1045.88***	380.58	207.65	25.0	1.67	0.46	0.39	0.64	0.60	0.29

[a] *$\theta = 4N_{ref}\mu$.*

[b] *N_1 = the resulting population size change from N_{ref} to population 1. N_2 = the resulting population size change from N_{ref} to population 2.*

[c] *F_1 and F_2 = the inbreeding coefficients (F) of population 1 and 2.*

[d] *m = the symmetrical migration rate between population 1 and 2, in 2Nref generations.*

[e] *T1 = time since divergence to present for one epoch models and time since divergence to T2 in two epoch model. T2 = time since T1 to present. Units in $2N_{ref}$ generations.*

** Models with the highest likelihood in bold.*

Table 2.2. *Demographic modelling performed using six cross-species comparisons (A. grahamae: AG1 and AG2 and A. lamarcki: AL1 and AL2) support histories of divergence with gene flow. Maximum likelihood estimates for each demographic scenario and each parameter is scaled by θ.*

Model	LogL	AIC	χ^2	θ[a]	N_1[b]	N_2[b]	F_1[c]	F_2[c]	m[d]	T1[e]	T2[e]
Between species (1) AG1 and AL1											
No mig	-500.02	1010.04	615.34	246.23	0.84	42.5	0.00	0.01	-	0.84	-
Sym mig	-432.32	876.64	330.27	94.41	2.09	2.88	0.00	0.02	0.10	3.21	-
Anc mig	-432.32	878.64	330.03	92.97	2.13	2.92	0.00	0.00	0.10	3.28	0.00
Sec cont	-426.92	**867.84***	307.21	201.16	0.87	76.8	0.15	0.36	0.27	1.29	0.17
Between species (2) AG1 and AL2											
No mig	-670.25	1350.50	1031.49	212.76	0.78	72.2	0.00	0.00	-	0.68	-
Sym mig	-578.08	**1168.16***	687.91	67.10	2.40	3.61	0.00	0.00	0.09	3.64	-
Anc mig	-578.15	1170.30	687.81	64.93	2.47	3.72	0.00	0.01	0.09	3.80	0.00
Sec cont	-576.35	**1166.70***	698.43	103.01	1.61	2.47	0.01	0.00	0.15	1.19	0.69
Between species (3) AG2 and AL1											
No mig	-277.28	564.56	229.23	174.10	0.77	42.6	0.02	0.01	-	0.96	-
Sym mig	-261.40	**534.80**	187.05	95.77	1.33	2.26	0.41	0.53	0.12	2.00	-
Anc mig	-261.37	536.74	187.54	74.17	1.69	2.87	0.33	0.52	0.11	2.91	0.04
Sec cont	-259.32	**532.64***	179.72	149.32	0.83	87.9	0.36	0.62	0.17	1.19	0.22
Between species (4) AG2 and AL2											
No mig	-401.24	812.48	479.69	159.48	0.69	41.8	0.02	0.00	-	0.79	-
Sym mig	-376.24	**764.48***	369.51	85.90	1.34	2.55	0.54	0.11	0.11	1.85	-
Anc mig	-375.68	765.36	389.22	67.73	1.65	3.16	0.51	0.01	0.11	2.65	0.06
Sec cont	-375.64	**765.28***	398.10	92.70	1.26	2.42	0.55	0.16	0.13	0.87	0.72

[a] *$\theta = 4N_{ref}\mu$.*

[b] *N_1 = the resulting population size change from N_{ref} to population 1. N_2 = the resulting population size change from N_{ref} to population 2.*

[c] *F_1 and F_2 = the inbreeding coefficients (F) of population 1 and 2.*

[d] *m = the symmetrical migration rate between population 1 and 2, in 2Nref generations.*

[e] *T1 = time since divergence to present for one epoch models and time since divergence to T2 in two epoch model. T2 = time since T1 to present. Units in $2N_{ref}$ generations.*

** Models with the highest likelihood in bold*

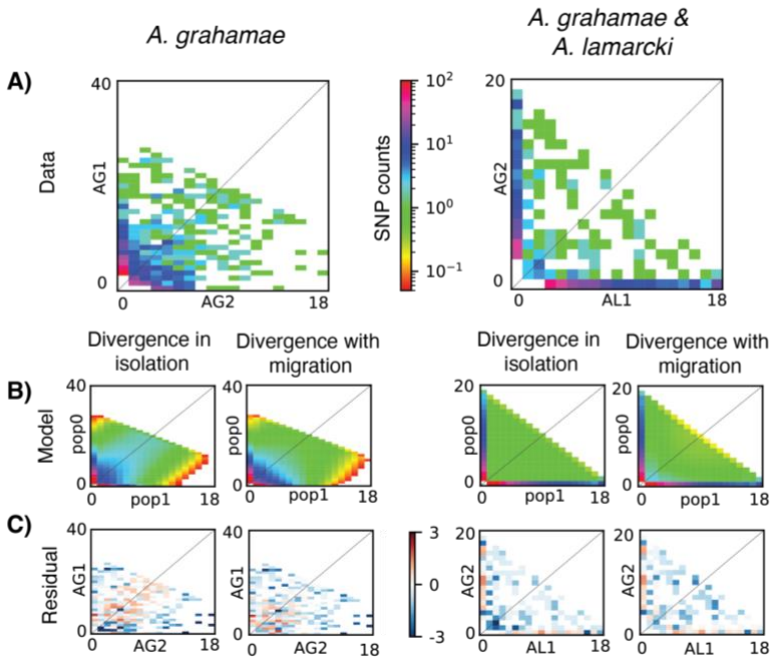


Figure 2.5. *Demographic analysis for cryptic taxa within species and between species shows that divergence with migration is more likely than divergence in isolation. A) The folded Joint Allele Frequency Spectrum (Data), B) two simulated JAFS from each model (divergence in isolation and divergence with migration) (Model), and C) their standardised residuals (Model – Data SNPs) are shown for (left panel) A. grahamae taxa (AG1 and AG2) and (right panel) between species (AG2 and AL1). Haplotypes from each population are represented on the x and y-axes of the JAFS and the colour scale represents the SNP counts corresponding to all haplotype frequency combinations between pairs of populations.*

2.5 Discussion

Mesophotic coral ecosystems harbour unique depth-specialist coral species, yet the ecology and evolution of these species remain almost completely unstudied. Given the assumed brooding reproductive mode within the *Agaricia* genus, we expected populations of the mesophotic-specialist species *A. grahamae* to be genetically structured over short spatial scales. Surprisingly, no horizontal spatial structuring was detected between reefs within Curaçao and Bonaire nor between the two islands (~40 km apart; Figures 2.2, 2.3 and RDA results) but appears at San Andrés (>1,000 km, Figure 2.3). Similarly, no horizontal spatial structure was found within Curaçao for the depth-generalist congener, *A. lamarcki* (Figure 2.4). Instead, we uncovered two sympatrically occurring and yet distinct cryptic taxa within each taxonomic species (Figures 2.2, 2.3 and 2.4) that were incompletely depth-partitioned in *A. lamarcki* but with no detectable depth or geographic segregation in *A. grahamae*. These cryptic taxa appear to be connected by historical and contemporary gene flow as indicated by the

presence of backcross individuals and through demographic analyses (Table 2.1, Figure 2.5, S-2.4 and S-T2.3). The divergence history of *A. grahamae* and *A. lamarcki* was also characterised by interspecific gene flow (Table 2.2, Figure 2.5 and S2.4), suggesting past semi-permeable boundaries between these species.

2.5.1 Lack of genetic structuring between reefs and islands

Contrary to our expectations of spatial genetic structure over small distances, genetic homogeneity was found for *A. grahamae* between Curaçao and Bonaire (<40 km) as evidenced by the RDA (see Results) and genetic structure results (Figure 2.3). Both cryptic taxa found within *A. grahamae* also occurred at San Andrés (>1,000 km away) and thus, it is unlikely that the two cryptic groups within *A. grahamae* were recently allopatrically formed. The congener, *A. lamarcki* was assayed only in Curaçao but also showed no genetic differentiation between the sampled reefs (RDA results, Figure 2.4). Genetic homogeneity within either island (Curaçao and Bonaire) may not be too surprising because reef communities are fairly continuous along leeward sides of the islands which could facilitate stepping-stone gene flow and occasional long-distance dispersal. However, Curaçao and Bonaire are separated by deep oceanic water (>500 m depth) with a north-west bound surface current and a west-east sub-surface counter current (Andrade, 2003), presenting a physical dispersal barrier. In contrast to our results (and consistent with limited dispersal), shallow occurring congeners *Agaricia agaricites* and *A. fragilis* were found to have localised structure (Brazeau et al., 2005; Bongaerts et al., 2017). Our results do, however, match to those described for mesophotic occurring *A. lamarcki* and *A. undata* (Gonzalez-Zapata et al., 2018; Hammerman et al., 2018) where populations did not exhibit local genetic structure (over similarly short distances: <40 km). Genetic subdivision in *A. grahamae* does emerge at larger spatial scales (AG2 individuals in San Andrés, Figure 2.3A). It is conceivable that reproductive strategies within *Agaricia* species are more variable than assumed and that the dispersal abilities within *A. lamarcki*, *A. grahamae* and *A. undata* are potentially greater than that of *A. agaricites* and *A. fragilis*. Taken together, these findings across studies highlight variability in spatial genetic structuring that can occur even among congeners. A comparative study on the spatial genetic structure at the same localities of *Agaricia* spp. would provide greater resolution to these hypotheses as well as studies on reproduction in *A. lamarcki* and *A. grahamae.*

The occurrence of the clone groups at most sites within *A. grahamae* and two of the sites within *A. lamarcki* are not surprising results as fission is common in *A. lamarcki* and in the congener *A. agaricites* (Hughes & Jackson, 1985). Their growth form (foliaceous/plating/encrusting) makes *Agaricia* spp. vulnerable to partial mortality from overgrowth by algae and sedimentation, which then results in subsequent fission where one colony becomes two or more colonies with separated tissue (Hughes & Jackson, 1980). At CR60, we confirmed this process by identifying clones from fission.

2.5.2 Distinct depth distributions for *A. lamarcki* but not *A. grahamae* taxa

Depth constitutes a strong environmental gradient for scleractinian corals, as it modulates light and other environmental conditions upon which corals depend (Bongaerts, Carmichael, et al., 2015). Although no depth-partitioning was observed for *A. grahamae* taxa between the upper (50 m) and lower (80 - 85 m) mesophotic zone at one site comparison in Curaçao and one in San Andrés, we did find a difference in the relative abundance of the cryptic taxa by depth in *A. lamarcki* (15 vs 50 m, Figure 2.4, $\chi^2 = 10.96$, df = 1, $p<0.01$). Given the exponential decay of irradiance with depth, the most extreme differences appear in first few metres thus comparisons between 15 m and the mesophotic are considerably greater than between differences within mesophotic zones (*e.g.*, between 50 and 85 m depth). The observed depth-differentiation within *A. lamarcki* and not *A. grahamae* follows these light transitions, although environmental factors such as nutrient availability, salinity, flow environment and temperature also vary between shallow and mesophotic depth zones (Dollar, 1982; Lesser et al., 2009; Bongaerts, Frade, et al., 2015) and could be contributing factors. Similar to the present study, a survey of *A. lamarcki* using ddRAD (Hammerman et al., 2018) also found two sympatric cryptic genetic groups but within Puerto Rico (sampled mostly between 10 – 20 m depth). At the population level, depth differentiation has been observed in Bermuda for the congener *A. fragilis*, where populations at 12 and 40 m were strongly differentiated (Bongaerts et al., 2017). Future surveys that can measure differences in microhabitats within the same depth profile may be informative for determining more precise environmental niches distinguishing cryptic taxa rather than depth alone.

In contrast to cnidarian host genotypes, we found almost complete depth partitioning of the symbiont profile within *A. lamarcki* (S3) which further supports the potential for depth-associated divergence. However, the symbiont profiles did not correspond directly to the different host taxa as expected due to algal symbiont specificity patterns seen within the genus, brooding taxa within the Caribbean (Bongaerts, Carmichael, et al., 2015) and other taxa (Prada et al., 2014; Thornhill et al., 2014). The symbiont profile found in individuals of *A. lamarcki* at mesophotic depths was the same profile shared by *A. grahamae* (S3, Bongaerts et al., 2013, Bongaerts, Carmichael, et al., 2015). Thus, this could indicate an ancestral association with this symbiont, more recent acquisition through host hybridisation, or potentially horizontal symbiont acquisition of this depth-specific endosymbiont (Quigley et al., 2018), which is indeed shown in found coral taxa (Bongaerts, Carmichael et al., 2015; Rowan & Knowlton, 1995). Another explanation for this disparity may be incomplete host-symbiont lineage sorting. Host divergence (between *A. lamarcki* taxa) could predate symbiont sorting due to retention in the maternal line. Further experimental work should investigate symbiont inheritance patterns and use modern methods of symbiont genotyping (amplicon sequencing) to better determine the relationship between host genotype and algal symbiont within these taxa.

2.5.3 Gene flow between cryptic taxa and species

Cryptic taxa are commonly found within genetic studies of scleractinians, yet until recently their divergence history has not been examined in detail in either shallow or mesophotic environments (but see Prada & Hellberg, 2020; Rippe et al., 2021). Often, cryptic coral taxa are closely related and sympatrically occurring and thus have the potential to interbreed. Here, we find evidence of two genetically distinct taxa within both species (Figures 2.2, 2.3 and 2.4) and backcross individuals between taxa (see Results, S-T2.4). An F1 hybrid was also found between *A. grahamae* and the "deep" *A. lamarcki* (Figure 2.3) which could suggest a continued low rate of recent interbreeding between species. Finally, demographic models that included gene flow during divergence (*i.e.*, continuous migration, ancient migration, and secondary contact) consistently had higher likelihoods than the null models of no migration for both between cryptic taxa within and between species (Figure 2.5, S-2.4, Tables 2.1 and 2.2).

Although results from *dadi* gave the greatest support to the two-epoch models of divergence in isolation followed by secondary contacts (Tables 2.1 and 2.2), if population expansions or

bottlenecks have occurred, divergence time can be over-estimated and results can favour the secondary contact model (Momigliano et al., 2021). As RAD datasets with *de novo* assemblies are prone to high error rates in low frequency SNP calling (Andrews et al., 2016), we did not include singletons and doubletons in the analyses and thus were unable to accurately model population size changes and differentiate between the different migration scenarios (*i.e.*, ancient migration vs. continuous migration vs. secondary contact). Thus, our inferences about timing and relative model support (among models including migration) should be interpreted cautiously. Nonetheless, models with no migration were consistently rejected and therefore we can confidently conclude that limited migration between distinct taxa has occurred between both cryptic taxa within and between species. Additionally, the relative amount of estimated gene flow scaled with divergence time, where migration was less between *A. lamarcki* and *A. grahamae* as compared to migration between less diverged taxa within each species. These results are consistent with hypothesis that genetic permeability scales with divergence time (Roux et al., 2016).

We were not able to confidently resolve whether the very early stages of divergence occurred with or without gene flow. However, our results and those of other studies (*e.g.*, *Madracis* spp., Frade et al., 2010; *Eunica felxuosa*, *Pocillopora damincornis*, Prada & Hellberg, 2020; *Agaricia fragilis*, Bongaerts et al., 2017; Prada & Hellberg, 2020 and *Montastraea cavernosa* and *Siderastrea siderea*, Rippe et al., 2021) confirm that low levels of gene flow connect such closely related taxa, and yet gene flow is insufficient to homogenise them. Without physical barriers between cryptic taxa, exogenous and/or endogenous barriers must maintain divergence. The occurrence of exogenous selection has been shown in many famous examples to maintain species barriers through disruptive selection in face of homogenising gene flow (*e.g.*, sticklebacks, Dean et al., 2019; Darwin's finches, Han et al., 2017; *Heliconious* butterflies, Merot et al., 2017; and cichlids, Poelstra et al., 2018). Among scleractinians there is considerable circumstantial evidence for depth-associated environmental attributes (including light, nutrient availability, temperature, water flow, etc.) providing strong exogenous selection that could contribute to maintaining divergence despite gene flow (Bongaerts et al., 2011, 2017; Carlon & Budd, 2002; Gorospe & Karl, 2015; van Oppen et al., 2011; Prada & Hellberg, 2013; Serrano et al., 2014). Consistently, depth partitioning is observed throughout the *Agaricia* genus, with each taxonomic species inhabiting a distinct depth profile yet each remaining sympatric at the edge of their depth range (Bongaerts et al.,

2013). Depth-associated factors appear to play a role in divergence-with-gene flow within *A. lamarcki* taxa, between *A. grahmae* and *A. lamarcki* but not within *A. grahamae* taxa.

Endogenous barriers may also be maintaining the cohesiveness of cryptic taxa and indeed the replicated pattern of genome-wide divergence (not just divergence at selected outlier loci) implicates isolating mechanisms that may not be solely limited to environmental factors. Pre-zygotic isolation is most likely to occur through either gametic incompatibilities or temporal and spatial isolation in spawning in organisms where one or both gametes spawn, such as in corals and other marine invertebrates. In the *Orbicella* coral genus, more closely related species experienced temporal differences in spawning time albeit with gametic compatibilities (in crossed experiments). More distantly related species had overlapping times with gametic incompatibilities (Knowlton et al., 1997; Levitan et al., 2004), thus demonstrating the interactions and development of reproductive barriers in corals. On the other hand, in Indo-Pacific closely-related *Acropora* species spawning times often overlap and interspecific gamete compatibility can be high, although in the presence of conspecific sperm the number of hybrid offspring produced is low (Willis et al., 2006). Post-zygotic isolation studies through experimental work in crosses of *Acropora* species have found equal or higher fitness F1 hybrids (compared to parentals) in parental habitats as well as greater fitness in F1 hybrids than parentals in higher temperature or environmentally variable habitats (Willis et al., 2006; Chan et al., 2019), but such fitness estimates for F2s and backcrosses remain unclear. Due to practical difficulties, there are few spawning and larval crossing experiments in many coral species (especially for brooders), therefore most endogenous pre- or post-zygotic barries have not been fully tested. The common occurrence of sympatric yet incompletely reproductively isolated genetic taxa within scleractinians is perplexing and suggests that common features contribute to this widespread phenomenon.

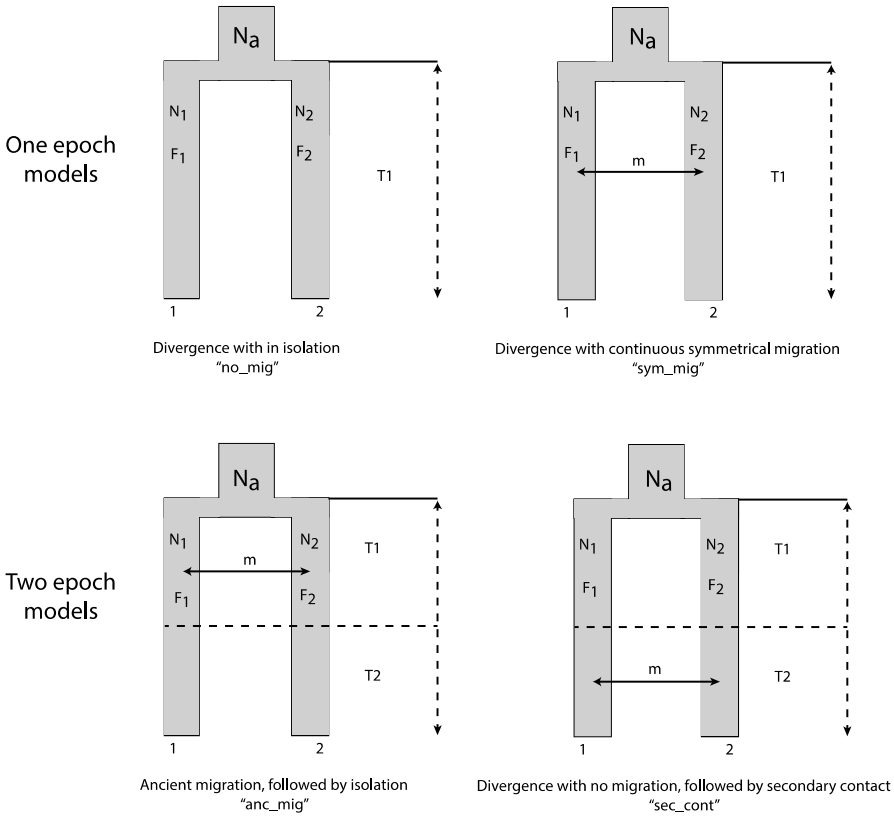
2.5.4 Conclusions

Here, we have shown that there is previously undescribed cryptic genetic diversity within *Agaricia* and that low levels of gene flow among taxa have characterised their divergence history. Thus, species boundaries within *Agaricia* appear to be semi-permeable. Inter-taxon gene flow stresses the importance of considering multiple closely related taxa in population genomic assessments, since erroneous conclusions could be drawn regarding assignment to

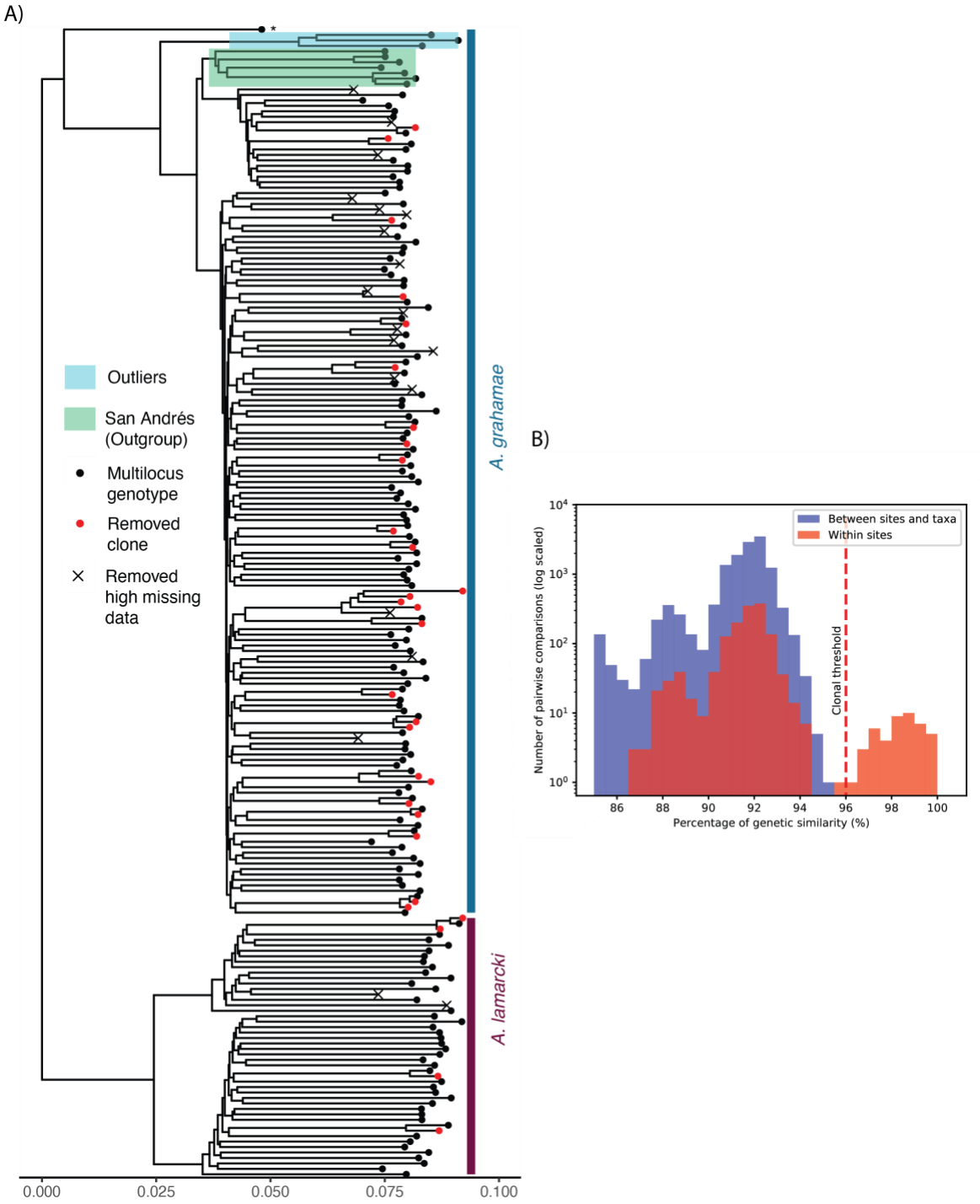
spatial population structure and species identity by purely relying on morphology to group individuals into taxa. Importantly introgression is likely to be common in scleractinian corals (Mao et al., 2018) and single species studies often ignore this important source of genetic diversity that could be adaptive in times of environmental change.

The lack of genetic structuring of mesophotic coral populations among reefs and islands indicates that mesophotic-specialist species may be more horizontally connected than we anticipated, which is important regarding their enhanced ability to recover from localised disturbances through replenishment from neighbouring reefs and islands. Pairing genetic data with hydrodynamic modelling approaches may help to resolve patterns of gene flow for regions of concern. The spatial genetic structure disparity between members of the genus may reflect undescribed differences in reproductive biology, and thus traits may be more variable and diverse within genera. This justifies the need to investigate species where localised dispersal has been assumed, especially those understudied in the mesophotic zone. This study draws attention to the lack of knowledge in the ecology and diversity of mesophotic corals. In light of recent reports of disturbances to mesophotic ecosystems and calls for the inclusion of mesophotic reefs into marine protected areas, understanding mesophotic coral connectivity should be a priority in future studies.

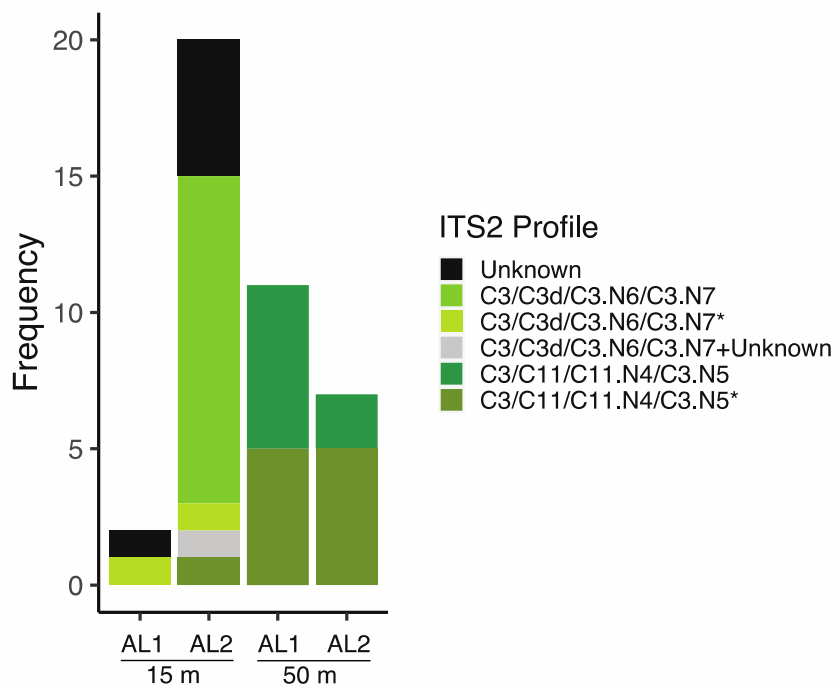
2.6 Supplementary material



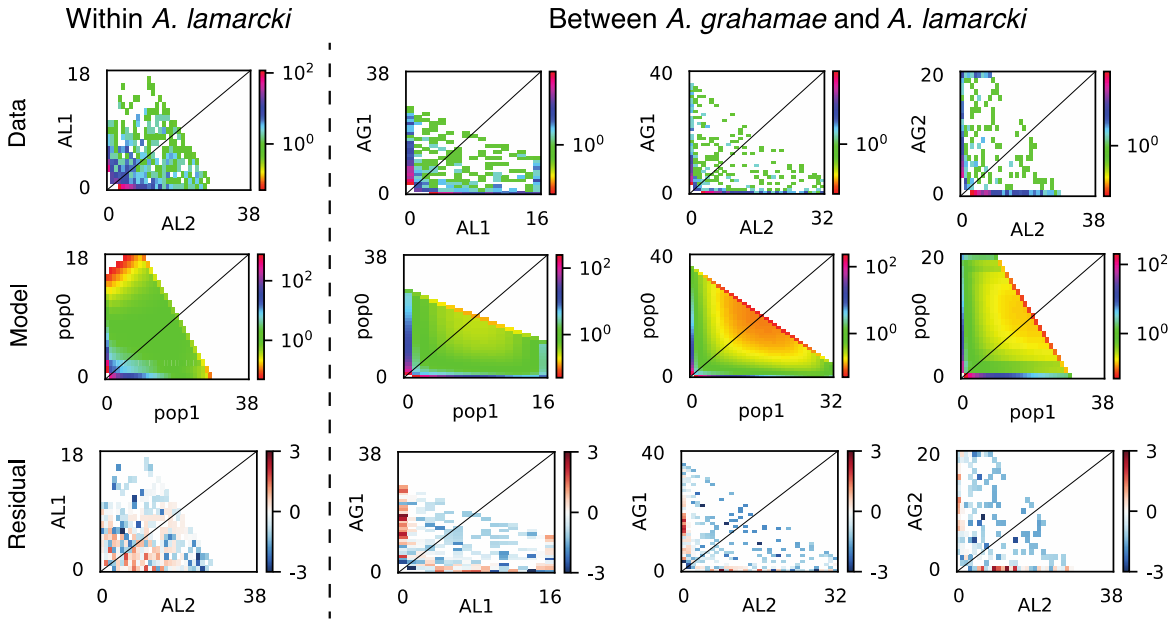
S-Figure 2.1. *Schematics of four demographic models used in dadi (Diffusion Approximation of Demographic Inference). The top row presents two, one epoch models: divergence in isolation and divergence with migration and the bottom row presents two, two epoch models: ancient migration and secondary contact. Parameters: Na = ancestral population size, N1 and N2 = population sizes after split of population 1 and 2, F1 and F2 = inbreeding within population 1 and 2, m = symmetrical migration between population 1 and 2, T1 = time between present and split in the one epoch models and between time spilt and T2 in two epoch models, T2 = time between T1 and present.*



S-Figure 2.2. *Genetic similarity of samples used to determine threshold between clonal genotypes and multilocus genotypes. A) Neighbour-joining tree of genetic distance where samples in red were deemed clones and removed. Samples with a cross were removed due to high missing data in filtered datasets. * indicates putative hybrid. B) Distribution of pairwise genetic similarity where the 96% clonal threshold is indicated by the dotted line. The number of pairwise comparisons on the y-axis are log-scaled for observation of clonal peak. The first peak to the left at ~86% represents between species comparisons, the next peak is between cryptic taxa comparisons, the third peak is within cryptic taxa comparisons and the final peak representing putative clonal genotypes.*



S-Figure 2.3. *Symbiont profiles for Agaricia lamarcki taxa show depth partitioning but not partitioning for genetic clade (AL1 and AL2). * indicates that different bands within the same profile were found. Unknown indicates a distinct unknown profile.*



S-Figure 2.4. *Demographic analysis using dadi within A. lamarcki (AL1 and AL2) and between A. lamarcki and A. grahamae. Data fitted for the divergence model with continuous migration. The folded Joint Allele Frequency Spectrum (Data), simulated JAFS (Model) and residuals (Model - Data).*

S-Table 2.1. *Samples sizes and description of locations and depths in which they were collected. Numbers in brackets are the number of unique multi-locus genotypes.*

Country	Site name	Site code	Latitude	Longitude	Depth (m)	*A. grah*	*A. lam*
Curacao	Playa Kalki	CK50	12.375	-69.158	50	12 (11)	4
	CARMABI Buoy 0	CR15	12.125	-68.974	15		9
		CR50			50	15 (14)	8 (5)
		CR60			60	32 (21)	
	Seaquarium	CS15	12.084	-68.898	15		9
		CS50			50	11 (10)	2
		CS80			75 – 90	9	
	Eastpoint Hamrak (2)	CF50	12.035	-68.791	50	12	
	Eastpoint Piedra Pretu (1)	CE15	12.043	-68.762	15		10 (9)
		CE50			50	9 (8)	8
Bonaire	Red Slave	BR50	12.027	-68.251	50	19 (14)	
	The Lake	BL50	12.107	-68.290	50	19 (17)	
	Karpata	BK50	12.219	-68.352	50	20	
					Total	158	50
					Total unique genotypes	137	46
San Andres, Colombia (outgroup)	Trampa Tortuga	ST65	12.543	-81.711	65	4	
	Cayo Bolivar	SC66	12.4	-81.467	66	1	
	Nirvana	SN60	12.5	-81.729	60	2	
		SN79			70	1	
		SN85			85	4	
					Total	12	
					Total in whole dataset	170	
					Total unique genotypes	148	

S-Table 2.2. *The number of SNPs/Loci and individuals removed and retained following each filtering step. "Sub" refers to subsampled dataset.*

Step	SNPs or Loci removed	Individuals removed	Loci retained	SNPs retained	Individuals retained
No filtering	-	-	130,890	686,608	226 (174 AG, 52 AL)
Removal of *Symbiodinaceae*	3, 984 loci	-	126,906	667,977	226
Removal of singletons and trimming contigs to 100bp	437,558 SNPs	-	-	230,419	226
Clone filtering	-	31	-	-	195 (148 AG, 47 AL)
Removal of non-Cnidaria	1, 904 loci	-	-	223,253	195
1. All individual dataset†	218,619 SNPs Outliers: 336 SNPs	34 (29 AG, 5 AL)	1,465	4,624 (all) 4,306 (neutral)	161 (119 AG, 42 AL)
2a. *A. grahamae* dataset†	220,149 SNPs	-	1,330 813 (10% missing sites)	3,104 (all)	118 (93 AG1, 21 AG2, 3 outliers, 1 putative hybrid)
2b. *A. grahamae* dataset† (no San Andres individuals)	220,385 SNPs, Outliers: 143 SNPs	8 outgroup, 1 putative hybrid, 3 outliers	2,228	2,868 (all), 2,725 (neutral)	106 (92 AG1, 14 AG2)
3. *A. lamarcki* dataset†	220,474 SNPs Outliers: 264 SNPs	-	1,328	2,779 (all), 2515 (neutral)	41 (14 AL1, 27 AL2)
4. AG1 and AG2 dadi dataset (1) ‡	-	123 (68 AG1)	2,204	2,204 Sub: 1,481 Masked: 908	38 (24 AG1, 14 AG2) Sub: 20, 9
5. AL1 and AL2 dadi dataset (2) ‡	-	123 (2 AL1, 1 AL2)	1,256	1,256 Sub: 1,211 Masked 1,025	38 (12 AL1, 26 AL2) Sub: 19, 9
6. AG1 and AL1 dadi dataset (3) ‡	-	125	2,434	2,434 Sub: 1,532 Masked: 887	36 (24 AG1, 12 AL1) Sub: 19, 8
7. AG1 and AL2 dadi dataset (4) ‡	-	111	1,956	1,956 Sub: 1,383 Masked: 856	50 (24 AG1, 26 AL2) Sub: 20, 16
8. AG2 and AL1 dadi dataset (5) ‡	-	135	1,827	1,827 Sub: 861 Masked: 572	26 (14 AG2, 12 AL1) Sub: 9, 9
9. AG2 and AL2 dadi dataset (6) ‡	-	121	1,617	1,617 Sub: 873 Masked: 641	40 (14 AG2, 26 AL2) Sub: 10, 19

† Removal of individuals with >50% missing sites, sites with <50% coverage across individuals, sites with depth <5, sites with a mean allele count of three, retaining only bi-allelic sites, removing outlier sites identified with PCADAPT for neutral datasets and then applying a max-missing 20% sites filter.
‡ Followed the same initial steps but without frequency filter, subset to individuals with no admixture and the highest coverage individuals for AG1 to reduce to a comparable sample size, then applied unreliable site filters (mean allele count of three, removal of outlier sites and 80% max-missing sites) and lastly thinning to one SNP per contig per contig where contigs are referred to as 'loci'.

S-Table 2.3. *Observed and expected heterozygosity within each taxon were significantly different, where each taxon has more homozygotes than expected under Hardy-Weinberg Expectations.*

Taxa	H_{obs}	H_{exp}	t	df	p
AG1	0.12	0.15	30.17	2589	<0.01
AG2	0.30	0.34	10.70	973	<0.01
AL1	0.27	0.32	19.62	1718	<0.01
AL2	0.23	0.27	21.90	2093	<0.01

S-Table 2.4. *Scaled likelihoods for each hybrid class between two previously assigned A. grahamae genetic groups: AG1 and AG2 using NEWHYBRIDS. Results are presented for 8 individuals out of 106 collected from Curaçao and Bonaire which had substantial backcrossing.*

Prior Assignment / Sample number	AG1†	AG2†	F1‡	F2‡	B-AG1§	B-AG2§	B-AG1-AG1¶	B-AG2-AG2¶
AG1 CS50_N4468	0.44	0	0	0	0.02	0	0.53	0
AG1 BL50_N6509	0.40	0	0	0.01	0.26	0	0.34	0
AG1 CR60_B5992	0.29	0	0	0.01	0.07	0	0.62	0
AG1 CS50_N4473	0.28	0	0	0	0.02	0	0.69	0
AG1 CR60_N5976	0.19	0	0	0.01	0.02	0	0.78	0
AG1 BK50_N6116	0.12	0	0	0.01	0.83	0	0.04	0
AG2 BL50_N6516	0	0.27	0	0	0	0.09	0	0.64
AG2 BR50_N6266	0	0.08	0	0	0	0.03	0	0.89

† *AG1 and AG2: pure parental classes (i.e., P1 and P2)*
‡ *F1 and F2: offspring of parental classes and offspring of two F1*
§ *B-AG1 and B-AG2: backcross from f1 into each parental class*
¶ *B-AG1-AG1 and B-AG2-AG2: second generation backcrosses into each parental*

Chapter 3: Fine scale reefscape genomics resolves cryptic taxa and dispersal distances in Agaricia corals

3.1 Abstract

Scleractinian corals support tropical coral reefs and are facing severe population loss and extinction risks due to global and local impacts. Measuring dispersal is essential for predicting population replenishment after disturbance and the spread of adaptive alleles. Dispersal in corals cannot be directly observed as it is conducted by microscopic larvae in the voluminous ocean. Genetic methods for estimating dispersal in corals are complicated by their complex and variable life history strategies with partially asexual populations, inbreeding, and overlapping generations. Additionally, cryptic coral taxa are common and can results in strong biases when measuring demographic processes. Here, I overcame these challenges using structure-from-motion photogrammetry to make spatially-explicit individual-based maps of the seafloor that were paired with reduced-representation genomic sequencing to analyse genetic variation across fine spatial scales and thus infer dispersal distances of both sexual and asexual propagules. Sampling was conducted on three species of the coral genus *Agaricia* (*A. agaricites*, *A. humilis* and *A. lamarcki*) at four locations and three depths (5, 10 and 20 m) across the leeward side of Curaçao (Southern Caribbean). Cryptic taxa were found in each species and were delineated following the 'genotypic cluster' definition. Localised dispersal (dispersal within metres) was discovered within *A. agaricites* and *A. humilis* taxa using isolation-by-distance analyses and kinship detection. However, no spatial signatures or kin were found within *A. lamarcki* taxa. Genetically identical colonies within all taxa were mostly in close proximity to each other (within 1 m) and generated by fission while sexually derived genotypic diversity was maintained across fine scales. Taxa within *A. agaricites* and *A. humilis* appear to mostly self-recruit and thus local scale protection is important for the maintenance of their populations; however, *A. lamarcki* taxa may be less reliant on fine scale self-recruitment due to likely greater dispersal capacities.

3.2 Introduction

Dispersal is a key process that structures genetic variation within populations. The dispersal capacity of a species can determine the short-term recovery of populations after disturbance through individual replenishment from neighbouring populations. Or, if environmental changes occur, populations can be rescued by adaptive gene flow, whereby selected genes are more easily spread through populations when dispersal is greater (Carlson et al., 2014). This evolutionary resilience is especially relevant for corals that are at risk from disturbances accelerated by climate change (Baird et al., 2008). In the Caribbean, coral populations have seen substantial declines and community shifts over the past decades (Gardner et al., 2003; Jackson et al., 2014; Schutte et al., 2010), including shifts from 'framework' species to 'weedy' species (Aronson et al., 2002; De Bakker et al., 2016; Knowlton, 2001; Robbart et al., 2004; Roff, 2020). It is important to understand dispersal capacities in dominant and different functional groups within this region because this provides insight into species- and location-specific demographic recovery dynamics over ecologically meaningful timescales (*i.e.*, whether populations are assisted by migrants or are self-recruiting).

One way to quantify ecologically relevant dispersal from indirect genetic methods is to use isolation-by-distance (IBD), which is a continuous decline in genetic similarity over geographic distance (Wright, 1943, 1946). The inverse slope of isolation-by-distance is Wright's neighbourhood size (*i.e.*, the number of effectively mating adults within an area) and if individual density can be independently estimated, then the synthetic descriptor of dispersal, dispersal variance (σ^2), can be estimated (Rousset, 1997, 2000). Dispersal variance is relevant to the migration-drift or migration-selection dynamics that shape genetic structure, and it may be used to parameterise a dispersal kernel (*i.e.*, the probability distribution of dispersal distances) (Broquet & Petit, 2009). Thus, this measure can be used in modelling situations to predict population replenishment and the adaptive spread of alleles, and thus will inform managers of relevant dispersal scales for marine spatial planning. Measuring genetic variation over spatial scales that are relevant to dispersal scales are more likely to yield accurate estimates as these scales are likely shaped by recent drift and dispersal (10σ - 50σ, Hardy & Vekemans, 1999; Slatkin, 1993) rather than historical events. Selection may also shape genetic structure across such scales and thus investigating any environmental causes for genetic structure is important, as is the removal of any loci under linked selection when measuring neutral patterns (*e.g.*, removal of outlier loci). Due to the non-Wright-Fisher nature of coral

populations (*i.e.*, asexual reproduction, inbreeding, and overlapping generations) using individual measures of genetic distance are more appropriate as this enables the relaxing of population equilibrium assumptions (*e.g.*, Rousset's *â*, Rousset, 2000) (Rousset, 1997; Vekemans & Hardy, 2004). Dispersal distances can also be validated by kinship analysis if parents and offspring are found within the sample.

Sexually derived dispersal abilities among species of corals vary considerably. Some species exhibit long range dispersal where pelagic larval durations can be days to weeks (*e.g.*, broadcasters) or more localised dispersal with larvae settling with hours or days (*e.g.*, brooders) (Carlon, 1999). Long-range dispersal abilities may lead to large effective population sizes which are more resistant to local disturbances through replenishment of propagules from distant unaffected locations, while short dispersal likely leads to small, isolated populations less likely to be rescued by migrants (Ronce, 2007). In corals, brooders, with likely short dispersal, reproduce more frequently (Carlon, 1999). Thus, persistence of populations with differing dispersal capacities is also related to reproduction rates and the severity and frequency of disturbances. Populations with limited dispersal may become more locally adapted as they are generally exposed to the same environment whereas populations with high dispersal are likely to experience many different environments and thus local adaptation is less likely (Ronce, 2007) but could still occur with phenotypic plasticity or at particular resistant loci despite gene flow across the genome (*e.g.*, spatially varying selection, Gagnaire et al., 2012).

Determining dispersal capacities in corals with genetic methods should be done cautiously. Inference into spatial genetic structure using population genetic structure (*i.e.*, F_{ST}) may not be relevant to gene flow over ecological timescales and stepping-stone gene flow may maintain genetic homogeneity (Whitlock & McCauley, 1999), but could be useful in that the occurrence of population structure will mean that dispersal was very low (< 1 migrant per generation, Waples & Gaggiotti, 2006) or has ceased, unless recent events have brought populations back into contact (*e.g.*, Pleistocene sea level changes, Ludt & Rocha, 2015). In broadcasters there may be no spatial genetic structure across large spatial scales (1000s of km) (Baums et al., 2010; Huang et al., 2018; Nakajima et al., 2010; Serrano et al., 2014; van Oppen et al., 2015) and in brooders there can be structure over kilometres (Casado-Amezúa et al., 2012; Goffredo et al., 2004). Assignment methods are sensitive to gene flow within a few generations but rely on whether the correct source populations have been sampled and may only describe singular events (Manel et al., 2005). Importantly, many previous studies have not incorporated the

occurrence of cryptic species and thus population structure inference and F_{ST} were affected (see Sheets et al., 2018).

Spatial-autocorrelation and isolation-by-distance methods reveal more general patterns. These methods have shown that in some brooders dispersal is likely to be restricted across tens of metres due to the decomposition of spatial autocorrelation occurring at this scale (McFadden & Aydin, 1996; Miller, 1998; Underwood et al., 2006). Similarly, dispersal may be restricted by tens of km in some species that broadcast (Palumbi et al., 2012; Underwood et al., 2009, 2020). Yet, dispersal could be similar in species with different modes (Miller & Ayre, 2008; Underwood et al., 2020), or highly disparate as predicted (*e.g.*, brooder <10 km and broadcasters >80 km Thomas et al., 2020), note that within species regional differences due to oceanography should be apparent. Despite these inferences on likely dispersal (where spatial autocorrelation stops is where drift is greater than migration), few studies of coral populations have quantified dispersal variance (but see Gazulla et al., 2021; Gorospe & Karl, 2013; Ledoux et al., 2010). Although many species fit the assumption that brooders harbour localised dispersal whereas broadcasters do not, there are enough exceptions that individual species should be assessed. Importantly we need to know what the exact spatial scales are for dispersal amongst different species as well as region specific environmental influences on dispersal between reefs (van Oppen et al., 2015). One issue is that if spatial genetic signatures are not seen, then dispersal variance cannot be estimated indirectly using isolation-by-distance. Indeed, high connectivity across the Great Barrier Reef is likely due to the continuous chain of reefs mostly <50 km apart (Almany et al., 2009). Genomic-level data and individual-based analyses, however, are able to detect more subtle spatial signatures. The majority of coral species in the Caribbean are brooders (Baird et al., 2009; Roff, 2020) and isolated populations are likely to require more assistance; thus it important to quantify dispersal in such species.

Dispersal within coral populations can occur through either sexual or asexual reproduction, and differing proclivities to these modes shapes the persistence of populations facing disturbance, disease, and environmental changes. Fragmentation of coral colonies from disturbance may increase the number of clones within a given area and colonies of only a few genotypes may cover large spatial areas (Baums et al., 2006; Gorospe & Karl, 2013). If the number of clones within a given area is high, populations could become highly locally adapted and genetically depauperate which reduces the capacity of populations to withstand disease outbreaks (*e.g.*, white band disease in the 1980s, Jackson et al., (2014), and now Stony Coral

Tissue Loss Disease, Estrada-Saldívar et al., (2020)) as well as changing environments (Ayre, 1985). Extreme cases are where populations are monoclonal (*i.e.*, one genotype) and thus these populations may have become sexually extinct due to a lack of self-fertilisation success and no other genotypes nearby (Baums et al., 2006; Reusch et al., 2000). Clonal propagation can be induced at fine-scales through benthic competition for space, physical damage, bleaching or damage from grazing predators, whereby partial mortality of colonies results in the separation of one colony into multiple and also when in contact tissue-separated colonies can fuse (Hughes & Jackson, 1980). Another mechanism of asexual propagation is the production of parthenogenetic larvae that are able to travel far distances likely benefitting populations when there are low densities of mates (Permata et al., 2000; Stoddart, 1983; Vollmer, 2018). Along with dispersal of sexual propagules, understanding the clonal propensities of different coral species is essential for predicting demographic and evolutionary responses.

In addition to clonality, wild coral populations are frequently characterised by inbreeding (Baums, 2008). Inbreeding can occur through selfing in hermaphrodites, which has been demonstrated in laboratory studies of corals (*e.g.*, Brazeau et al., 1998), or may be a consequence of limited dispersal in small populations, more likely of brooders. Broadcasters are more commonly hermaphrodites and brooders gonochoric (Baird et al., 2009), and thus inbreeding is possible in both modes. Inbreeding is known generally to be detrimental to populations as it leads to the surfacing of deleterious recessive mutations. However, consistent inbreeding will lead to purging of recessive mutations from the population, increasing the populations local fitness. For example, in plants, rates of self-fertilisation are negatively correlated with inbreeding depression (Charlesworth & Charlesworth, 1987). There is evidence that inbreeding depression still affects species with regular selfing, but generally at later life stages (Husband & Schemske, 1996). The combination of inbreeding and clonality can be an evolutionary robust strategy for preserving local adapted haplotypes and genotypes (Smith, 1977) as well as colonising new or recently disturbed habitat through establishing populations with a few initial individuals (Olsen et al., 2020). Crucially both plants and sessile marine invertebrates appear to commonly inbreed (Olsen et al., 2020) and thus it is likely a symptom of restricted gamete dispersal mechanisms and important to measure for all coral species.

Determining spatial genetic patterns in corals can be difficult due to the common occurrence of cryptic genetic substructure. As genomic sequencing has become increasingly accessible, more cases are being uncovered in population genomic studies of corals and some of these

genetically divergent clusters are being called cryptic species or cryptic taxa (Bongaerts, Cooke, et al., 2021; Matias et al., 2022; Rippe et al., 2021; Chapter 2). Cryptic taxa are morphologically similar (or indistinguishable) genetically divergent groups. Often cryptic taxa are associated with depth (Prada & Hellberg, 2020) or other habitat delineations (Matias et al., 2022; Tisthammer & Richmond, 2018; van Oppen et al., 2018). In particular, genetic structure related to depth partitioning is repeated at different locations when comparing mesophotic to shallow populations (Bongaerts et al., 2017; Serrano et al., 2016; Chapter 2). Some of these ecologically partitioned, partially divergent groups also exhibit hybridisation or gene flow between them (Matias et al., 2022; Prada & Hellberg, 2020; Rippe et al., 2021; Chapter 2), which could be bridges for adaptive gene flow and genetic rescue. However, obvious ecological boundaries between taxa are not always seen (Johnston et al., 2022; Ladner & Palumbi, 2012; Oury et al., 2020; Chapter 1) and hierarchical structure is shown (Bongaerts, Cooke, et al., 2021; Gélin et al., 2018; van Oppen et al., 2018). Understanding the cause of cryptic genetic structure (*i.e.*, demographic, or selective processes) is important for studies interested in measuring demographic processes and thus investigators should first screen for cryptic taxa and treat these separately.

In order to measure individual spatial distances, sampling strategies require detailed spatial mapping. Structure-from-motion photogrammetry uses imagery to create virtual 3D models of stationary structure and can be used for fine-scale mapping of the seafloor. The benefits of this technique in underwater research are that precise mapping of samples (*e.g.*, specific coral colonies or other benthic organisms) can be achieved *in silico* allowing for substantial increases in sampling size and more accurate estimates of spatial distances and depths (*i.e.*, applications of reefscape genomics, Bongaerts, Dubé, et al., 2021). Most population genetic studies on coral (or other marine taxa) have not been spatially explicit down to the individual level (*i.e.*, generally grouping individuals into a population at a particular location) and have assessed more broad scale patterns (but see Dubé et al., 2020; Gorospe & Karl, 2013; Ledoux et al., 2010; Miller & Ayre, 2008; Underwood et al., 2006). Multiple processes are at work simultaneously at different spatial scales (Cowen & Sponaugle, 2009) and often broad scale patterns can be at odds with fine scale patterns (Gorospe & Karl, 2013). Using photogrammetry is a novel approach for creating spatially explicit individual genetic data. Through harnessing this technology, we can build a more accurate picture on how processes such as dispersal, inbreeding and clonality shape the genetic variation of populations and taxa across fine scales.

The Atlantic coral genus *Agaricia* harbours species occurring over the entire depth range available for zooxanthellate corals. Species at shallow depths (< 30 m) exhibit varied colony morphologies, *i.e.*, domed, plating, foliose and encrusting and species in the mesophotic (> 30 m) harbour encrusting or plating morphologies. The genus is described as having a 'weedy' life-history due to their ability to colonise disturbed areas (Darling et al., 2012), which has been exemplified by relative increases in abundance while total abundances of hard corals decline in the Caribbean (De Bakker et al., 2016). Morphologically cryptic but genetically divergent groups have been found within species that occur abundantly at mesophotic depths either partitioned by depth, *A. fragilis* (Bongaerts et al., 2017), *A. lamarcki* (in Curaçao, Chapter 2) or without any obvious environmental or spatial pattern *A. lamarcki* (in Puerto Rico; Hammerman et al., 2018), *A. grahamae* (Chapter 2). However, genomic methods have not been applied to congeners that are more common at shallow depths, *i.e.*, *A. humilis*, *A. agaricites* and *A. tenuifolia*. Interestingly, disparate spatial genetic structures have been seen over similar distances between *A. agaricites* (structuring within 10 km, Brazeau et al., 2005) and *A. fragilis*, *A. lamarcki*, *A. grahamae* and *A. undata* (no structure over >20 km but some occurring from 10s to 1000s of km, Bongaerts et al., 2017; Gonzalez-Zapata et al., 2018; Chapter 2). This highlights that there could be reproductive differences among the shallow occurring and mesophotic occurring species albeit comparisons are between different regions and using different makers (*A. agaricites* study based on amplified fragment length polymorphisms (AFLP) compared to all others using genomic methods). Therefore, in this study genetic variation was assessed using the same methodology across the same spatial scales at the same locations and depths (5, 10 and 20 m) across all *Agaricia* spp. found within the leeward side of Curaçao.

The overarching aim within this Chapter was to assess dispersal of asexual and sexual propagules comparatively among *Agaricia* spp.. Taxonomic species and cryptic taxa within them were delineated and then treated separately in subsequent analyses. Then whether there was an effect of depth beyond the spatial pattern (*i.e.*, isolation-by-resistance) was assessed. To measure dispersal capacities isolation-by-distance analyses were conducted and indirect genetically derived dispersal variances (with independent estimates of individual densities) were estimated. To corroborate measures, dispersal variances were compared with direct measures of dispersal distances from kinship analyses. Distances between clones were also compared amongst taxa. I predicted that there could be asexual propagation from three mechanisms, fission of a colony into multiple colonies at fine scales (centimetres to metres),

breakage from storms and reattachment at fine to moderate scales (metres to 10s of m) and asexual parthenogenic larvae at scales from moderate to larger scales (10s of m to 100s of m). Additionally, levels of inbreeding were compared amongst all species and taxa to assess whether inbreeding was associated with dispersal capacity. Thus, this study measured ecologically applicable demographic information for species within the genus, *Agaricia*, and is the most extensive study of dispersal in corals with co-distributed species and taxa, and sampling over depth as well as space to date.

3.3 Methods

3.3.1 Study sites and sample collection

Specimens of *Agaricia* spp. were collected during Feb – April 2019 from four locations on the leeward side of Curaçao, an island in the Southern Caribbean (Figure 3.1). Each location is roughly 10 km apart distributed from the north to the south of the island: West Point (Playa Kalki), Cas Abao, Snake Bay and Seaquarium. Tissue sampling was conducted on every *Agaricia* spp. colony within 25 m length (across reef slope) x 2 m width (following reef slope) plots. These plots were set up as permanent plots as part of the CoralScape long-term monitoring project and which are imaged and reconstructed into virtual 3D-representations of the reef (Bongaerts, Dubé, et al., 2021). At three locations, samples were collected from plots each at a different depth profile (5, 10 and 20 m); these plots being parallel to each other (see sampling design, Figure 3.1). At Seaquarium, samples were taken at two depths (12 m and 20 m), the first plot was created at 12 m due to the coral community not occurring until this depth (because of a breakwater structure). For convenience, the 12 m Seaquarium samples were grouped together with the 10 m in figures to match the other locations. Sampling was conducted using SCUBA while recording with a Paralenz video camera attached to the mask for a Point-of-View angle. Each sampled colony was photographed using an Olympus-TG5 in housing with a flash diffuser. Two types of photographs were taken: one of the whole colony using the "wide" underwater setting and one of the corallites using the "underwater microscope" setting with ruler scales and white tape to white-balance the photographs. The videos (with some aid of the photographs) were used to map colonies within the virtual constructs of reef in *silico*. The photographs were used to first identify samples to taxonomic species, but the results of the genetic data were used for the final species identifcation. Four samples of live skeleton ~2 mm^2

were taken from each colony for two different preservation solutions and with one back-up of each. Each piece was preserved in either 99% ethanol or 20% salt saturated EDTA DMSO. Samples were collected under the CITES Curaçao permits of the CARMABI foundation. Four extracted gDNA samples per *A. lamarcki* cryptic taxa (AL1, AL2) and two individuals per *A. grahamae* cryptic taxa (AG1, AG2) were also included for assignment of taxa from Chapter 2.

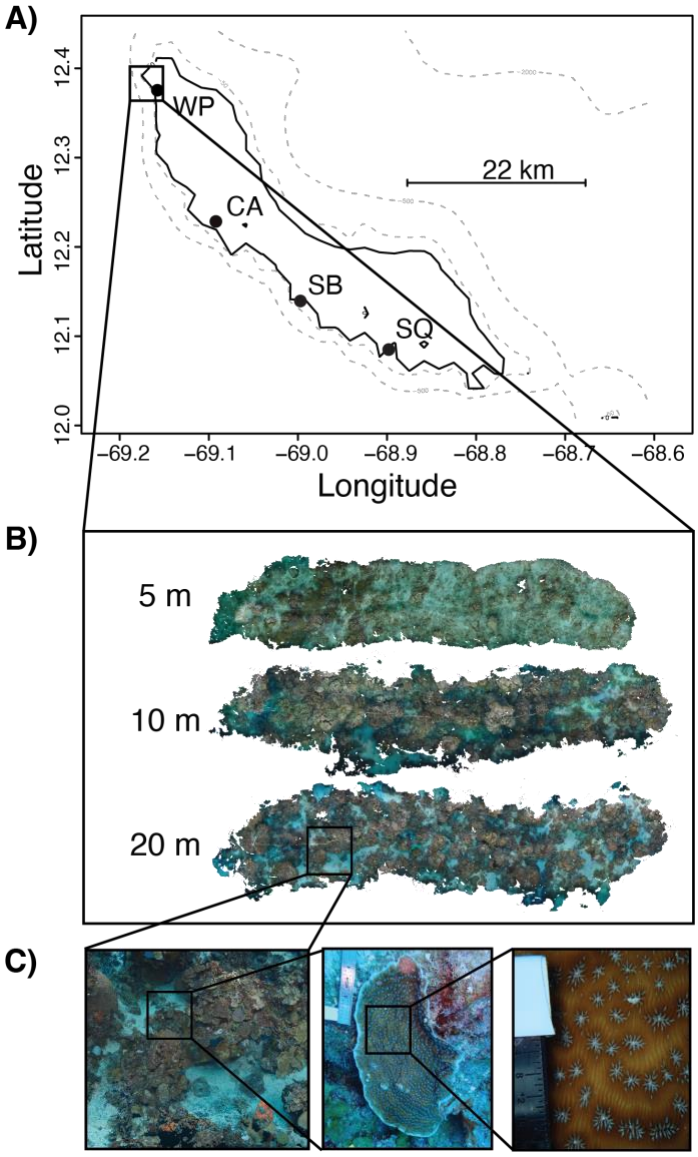


Figure 3.1. *Hierarchical sampling design for Agaricia spp. in Curaçao (Southern Caribbean). A) Four locations on the leeward side were chosen, West Point (WP), Cas Abao (CA), Snake Bay (SB) and Seaquarium (SQ). B) Within WP, CA, and SB sampling was conducted within three 3D-imaged plots (25 m length x 4 m width) within depth zones 5, 10, and 20 m, at SQ two were made at 12 and 20 m. C) Genotyped colonies were annotated within the 3D plots, these colonies were also photographed at the colony scale and corallite scale.*

3.3.2 Structure-from-motion photogrammetry, point cloud reconstruction and point annotations

Plots were imaged at two intervals per year, roughly six months apart (Feb – Mar and Sep – Oct) over a three-year period (2019 - 2021). Photographs were taken while SCUBA diving using a Canon 5DmIII (22 Mpx) or Canon 5DsR DSLR camera (50 Mpx) with a 24mm prime lens and four Inon Z-330 strobes on a mounted aluminium frame, using sonar to maintain a fixed altitude of 1.3m above the substrate. Plots used for analysis were from either early or late 2020, due to improved reconstructions from the use of an increased camera resolution compared to the 2019 periods when the samples were collected. Dense point cloud reconstruction was conducted in AgiSoft MetaShape Professional v.1.8 (Agisoft LLC., St Petersberg, Russia) and exported as point cloud PLY files. Scaling, orientation and alignment (of subsequent timepoints) was done in Viscore (Fox et al., 2019; Petrovic et al., 2014), using coded targets and manual depth measurements. PLY files were decimated and imported into CloudCompare v.2.11 (*Cloud Compare,* 2021) for annotation. Individual points representing the centre of each colony were annotated with the colony sample name and two additional points per colony were annotated at the longest edge of each colony. Locating the genotyped colonies was achieved by watching the Paralenz videos that were recorded during sampling and matching the location of the colony in the video with the location of colony in the 3D point cloud.

Most samples were found digitally within the plots; however, some samples were not found due to missing data in parts of the model, missing videos or photos, or the individual disappearing (the plot annotated was imaged one year after sample collection). These missing individuals further reduced the number of genotyped individuals available for spatial analysis.

3.3.3 DNA extractions and library preparation

Extractions of genomic DNA were carried out using Wayne's method (Wilson et al., 2002), which is a salt extraction and has been optimised for coral. Concentrations of gDNA were assessed on a Qubit flurometer or by PicoGreen and quality of the gDNA was assessed for a subset of the samples by Gel Electrophoresis. Samples were then randomised into plates and normalised to be 200ng each at 5ng per μl. The library preparation for RADseq followed a fusion of GBS and ddRAD methods using *PstI* and *MspI* restriction enzymes designed by

Hereward et al. (2020). This method is based on Elshire et al. (2011) with 96 forward adaptors from Poland et al. (2012), 12 reverse adaptors from Peterson et al. (2012), and with use of golay barcodes from Caporaso et al. (2012). This protocol uses variable length MTC codes for improved reverse read quality and allows duplex indexing for increasing performance of parallel sequencing. Adaptors were ligated to digested gDNA with three different 96-well plates of reverse barcodes and one 96-well plate of forward barcodes so that each sample had a unique forward and reverse barcode combination. Size selection was then conducted on 96-sample pools using a Blue Pippin (Sage Science) for lengths between 300-400bp. Then each pool was PCR amplified to add the Illumina indices to the adaptors. The PCR products were assessed for quantity within desired length range and quality purposes using a BioAnalyzer (Shimadzu). Three PCR products were normalised, pooled and bead cleaned to create the final library. Libraries were sent to the Australian Genome Research Facility (AGRF) for paired-end sequencing on a NovaSeq SP lane, 300 cycle for 279 samples per lane. A total of 1,104 unique colonies were sequenced and due to low reads for some samples, an additional 240 samples were sequenced and were comprised of resequencing failed samples and adding technical replicates (new RADseq library replicates of the same gDNA extraction).

3.3.4 Read clustering, de novo mapping and quality control

Samples were demultiplexed using stacks v.2.4 (Catchen et al., 2013). Then a *de novo* assembly was created using ipyrad v.0.9.67 (Eaton & Overcast, 2017) with all standard settings, except for minimum depth statistical and majority of 10, strict filtering of adaptors and primers, and a minimum of four samples per locus and outputting VCF and loci files.

Basic filtering was performed on the VCF file using VCFtools v0.1.16 (Danecek et al., 2011). First, filtering for bi-allelic sites with a minimum allele count of three was conducted. Reads with low and high depths were removed with maximum depth three times higher than the highest read locus average and a minimum depth of five. SNPs that were present for >50% individuals were retained. Contamination and endosymbionts were found through using blastn searches on a fasta file of RAD loci. Reads that matched e^{-15} known Symbiodiniaceae, *Cladocopium* genome (Liu et al., 2018) and nextRAD Symbiodiniaceae isolates from *Agaricia* spp. (Bongaerts et al., 2017) were removed. Reads that matched e^{-4} nt-17-Apr-2014 NCBI database and were non-scleractinians were removed and were found on a blastn search using the Galaxy public server at usegalaxy.org (Afgan et al., 2018).

Final filtering on the VCF file was performed with VCFtools. Individuals that had >50% missing data were removed (202 individuals) and four missing data thresholds were used for SNPs: 5, 10, 20 and 50% for consensus regarding genetic structuring results. After population structure analysis identified taxonomic species groups, these filters were applied for the three separate datasets for the three taxonomic species: *Agaricia agaricites*, *A. humilis* and *A. lamarcki*. Taxonomic species and taxa (putative cryptic species within species) are defined and delimitated within Appendix 1 based upon Mallet (1995) "the genotypic cluster" definition. Briefly, these definitions are that species (used if matches the morphological descriptions of taxonomic species) and taxa (any group within the taxonomic species) form distinct genetic groups that contain individuals that can be fully assigned to the groups, and that the groups are differentiated before spatial structure and are not related to spatial structure (*i.e.*, groups are sympatric). The species datasets were separated from the initial dataset with 'basic filtering' applied in order to obtain more species-specific loci that may have been filtered out previously and an additional step of filtering for monomorphic sites was performed because these are produced when individuals with alternative alleles are removed. For each species dataset, PCAdapt v.4.3.3 (Luu et al., 2017) was used to find outliers (MAF>0.05, 5% FDR) using the consensus of qvalues, Benjamin-Hochberg procedure and Bonferroni correction. Outliers were assessed for each species separately and then removed from each species dataset as well as from the 'all-individuals' dataset as the focus of the present investigation is on describing demographic (neutral) processes. Unlinked datasets were created by randomly selecting one SNP per RAD contig. Genetic structuring results were consistent across these thresholds and thus the 20% missing dataset was used for all other analyses.

3.3.5 Clone evaluation and distribution

Clones were identified for each taxonomic species separately by creating a pairwise genetic distance matrix using Hamming's distance. Pairwise distances were then structured using agglomerative hierarchical clustering with *scipy.cluster.hierachy.linkage()*, using the scipy package v.1.73 (clustering approach from Müllner, 2011). The breaks in distribution of pairwise similarities were used to denote a threshold for clonality. Technical replicates for each taxonomic species were included and used as a reference for comparison of true clones in the case of sequencing errors or somatic mutations diminishing similarity. Two separate datasets were kept, one "with clones" and another "no clones". Genotypic richness was calculated as

the proportion of unique multilocus genotypes (G) relative to number of samples (N), expressed as R = (G-1) / (N-1). For all taxa, the number and distribution of clones and the spatial distance between clones was assessed in order to understand the mechanisms that create clones and compare any differences among taxa.

3.3.6 Spatial analyses

Because both space and depth can contribute to spatial structure, I used Redundancy Analysis (RDA) (a multivariate regression that can include multiple predictors) to assess the relative effects of spatial distance and depth distance on genetic variation. To measure effective dispersal, I used the slopes of isolation-by-distance (IBD) analyses (log geographic distance versus Rousset's $\hat{a}$, Rousset, 2000). Rousset's $\hat{a}$ is used because it is an individual genetic distance measure that linearly scales with log geographic distance under theoretical assumptions of IBD. For calculating distances for both analyses, coordinates were taken from the central point of each colony within each of the 11 photogrammetry plots. Each plot was constructed in three-dimensions with its own arbitrary local coordinate system. For RDA, the 3D plot constructs were oriented so that decreases in Z reflected decreases in metres below sea-level (*i.e.*, oriented to reflect the real slope of the reefs). For IBD regressions (methods from Rousset, 2000) horizontal planes were fit to each of the 3D plot constructs and coordinates were rotated so that the previously fit horizontal plane reflected the XY axis (*i.e.*, reducing the angle of elevation of the plot to 0°), using CloudCompare. This reduced the deviation in Z-coordinates within the plot (although it did not eliminate it) so that only the XY coordinates could be used to reflect geographic distances in 2-Dimensional space. All plots (for both analyses) were then rotated about the Azimuth (or about the Z-axis) using Euler's rotation theorem, so that plots at each depth within locations were parallel to each other. The X-axis corresponded to the plot's length-wise horizontal axis (~0 – 25 m) and Y-axis corresponded the plot's width-wise horizontal axis (~0 – 4 m) (Figure 3.2). For standardisation and clarity, the minimum for both the X and Y coordinates were added to all equivalent XY coordinates within each plot so that coordinates started from 0. Coordinates were scaled to reflect real distances in metres. Distances between plots (within locations) were only available for 10 – 20 m plots in West Point and Snake Bay. I averaged these distances to estimate the 10 – 20 m depth distance for the other locations (Cas Abao and Seaquarium) and then used a rough estimate of 30 m for the distance in Y between 5 - 10 m depth plots for all locations. These average distances between plots were subtracted from Y-coordinates of the deeper plot (*i.e.*,

Y-coordinate for 5 m > 10 m > 20 m depth plots). Distances were calculated between locations with the latitude and longitude points (one point for each location) using the Haversine formula. These distances were added to the X-coordinate of each plot by setting West Point (the northern most site) as the origin (coordinates kept the same) and to each location adding the distance from West Point to that location. Due to the distances between the locations being much larger than the distances within or between the plots (0.01 – 25 m) and the plots not being oriented to the actual latitude and longitude coordinates, adding to just the X-coordinate reflects a close enough measure to the real distance and position of the plots to not affect the statistics.

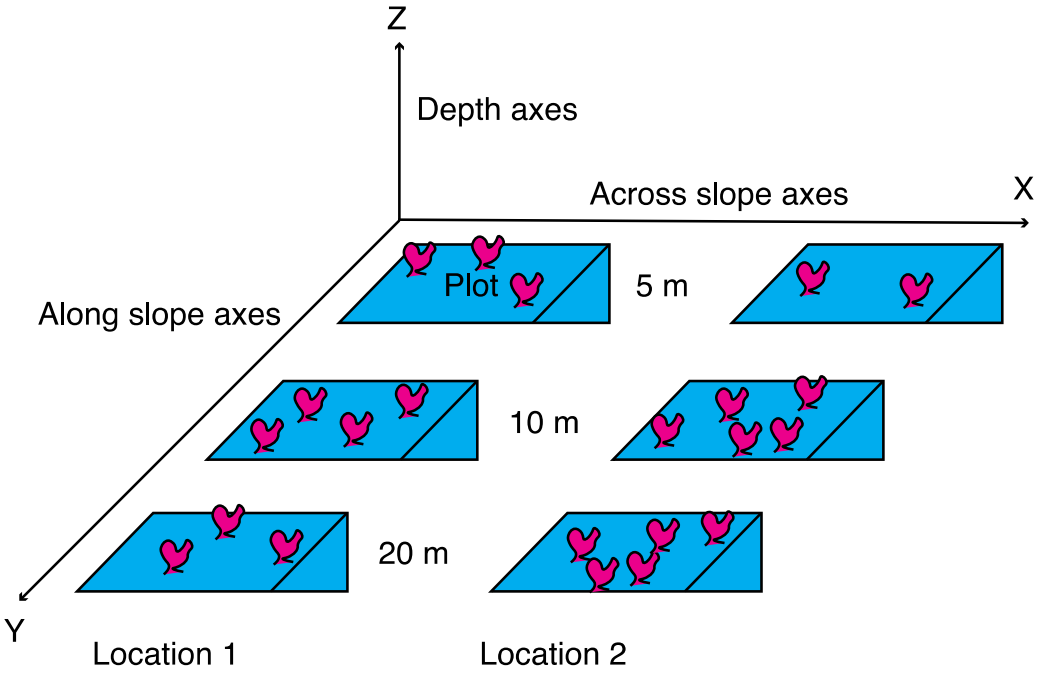


Figure 3.2. *Schematic of the arrangement of the coordinates for the 3D plots with referenced colonies of Agaricia spp. for the spatial analyses.*

RDA was used to assess and compare the effects of geographic distance and depth on genetic distance. The RDA was performed before IBD analyses in order to test for any isolation-by-environment (depth), as this affects how the data should be treated when measuring the IBD slopes, *i.e.*, including all depths together or using separate analyses per depth. Note that the Y-axis of geographic distance (horizontal distance between depths of the same location) is mostly correlated with the Z-axis (reflecting depth below sea-level) and thus exploring depth vs. spatial effects on genetic structure across short distances is limited however this correlation is unavoidable. The XY-coordinates were transformed to be orthogonal through Principal Components Analysis (PCA). PC1xy and PC2xy were used as predictors reflecting geographic

distance and the Z-coordinate was used as an additional parameter but was not transformed because it is inherently orthogonal to the horizontal plane. Models were set up so that variance explained by XY-coordinates were considered before Z. Individual genetic distances ($\hat{a}$, Rousset, 2000) were transformed into two PCoA axes for the response matrix of the model. Separate RDAs were run on each taxon using the *rda()* function in the R package, vegan v.2.6-2 (Oksanen et al., 2018). For each taxon, the full model was first explored (eq 1).

$$PCoA1_{\hat{a}} \times PCoA2_{\hat{a}} \sim PC1_{XY} \times PC2_{XY} \times Z \quad (1)$$

Interaction terms were included to allow for geographic distance slopes to differ by depth classes. Then the Δ Akaike's Information Criterion (AIC) between models after removing terms were compared with the *step()* function. Terms were removed when the AIC of the model was improved (lowering the AIC value) or when removing the term made no difference in AIC (*i.e.*, <Δ2AIC), preferencing the simpler models. When the *step()* function suggested models including non-significant terms then forward model selection was applied with the *ordi2step()* function. The proportion of variance confounded between the terms was explored by partitioning out the variance explained by multiple terms and leaving one term in as the explanatory variable. This was to assess particularly how confounded the PC2xy and Z-coordinates were in order to assess whether a depth distance could be evaluated separately from geographic distance.

To measure IBD slopes, I regressed Rousset's $\hat{a}$ and estimated log geographic distances (from transformed XY coordinates – reflecting a 2D-surface). Distances were calculated, and IBD-analyses were implemented using GENEPOP v.4.7.5 (Rousset, 2008). The regression was run using GENEPOP with the *ibd()* function in R. IBD analysis was run for each taxon for two different scales: 'Within' locations and 'All' locations. This was achieved by varying the minimum and maximum distances between pairs. Because the dispersal variance is unknown, assessing IBD at different scales is best practice. For the 'Within' analyses distances between 0.001 and 100 m were used (including within and between depth distances at a particular location) and for 'All' the scale was set to 0.001 and 100000 m. Approximate Bayesian Computation (ABC) bootstrapping was used to get confidence intervals of the parameter estimates. P-values were calculated for the slope (using one tailed-tests in both directions) and were assessed for a threshold of 0.05 significance and that confidence intervals did not span 0.

3.3.7 Neighbourhoods, individual density, and dispersal variance

A neighbourhood (Wright's Neighbourhood, Wright 1943) is defined by the number of effectively mating individuals within a given area and can be derived by the slope of IBD following Rousset (2000) (eq 2 and 3),

$$\hat{a} = a + bln(d) \quad (2)$$

$$b = \frac{1}{4D\pi\sigma^2} \quad (3)$$

where d is the geographic distance and *D* is the density, *i.e.*, the number of the individuals per m^2. Two densities were calculated, one using census density (the actual density of multi locus genotypes) and the other using an effective density based on effective population size (N_e), derived from the kinship analysis (see Section 3.2.8). To calculate census density some assumptions were made to account for error. For each plot I attempted to sample all the *Agaricia* spp. colonies within a 50 m^2 area, but at least in the plots with a large number of individuals some were either missed or purposely not sampled due to time constraints. I used a conservative discovery rate of 0.7 because of the likelihood of missing colonies when sampling. The total genotyping rate for all colonies collected was 0.66, and colonies not annotated due to not be found in the models varied among taxa and these rates were incorporated into the density calculation. Once the density was calculated, the dispersal variance of 2D dispersal distances was estimated following Rousset (2000) (eq 4).

$$\sigma^2 = \frac{1}{4D\pi b} \quad (4)$$

The dispersal variance (σ^2) is not the variance in Euclidean dispersal distances but of the distribution of axial dispersal distances (*i.e.*, non-central second moment), and thus can be used to characterise the shape of the distribution of dispersal distances if the distribution family is known (this can be approximated by measuring parent-offspring distances). These estimates were only calculated for instances when the slope of IBD was statistically significant. I assumed a negative exponential for the dispersal distribution (eq 4) as it is a common distribution. However, there could be a more appropriate bi-modal distribution as occasional long-distance dispersal has been observed in brooders (Underwood et al., 2006). Using the cumulative distribution function (*i.e.*, the integral of the probability distribution function, eq 5 - 9), I estimated the distance (d) in which 95% of all dispersal events should have occurred within.

$$P(d) = \frac{1}{\sigma}\exp\left(-\frac{d}{\sigma}\right) \quad (5)$$

$$CDF(d) = \int_0^d \frac{1}{\sigma}\exp\left(-\frac{x}{\sigma}\right)dx \quad (6)$$

$$\int_0^d \frac{1}{\sigma} \exp\left(-\frac{x}{\sigma}\right) dx = 1/\sigma[-\sigma \exp\left(-\frac{x}{\sigma}\right)]_{x=0}^{x=d} \quad (7)$$

Substituting for x = 0 and x = d,

$$CDF(d) = [-\exp\left(-\frac{d}{\sigma}\right) - (-\exp\left(-\frac{0}{\sigma}\right)] \quad (8)$$

$$CDF(d) = 1 - \exp\left(-\frac{d}{\sigma}\right) \quad (9)$$

3.3.8 Kinship

Kinship analysis was conducted to confirm theoretical estimates of dispersal from IBD by assessing the distribution of parent-offspring and sibling distances. Kinship analysis was conducted within COLONY v.2.0.6.8 (Jones & Wang, 2010). COLONY uses a full pedigree maximum-likelihood approach to jointly estimate both parentage and sibship and only 60 – 200 SNPs are required to obtain accurate estimates of kin (Flanagan & Jones, 2019). Parameters in the analyses included: a polygamous mating system, and inbreeding. A separate analysis was run for each taxon. For AA2 these were split into four separate analyses corresponding to each location due the large number of individuals slowing down runtime. All individuals were considered as potential mothers, fathers, and offspring, due to colonies potentially being hermaphroditic (some sources report *Agaricia* spp. as either hermaphroditic or gonochoric) (Baird et al., 2009; Fadlallah, 1983) and because colony size is not directly correlated with age (Hughes & Jackson, 1980). I estimated potential thresholds of parents (mothers and fathers) being within the sample by making some assumptions. Because of the results of the dispersal variance, I first assumed a probability of 1 then reduced this by adjusting for the genotyping rate for the entire dataset (0.66) and a discovery rate from (0.7). Given these, probabilities that potential parents were included in the sample used for COLONY analysis were from 0.5, 0.2, 0.1 and 0.05 and were assessed for consistent results. If parent-offspring or sibling pairs were found, distances between them were calculated for the comparison with dispersal variance. Effective population size (N_e) was calculated using the estimate from Wang (2009).

3.4 Results

3.4.1 Plot annotations and sequencing

After *de novo* construction of RAD loci, 619,484 polymorphic sites and 57,960 loci were found within 970 individuals and were mostly within 285 bp but to up 391 bp in length. Basic filtering with VCFtools (minimum allele count = 3; bi-allelic sites, 50% maximum missing data sites, minimum depth of 5) yielded 40,044 sites. Symbiont loci matching the *Cladocopoum* spp. genome were removed (n = 15,607) and as well as the nextRAD isolates (n = 5,552) and other non-Cnidarian taxa (n = 3, 243). Individuals that had high missing sites (>50%) were removed (n = 203), then more stringent filters for sites were used (20% maximum missing) for the all-individual analysis dataset contained 767 individuals, 15,659 sites and 919 loci. Population structure analyses of the dataset including all individuals found three highly divergent clusters that assigned individuals to three groups, corresponding to the three taxonomic species: *Agaricia agaricites, A. humilis* and *A. lamarcki*. Thus, species datasets were separated based upon Admixture assignments and PC scores (see Appendix 1). Initial yields for each species comprised: 512 individuals of *A. agaricites* with 10,205 sites and 1,606 loci, 142 individuals of *A. humilis* with 3,669 sites and 1,282 loci and with the addition of samples from Chapter 2, there were 113 individuals of *A. lamarcki* (and 4 *A. grahamae* samples) and 10,736 sites with 941 loci. After removing clones (see Appendix 1 for clone thresholds) and PC outliers (using pcadapt) and thinning to one SNP per RAD locus: the putatively 'neutral' and 'unlinked' datasets were used for taxa evaluation and spatial analysis (see Table S1 specific analysis numbers of individuals and SNP loci). Each species comprised of multiple taxa. Here 'taxa' refers to putative cryptic taxa within taxonomic species (see Methods and Appendix 1 for definitions and taxa evaluation) and these terms will be used throughout. Within *A. agaricites* two taxa named: "AA1" and "AA2", within *A. humilis*, three taxa were found and named: "AH1", "AH2" and "AH3" and within *A. lamarcki* the same two taxa were found as in Chapter 2 and were named as the same, "AL1" and "AL2" (Figure 3.3). Further spatial genetic structure was found within AA2 (thus showing K = 4 in Figure 3.3) and within AH1 taxa (thus showing K = 4).

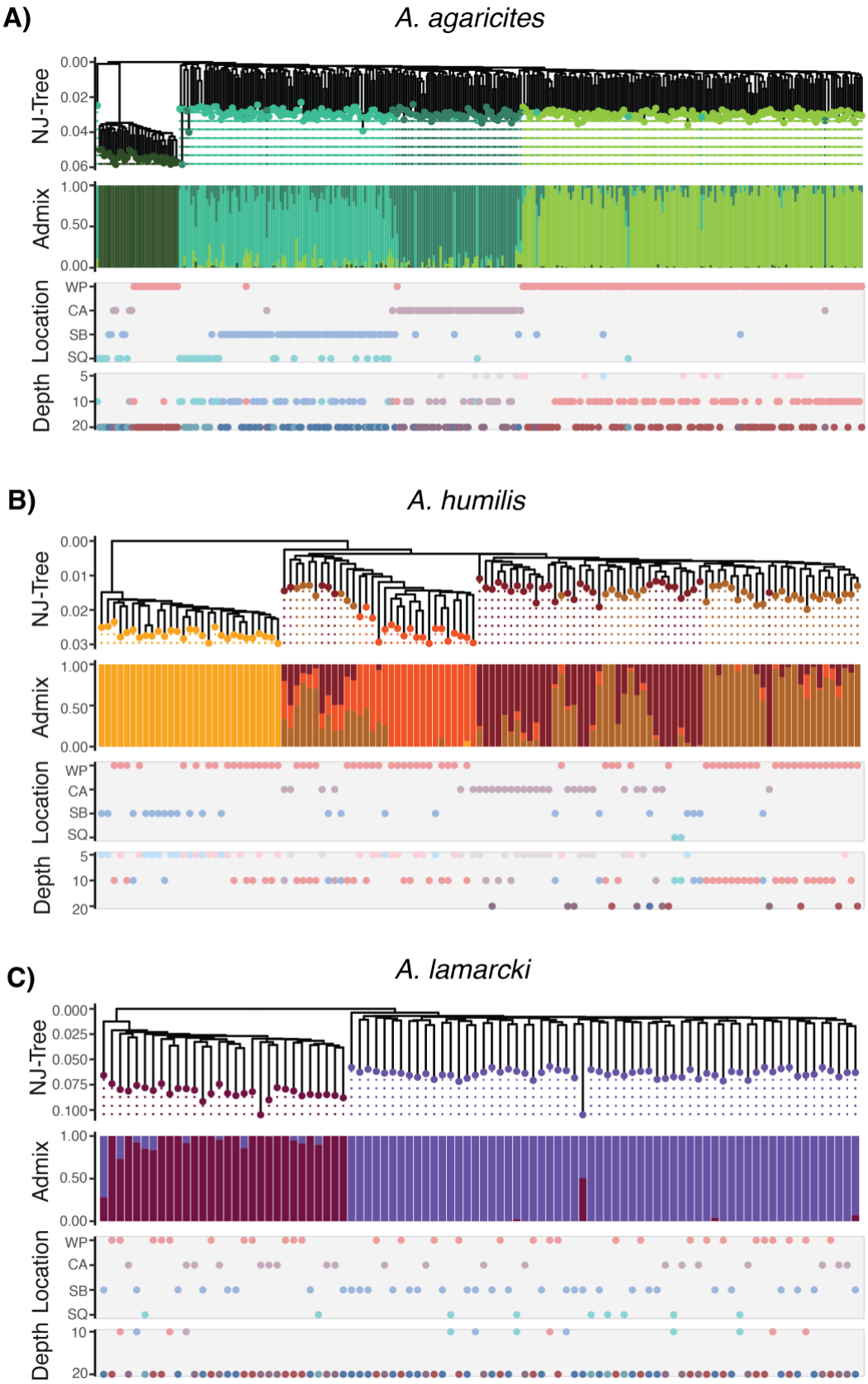


Figure 3.3. *Summary plots of the genetic and spatial data for Agaricia corals collected in Curaçao. Each panel contains a Neighbour-Joining Tree, an ADMIXTURE plot and dot plots representing where samples were collected (the location and depth). Colours in the genetic plots represent genetic clusters and in the dot plots represent the locations and location/depth. A) A. agaricites B) A. humilis and C) A. lamarcki.*

A reduced number of samples were used spatial analyses than the genetic assignment methods, Figure 3.4 shows the composition of species and taxa at locations and depths for the spatial analysis datasets.

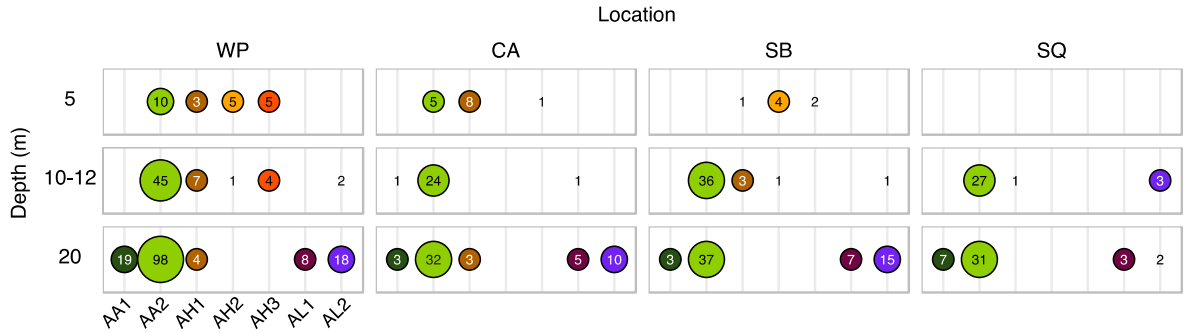


Figure 3.4. *Abundances and distribution of samples of coral taxa within Agaricia that were spatially located within virtual plots and represent multi-locus genotypes (MLGs). Numbers indicate the number of colonies within that plot.*

3.4.2 Clone distribution and genetic diversity

Most of the clone groups comprised of dyads (Table 3.1, Figure 3.5) and *A. agaricites* taxa (AA1, AA2) had lower genotypic richness (*i.e.*, more clones relative to unique genotypes) than the other taxa with *A. lamarcki* and *A. humilis* exhibiting similar proportions of clones. AA2 had the highest number of clones with many groups of 2, 3 and a few within groups from 4 to 7 clonal colonies. Clonal spread within plots was very limited with most clones under 1 m distant (Figure 3.6), and only two clone groups were between 10 and 30 m distant, one within AH1 and one within AH3.

Table 3.1. *Abundances of clones and clone groups amongst taxa. Genotypic richness is the proportion of multi-locus genotypes (MLGs) out of all colonies sampled.*

Taxa	*#MLGs*	*#Samples*	*Genotypic Richness*
AA1	34	46	0.733
AA2	299	462	0.646
AH1	72	88	0.816
AH2	29	31	0.933
AH3	20	22	0.905
AL1	31	34	0.909
AL2	62	69	0.897

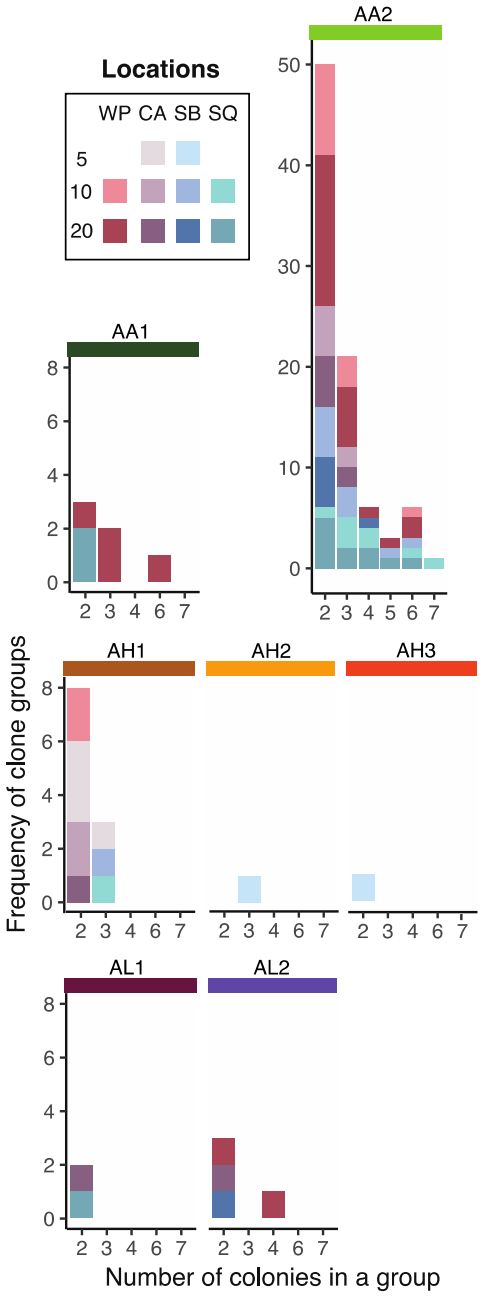


Figure 3.5. *Frequency and distribution in abundance of different clone groups for each Agaricia spp. taxon at four sites and three depths within Curaçao.*

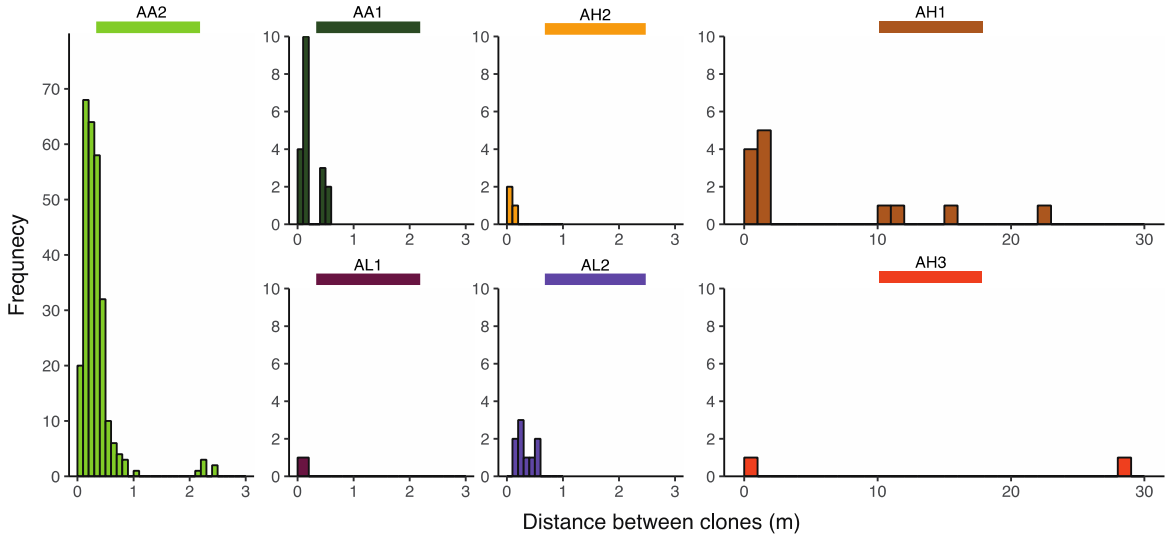


Figure 3.6. *Distributions of the spatial distances between clones for Agaricia spp. taxa at four sites and three depths within Curaçao. Frequency refers to the number of clones. Clone distances within all locations and depths are all combined.*

Allele frequency differences between cryptic taxa within species were moderate to high (F_{ST} = 0.18 – 0.60, Table 3.2) and inbreeding appeared to occur within taxa (Table 3.2). Taxa within *A. agaricites* and *A. humilis* exhibited population structure among locations, yet *A. lamarcki* taxa did not (see F_{ST}, Table 3.3). Given that *A. agaricites* and *A. humilis* experienced population subdivision, inbreeding was estimated for within each location, where all taxa showed inbreeding, except AA1 of *A. agaricites* (see F_{IS} for *A. agaricites*/*A. humilis* and F_{IT} for *A. lamarcki*, Table 3.3). However, these levels of inbreeding were low for AA2 and *A. lamarcki* taxa. Each taxon within *A. humilis* had exceedingly high levels of inbreeding, with AH3, the least abundant group that harbours admixed individuals with AH1, presenting an average inbreeding score within populations of 0.8.

Table 3.2. *Fixation statistics, F_{ST} and F_{IS} of Agaricia spp. corals with taxa treated as the population.*

	F_{ST}	F_{IS}	F_{ST} b/w Taxa		
A. agaricites	0.34	0.05		0.34	
A. humilis	0.54	0.66	1-2: 0.60	1-3: 0.41	2-3: 0.53
A. lamarcki	0.18	0.04		0.18	

Table 3.3. *Bootstrapped 95% confidence intervals for heterozygosity statistics, including observed and expected heterozygosity and F-statistics: F_{ST} F_{IT}, F_{IS}. Locations (WP, CA, SB and SQ) are treated as populations for each taxon. Where observed heterozygosity is different to expected heterozygosity (i.e., confidence intervals do not overlap) and F-statistics is different to zero (i.e., confidence intervals do not overlap with 0), the statistics are in bold.*

	H_{obs}	$H_{exp(total)}$	F_{ST}	F_{IT}	F_{IS}
AA1	0.22 (0.21, 0.24)	0.25 (0.23, 0.26)	**0.09 (0.08, 0.11)**	**0.09 (0.06, 0.12)**	0.00 (-0.03, 0.03)
AA2	0.13 (0.12, 0.14)	0.14 (0.13, 0.15)	**0.04 (0.04, 0.04)**	**0.07 (0.05, 0.08)**	**0.03 (0.02, 0.03)**
AH1	**0.08 (0.07, 0.08)**	**0.21 (0.20, 0.22)**	**0.08 (0.07, 0.09)**	**0.64 (0.60, 0.68)**	**0.61 (0.57, 0.64)**
AH2	**0.13 (0.12, 0.14)**	**0.29, 0.27, 0.30)**	**0.06 (0.05, 0.08)**	**0.55 (0.52, 0.59)**	**0.52 (0.49, 0.56)**
AH3	**0.06 (0.05, 0.07)**	**0.34 (0.32, 0.35)**	**0.12 (0.08, 0.15)**	**0.82 (0.79, 0.85)**	**0.80 (0.76, 0.83)**
AL1	0.21 (0.19, 0.22)	0.21 (0.20, 0.23)	-0.00 (-0.01, 0.00)	**0.03 (0.00, 0.07)**	**0.04 (0.01, 0.07)**
AL2	0.16 (0.15, 0.17)	0.17 (0.16, 0.18)	-0.00 (-0.01, 0.00)	**0.05 (0.02, 0.07)**	**0.05 (0.02, 0.08)**

3.4.3 Genetic differentiation by distance and depth

Redundancy Analysis (RDA) was used to assess the explanatory power of geographic distance and depth on genetic distances for each taxon between all plots. Geographic distance was reflected by transformed, orthogonal *XY*-coordinates ($PC1_{xy}$ and $PC2_{xy}$) and depth was reflected by the *Z*-coordinate. Genetic distances reflect Principal Coordinate Analysis axes (*PCoA1*, *PCoA2)*. Geographic distance explained most of the variation in genetic distance for both *A. agaricites* and *A. humilis* taxa (Table 3.4). For AA1, AA2 (within *A. agaricites*), AH2, and AH3 (within *A. humilis*) variation in $PC1_{xy}$ corresponding to the *X*-axis (*i.e.*, between location geographic distances and the horizontal axis within a plot) was the only significant term in explaining genetic distances. The *Z*-coordinate was significant in explaining some of the variation within AH1 (*A. humilis* taxon) along with $PC1_{xy}$ explaining most of the constrained axes' variance. However, the *Z*-coordinate only explained slightly more of the variation than $PC2_{xy}$ (~ 1% difference of explained variation), thus both models including either *Z* or $PC2_{xy}$ explained similar levels of variance. None of the explanatory variables explained significant variation within *A. lamarcki* taxa.

Table 3.4. *The first axis of geographic distance (PC1xy) explained most variation within genetic distance for coral taxa within Agaricia spp. at locations distributed along the leeward side of Curaçao using Redundancy Analyses (RDA). Genetic distance in one taxon, AH1 was also secondarily explained by depth (Z), but this effect was marginal and similar to the variation explained by the second axis of geographic distance (PC2xy).*

Taxa	Axes	Var	% var	F	Pr(>F)	Global model
AA1	*PC1xy*	0.0023	48.94	26.03	0.001***	F = 26.03, p < 0.001, adjR^2 = 0.46
AA2	*PC1xy*	0.0007	35.56	144.39	0.001***	F = 144.39, p < 0.001, adjR^2 = 0.37
AH1	*PC1xy*	0.15	35.71	20.23	0.001***	F = 11.82, p < 0.001, adjR^2 = 0.38
	Z	0.02	4.76	3.32	0.036*	
AH2	*PC1xy*	0.1804	40.17	7.67	0.003**	F = 7.67, p < 0.001, adjR2 = 0.25
AH3	*PC1xy*	2.3757	36.00	6.19	0.006**	F= 6.19, p = 0.007, adjR^2 = 0.30
AL1	*PC1xy*	0.0003	10.71	1.94	0.173	F = 1.94, p = 0.185, adjR^2 = 0.05
AL2	*PC1xy*	0.0000	0.02	0.01	0.992	F = 0.01, p = 0.991, adjR2 = 0.00

Taxa within species exhibited varied patterns of isolation-by-distance (Table 3.5). Taxa within *A. agaricites* and *A. humilis* exhibited isolation-by-distance across all locations (between 0.01 – 100 km distance) (Table 5, Figure 7). AA1 exhibited IBD within plots (0.01 – 100 m distance) however while the slope for AA2 for within plots was significant, the lower confidence interval included 0, and thus was not considered statistically significant (Table 3.5). *A. lamarcki* taxa did not exhibit IBD at any scale (Table 3.5, Figure 3.7).

Table 3.5. *Isolation-by-distance results (â = a + bln(geodist)) for each taxon of Agaricia spp. at two scales, "Within" considered all distances between 0.01 – 100 m and "All" between 0.01 – 100,000 m. ABC bootstraps were used for 95% confidence intervals (CIs) and significance of slope was assessed.*

Taxa	***n***	***Scale***	***Intercept (95% CIs)***	***Slope (95% CIs)***	***Slope* p**
A. agaricites					
AA1	30	**Within**	**-0.064 (-0.096, -0.028)**	**0.030 (0.022, 0.040)**	**< 0.001*** **
		All	**-0.032 (-0.063, 0.003)**	**0.013 (0.010, 0.016)**	**< 0.001*** **
AA2	247	**Within**	**0.016 (0.004, 0.030)**	**0.001 (0.000, 0.0003)**	**< 0.030***
		All	**0.005 (-0.007, 0.018)**	**0.006 (0.006, 0.007)**	**< 0.001*** **
A. humilis					
AH1	37	Within	1.597 (1.393, 1.778)	-0.024 (-0.044, -0.004)	0.989
		All	**1.479 (1.287, 1.647)**	**0.029 (0.022, 0.037)**	**< 0.001*** **
AH2	21	Within	1.400 (1.227, 1.563)	-0.005 (-0.032, 0.022)	0.648
		All	**1.361 (1.197, 1.512)**	**0.013 (0.008, 0.020)**	**< 0.001*** **
AH3	13	Within	5.002 (4.009, 6.028)	-0.026 (-0.096, 0.044)	0.767
		All	**4.738 (3.795, 5.719)**	**0.150 (0.105, 0.201)**	**< 0.001*** **
A. lamarcki					
AL1	20	Within	0.056 (0.016, 0.100)	0.010 (-0.003, 0.024)	0.064
		All	0.072 (0.034, 0.113)	0.000 (-0.001, 0.002)	0.312
AL2	47	Within	0.060 (0.031, 0.091)	0.003 (-0.003, 0.010)	0.177
		All	0.067 (0.040, 0.96)	0.000 (-0.001, 0.001)	0.791

Significance codes: - = 0.06, * ≤ 0.05 & > 0.01, ** < 0.01& > 0.001, *** ≤ 0.001

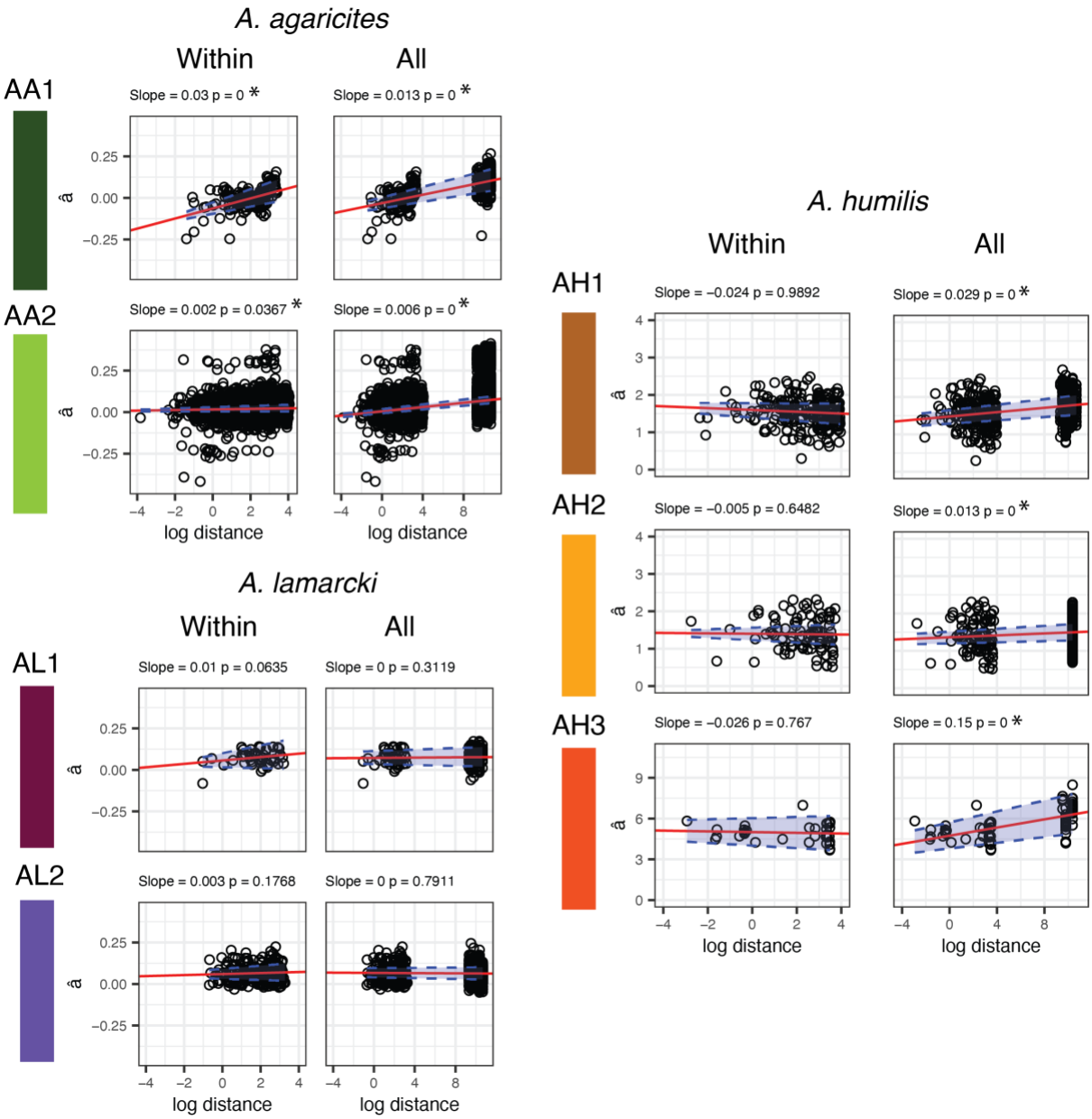


Figure 3.7. *Isolation-by-distance plots for taxa within A. agaricites, A. humilis and A. lamarcki across four locations and three depths at two scales. Within refers to scales between 0.01 – 100 m (within locations) and all refers to scales between 0.01 – 100,000 m (between all locations and depths). The Y-axis for each plot is individual genetic distance (â) and the X-axis is the natural logarithm of spatial distance.*

3.4.4 Neighbourhoods, individual density, and dispersal variance

Neighbourhood sizes were varied but all estimates of σ^2 were within metres and became smaller when using effective population size estimates compared to census density estimates (Table 3.6). Occasional long-distance dispersal may be likely due to the assignment method results, where some individuals assigned to a different location from where they were collected

(Figure 3.3). Within both *A. agaricites* and *A. humilis* taxa 95% of dispersal events are likely to have occurred within several metres (Table 3.6).

Table 3.6. *Neighbourhoods, individual densities, and dispersal variance (σ^2) for taxa within Agaricia agaricites and A. humilis. Densities were calculated with two methods; D was estimated as the census number of unique MLGs per m^2 whereas D_e is the effective density using estimates of N_e from kinship analyses (COLONY). The cumulative density function (CDF) was used to calculate the distance where 95% of dispersal events should have occurred when considering a negative exponential dispersal probability distribution.*

Taxa	Scale	Neighbourhood (95% CIs)	D (per m^2)	D_e (per m^2)	σ^2 with D	σ^2 with D_e	d (m), CDF(d) = 0.95
AA1	Within	33 (25, 46)	0.25	0.41	10.50	6.45	5.91 (*D*) 7.62 (D_e)
	All	80 (63, 102)	-	-	25.20	14.90	15.04 (*D*) 11.58 (D_e)
AA2	All	155 (139, 174)	1.17	3.61	10.56	3.69	9.74 (*D*) 5.74 (D_e)
AH1	All	79 (63, 102)	0.38	0.73	16.56	3.76	12.19 (*D*) 5.80 (D_e)
AH2	All	77 (51, 130)	0.31	1.57	19.80	3.88	13.33 (*D*) 5.92 (D_e)
AH3	All	7 (5, 10)	0.18	-	2.92	-	5.12 (*D*)

3.4.5 Kinship

Kinship was estimated in order to define the likely dispersal distribution and validate the dispersal variance by creating a distribution of parent-offspring distances. Relatives were identified in both *A. agaricites* taxa and *A. humilis* taxa; however, no relatives were found in *A. lamarcki* taxa consistent with the no IBD signal (Table 3.7). Three pairs of parent-offspring were found within *A. agaricites* taxa, two in AA1 and one in AA2 (Table 3.7). Due to the lack of parent-offspring dyads the dispersal distribution could not be accurately characterised.

Table 3.7. *Number of kin identified with COLONY for each taxon within Agaricia spp. (A. agaricites, A. humilis and A. lamarcki) within locations across the leeward side of Curaçao. Inbreeding was estimated using a maximum likelihood estimate, alpha was the inbreeding measure used for the effective population size estimate (N_e). N_e was calculated using Wang (2009) and 'prob' is the probability used for how likely it is parents are included in the sample for this particular result.*

Taxa	Site	#Parent offspring	#Full siblings	#Half siblings	alpha	Ne (95% CI)	Inbreeding (MLE)	Prob
AA1	All	2	2	10	0.04	82 (51, 146)	0.044	0.2
AA2	WP	1	5	15	0.02	665 (472, 1031)	0.019	0.2
AA2	CA	0	3	5	0.01	365 (225, 842)	0.012	0.2
AA2	SB	0	4	5	0.02	543 (346, 1267)	0.029	0.2
AA2	SQ	0	3	1	0.00	232 (125, 1083)	0.006	0.2
AH1	All	0	2	8	0.62	183 (111, 382)	0.624	0.1
AH2	All	0	1	1	0.53	157 (76, 1225)	0.525	0.1
AH3	All	0	0	1	0.80	124 (39, Inf)	0.798	0.1
AL1	All	0	0	0	-	-	0.048	0.05
AL2	All	0	0	0	-	-	0.055	0.05

Spatial distances between related colonies were calculated where possible. All genotyped individuals were included in the kinship analyses and some of the colonies included were not spatially mapped but a range of possible distances could be estimated by whether pairs were within or between plots. Only two pairs of full-sibs not spatially mapped were between plots at the same location, one from AA1 (10 – 20 m, WP) and one from AH1 (5 – 10 m, CA). All other pairs were found within plots and parent-offspring distances were all under 3 m (Figure 3.8).

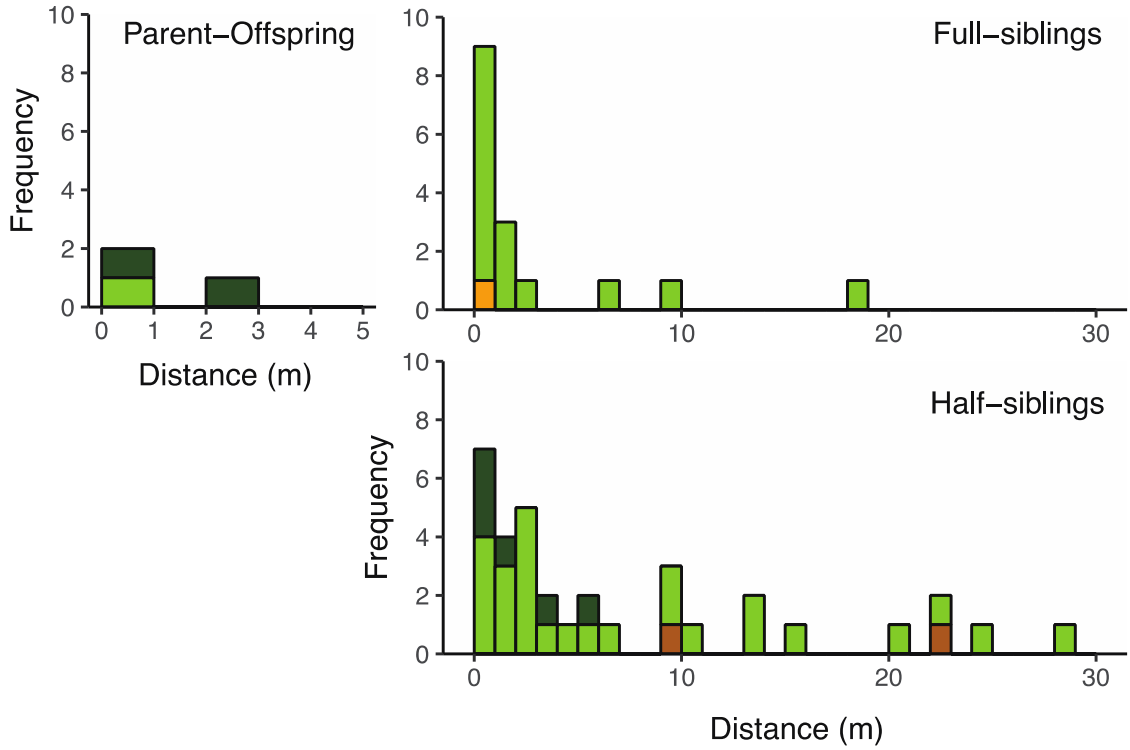


Figure 3.8. *Distribution of spatial distances between kin (parent-offspring, full and half siblings) within Agaricia spp. taxa across four locations and three depths within Curaçao.*

3.5 Discussion

This study provides one of the few examples of comparative dispersal measurements for scleractinian corals across fine scales, including estimates of dispersal variance. Estimating demographic patterns within corals can be difficult due to cryptic genetic substructure, irregular natural histories, and knowing which spatial scale is relevant for particular processes, such as dispersal. Starting with the spatial scales that are relevant to dispersal distances, which are less likely to have had other historical processes affect the genetic signatures, more accurate

measures of dispersal can be estimated. Here, the 'genotypic cluster' definition was applied to delineate cryptic taxa within species of *Agaricia*. The discovery of such cryptic taxa within each species exemplifies how widespread this phenomenon is. It is likely there are many more cryptic taxa within other coral species that have not yet been genetically examined, which will have implications for estimating population sizes and environmental tolerances. Taxa within species showed some depth partitioning, however within taxa, spatial scale was a stronger driver of genetic variation than depth (Table 3.4). Following our predictions, isolation-by-distance (IBD) patterns were found within *A. agaricites* and *A. humilis* taxa but not *A. lamarcki* taxa (Tables 3.4 and 3.5), highlighting stark differences in the life histories of congeners assumed to be ecologically equivalent (Darling et al., 2012). Dispersal of sexually derived propagules were found to be limited in taxa within *A. agaricites* and *A. humilis* (across metres, Table 3.6), and thus populations are likely mostly self-recruiting. Most clones were found within 1 m and thus it is likely that they were formed from partial mortality (*i.e.*, larger colonies separating into multiple colonies) (Figure 3.6), and genotypic diversity was maintained across fine scales. This study demonstrates a novel application of Structure-from-motion photogrammetry for spatially explicit population genomic studies on benthic organisms in measuring demographic patterns. Here, I elucidate patterns of clonal propagation, population densities, effective population sizes and dispersal estimates, revealing disparity in the demography of *Agaricia* species and highlighting the importance of local-scale processes in coral populations.

3.5.1 Localised dispersal implications and disparity among congeners

The shallow taxa, *A. agaricites* and *A. humilis* exhibit localised dispersal and spatial genetic structure among locations, whereas spatial genetic structure was not detected within *A. lamarcki* taxa when measured at the same spatial resolution and scale (Tables 3.4 and 3.5, Figure 3.7). Studies on brooding corals have found similar results to our findings within *A. agaricites* and *A. humilis* with dispersal likely across metres (Gorospe & Karl, 2013; Ledoux et al., 2010). Moderate to regional genetic homogeneity (10 – 250 km) appears more commonplace in broadcasters than brooders (Baums et al., 2010; Nakajima et al., 2010; Serrano et al., 2014; Underwood et al., 2009; van Oppen et al., 2015). There are examples where presumed brooders appear to have structure over greater distances. For example, *Favia fragum* presented no genetic structure >10 km within islands and structure was only shown at the Caribbean-wide scale using a mitochondrial and a nuclear marker (100s to 1000s km,

Goodbody-Gringley et al., 2010) and *Porites astreoides* had little genetic variation differentiation across a scale of >1,700 km using microsatellites (Serrano et al., 2016). However, these studies used markers with less intraspecific resolution and did not estimate dispersal directly and thus genetic homogeneity may have been maintained due to stepping-stone gene flow or insufficient time to reach drift-mutation equilibrium. Thus, it is important to test genetic homogeneity and estimate dispersal using individual-based approaches and using genome-level variation. Within *A. agaricites* there were a few individuals that were genetically assigned to a different location from the one they were collected (Figure 3.3), which could mean that they exhibit occasional long-range dispersal >20 km similar to likely occasional dispersal predicted for *Seriatopora hystrix* (Underwood et al., 2006). Both *A. agaricites* and *A. humilis* taxa appear to exhibit highly localised dispersal and thus most likely their populations rely on self-recruitment, and disturbances could have large effects on populations that are only marginally isolated.

Local dispersal could facilitate local adaption to different depths and in the extreme lead to speciation (*e.g.,* parapatric speciation, Bird et al., 2012). One taxon within *A. humilis* demonstrated a slight effect of depth (or the spatial separation of depths) on genetic variation (Table 3.5). Limited dispersal in *A. agaricites* and *A. humilis* taxa thus may have enabled such divergence. However, for *A. lamarcki* and *A. grahamae* taxa, their dispersal capabilities do not appear to be restricted by depth distances (current study and Chapter 2), so conditions of the formation of these taxa remain speculative and potentially variable among species. Discrepancy of dispersal among congeners represent very different evolutionary strategies that need to be considered separately. For example, rates of adaptation will vary with dispersal variances and effective population sizes (Broquet & Petit, 2009; Ronce, 2007). The stark differences in likely dispersal among species (within this study and Chapter 2) similarly reflect the deep bifurcation of divergence between the mesophotic-occurring plating species (*A. lamarcki*, *A. grahamae*, *A. fragilis,* and *A. undata*) and the shallower-occurring species (*A. agaricites*, *A. humilis* and *A. tenuifolia*) (Gijsbers et al., 2022). When implementing conservation strategies, measuring dispersal capabilities among multiple species is essential. Dispersal potential: informs development of the confines and distribution of Marine Protected Areas (MPAs) (Jones et al., 2009), guides assisted gene flow or outplanting to rescue populations (Hagedorn et al., 2021), and enables predictions of demographic recovery and adaptation rates (Hoffmann et al., 2021).

3.5.2 Clonal propagation at the finest scale

Clonal propagation in corals occurs naturally from disturbance or intrinsically (*i.e.*, generation of asexual propagules) but our understanding of its dynamics across fine scales in many species (and growth forms) of coral are unknown. Asexual reproduction within *Agaricia* corals is mostly distributed at the finest scale (~1 m), with an abundance of such finely distributed clones within *A. agaricites* taxa (Figure 6), which has diverse morphologies of foliose unifacial and bifacial fronds. The process that likely creates these clones is partial mortality separating one colony into multiple smaller colonies. Hughes & Jackson (1985) documented this process of partial mortality in *Agaricia* spp. and other foliaceous corals in Jamaica and found that many of the largest colonies decreased in area by >90% over three years due to disturbance. Thus, size is not always a reliable estimate of age in coral colonies that undergo this process (Hughes & Jackson, 1980), hence in the present study, I did not use size as a proxy for age for kinship analysis (*e.g.*, juvenile and adult). Partial mortality appears a useful strategy when facing frequent disturbance as larger colonies divide instead of suffering from complete mortality and this process along with frequent reproduction (in brooders) may have led to the relative increase and dominance of these corals after the multitude of disturbances the Caribbean has suffered in recent decades (De Bakker et al., 2016).

Regarding clonal comparisons amongst species, we found higher genotypic richness within both *A. humilis* and *A. lamarcki* compared to *A. agaricites* taxa (Table 3.1, Figure 3.5). For *A. humilis*, partial mortality is less likely because colonies are not easily broken from physical force (due to domed/encrusting morphologies). Also, small colonies are less likely to be hit by a disturbance and when they are affected, they are more likely to suffer from complete mortality than partial (Hughes & Jackson, 1985). Clonal colonies within *A. humilis* were also found between distances of 10 – 30 m (Figure 3.6), which could represent breakage and subsequent reattachment from storms or asexual larvae. Although it is possible for water movement to carry a dislodged coral 10s of metres, due to the size of the colonies, survival after dislodgement seems unlikely. Asexual parthenogenesis may be possible (Permata et al., 2000) but given only two occurrences of such clonal dispersal this is rather speculative and direct observation is required to substantiate this hypothesis. Regarding *A. lamarcki*, the lack of clones in comparison to *A. agaricites* despite their similar morphologies could in part be explained by the depth and habitat in which they are found. Most of the *A. lamarcki* colonies were found in cryptic locations (*i.e.*, at the bottom of outcrops and under ledges, see Chapter

4) and thus may be less affected by physical abrasion and potentially competition. At deeper depths, *A. lamarcki* starts to become more abundant and can dominate coral cover in some areas (Frade et al., 2019), at >20 m depth storm damage is also less likely and competitive sponges become more dominant (Hughes & Jackson, 1985; Lesser et al., 2009). *A. lamarcki* has been described as having a stationary life history strategy compared to a mobile *A. agaricites* due to their divergent patterns of propagation (*i.e.*, over time colonies of *A. agaricites* appear to move around whereas *A. lamarcki* stay in the same place) (Hughes & Jackson, 1985). These findings presented add substance to the early works on *Agaricia* corals that *A. agaricites* and *A. lamarcki* have divergent clonal propensities and produce separated colonies from partial morality.

Despite asexual reproduction being common within *A. agaricites*, the number of genetically distinct, multi-locus genotype colonies within close proximity to each other is high and thus reefs or small areas within reefs are not dominated by a few clonal genotypes (S-3.1). Such a pattern also means that the isolation-by-distance analyses were not affected by clonal reproduction. The average genotypic richness for *Acropora* spp., which is known to form beds of continuous colonies across large areas, across studies is, R = 0.62, whereas for massive growth forms richness is high, R = 0.89 (averaged from Baums, 2008). Although, genotypic richness within species and growth forms is highly variable, and it can range anywhere from monoclonal populations to full genotypic richness. Such variation is likely due to habitat characteristics (*i.e.*, disturbance level) (Baums et al., 2006). Here, the *A. agaricites* taxon with lowest genotypic richness was similar to that of the average genotypic richness of *Acropora* spp. (R = 0.65 reported here) and thus could be a common richness characteristic of corals with high clonal propensities. However, it is difficult to directly compare genotypic richness estimates among many other population genetic studies due to the sampling technique. For example, many studies will often sample colonies a given distance away (Gorospe et al., 2015). But here, we attempted to sample everything within a given area that was tissue unconnected, with some instances very clearly being formed by partial mortality as the recently deceased skeleton could be seen. Clonal dispersal within *Agaricia* corals appears (within metres) and partial mortality likely propagates the number of colonies of *A. agaricites* across reefs, but sexual reproduction maintains genotypic diversity within small areas.

3.5.3 Inbreeding found within all *Agaricia* spp. despite divergent dispersal capacities

Inbreeding appears common in many coral populations (Baums, 2008), but the specific causes and consequences of it are unknown. Low but significant levels of inbreeding were detected within *A. agaricites* and *A. lamarcki* taxa, yet within *A. humilis* taxa inbreeding levels are exceedingly high (Table 3.3). When spatial genetic structure was accounted for within *A. agaricites* taxon, AA1, then no inbreeding was detected (Table 3.3). Despite displaying divergent dispersal strategies, *A. agaricites* and *A. lamarcki* taxa show similar levels of inbreeding. Inbreeding was expected within *A. agaricites* due to local dispersal however this was not expected to be as high for *A. lamarcki*. Although, other coral species with high dispersal capabilities also have shown inbreeding (Baums et al., 2006; Foster et al., 2007). *Agaricia* spp. have been reported to have mixed sexual systems of both gonochoric and hermaphroditic colonies (Baird et al., 2009; Fadlallah, 1983; Harrison, 2011) and self-fertilisation has been reported in *A. agaricites* to be up to 38% in natural conditions (Gleason et al., 2001). *A. lamarcki* could be occasionally self-fertilising when there is mate limitation, thus explaining the low levels of inbreeding despite panmixia across the leeward reefs of Curaçao (Tables 3.3, 3.4 and 3.5, Figure 3.7). The high levels of inbreeding within *A. humilis* taxa could be of concern and lead to inbreeding depression. *A. humilis* colonies mostly occur at shallow depths (5 – 10 m) with high disturbance, are patchily distributed among distant outcrops separated by sand, and represent small colonies (~ a few cm^2), thus it is likely that when densities decrease, they may also benefit through self-fertilisation, particularly, given how limited sperm dispersal is in another brooder (~5 m, Warner et al., 2016). These high levels of inbreeding could suggest that instead of being ecologically and genetically divergent taxa, cryptic genetic structure is formed by local kin-groups, as seen as chaotic genetic patchiness patterns (see Cros et al., 2020). However, the kinship analysis incorporated inbreeding and did not detect such high levels of kinship among individuals within each taxon (Table 3.7) and such groups are replicated across locations at further distances (10s of km) than likely dispersal distances (metres). Each *Agaricia* spp. shows different patterns of inbreeding which warrants further exploration into self-fertilisation rates in both *A. lamarcki* and *A. humilis* and the potential fitness costs associated with high inbreeding levels in *A. humilis* taxa.

Two of the taxa within *A. humilis* display a continuous spread of admixture proportions between them and could also have increased hybridisation rates among taxa due to their limited

densities. Detecting evidence of both inbreeding and hybridisation among these taxa is interesting and hybridisation amongst inbred taxa could potentially provide an evolutionary rescue strategy that preserves populations in low densities by reducing risks from inbreeding depression through heterosis (Charlesworth & Willis, 2009). On the IUCN red list as of March 2023, *A. humilis* and *A. lamarcki* are reported as critically endangered, and *A. agaricites* is vulnerable due to recent declines and susceptibility to threats. Here, I suggest that monitoring populations of each *A. humilis* taxon in Curaçao may be important for both local and global conservation of *A. humilis* taxa given such high inbreeding levels and such monitoring could also inform the interplay between hybridisation and inbreeding in natural coral populations.

3.5.4 Cryptic taxa should be delineated following a consistent framework and depth likely drives ecological divergence

Cryptic taxa are commonly found in population genomic studies of corals but are rarely delineated following an explicit framework and demographic differences among such taxa are not often reported. Based upon the genotypic cluster definition by Mallet (1995), I identified taxa within *Agaricia* corals which are sympatric and with some taxa exhibiting depth partitioning (Figure 3.3). According to assignment methods, no intermediates exist between *A. agaricites* taxa and regarding *A. lamarcki* taxa the number of admixed individuals and their admixture levels are low, and thus these taxa represent discrete genetic entities (Figure 3.3 and see Appendix 1). Presence of low levels of gene flow were detected among both sets of taxa and species within *A. lamarcki* and *A. grahamae* (Chapter 2), and therefore it is not surprising that admixed individuals between taxa within both *A. lamarcki* and *A. humilis* species were observed. AH1 and AH3 form a more continuous cline of differentiation. However, whether the integrity of these groups will be maintained or whether they will homogenise into a single taxon is unknown. For example, the integrity of AH3 (the smaller group) may not be able to withstand the ongoing gene flow with AH1 due to the intermediates being of similar abundances to pure AH3. This example of intermixing is interesting due to the high inbreeding values within *A. humilis* taxa as mentioned previously. Inbreeding levels may be slightly elevated by introgressed alleles from hybridisation amongst taxa and be an artefact of F_{IS} (*i.e.*, previously fully homozygous loci in one taxon now presenting low levels of heterozygosity due to introgression of the alternate allele) despite genome-wide increases in heterozygosity. It is likely that inbreeding and hybridisation occur because of the same proximal cause, low densities (*i.e.*, in the absence of mates, hybridisation occurs more readily, Willis et al., 1997).

A particularly important finding that justifies the separation of these taxa is that spatial genetic structure signatures appeared once these taxa were treated separately (in *A. agaricites* and *A. humilis* taxa). It is likely that cryptic taxa exist within *Agaricia* spp. corals, and it could be that disruptive selection for specific depths or other ecological preferences that drives divergence despite likely gene flow among them.

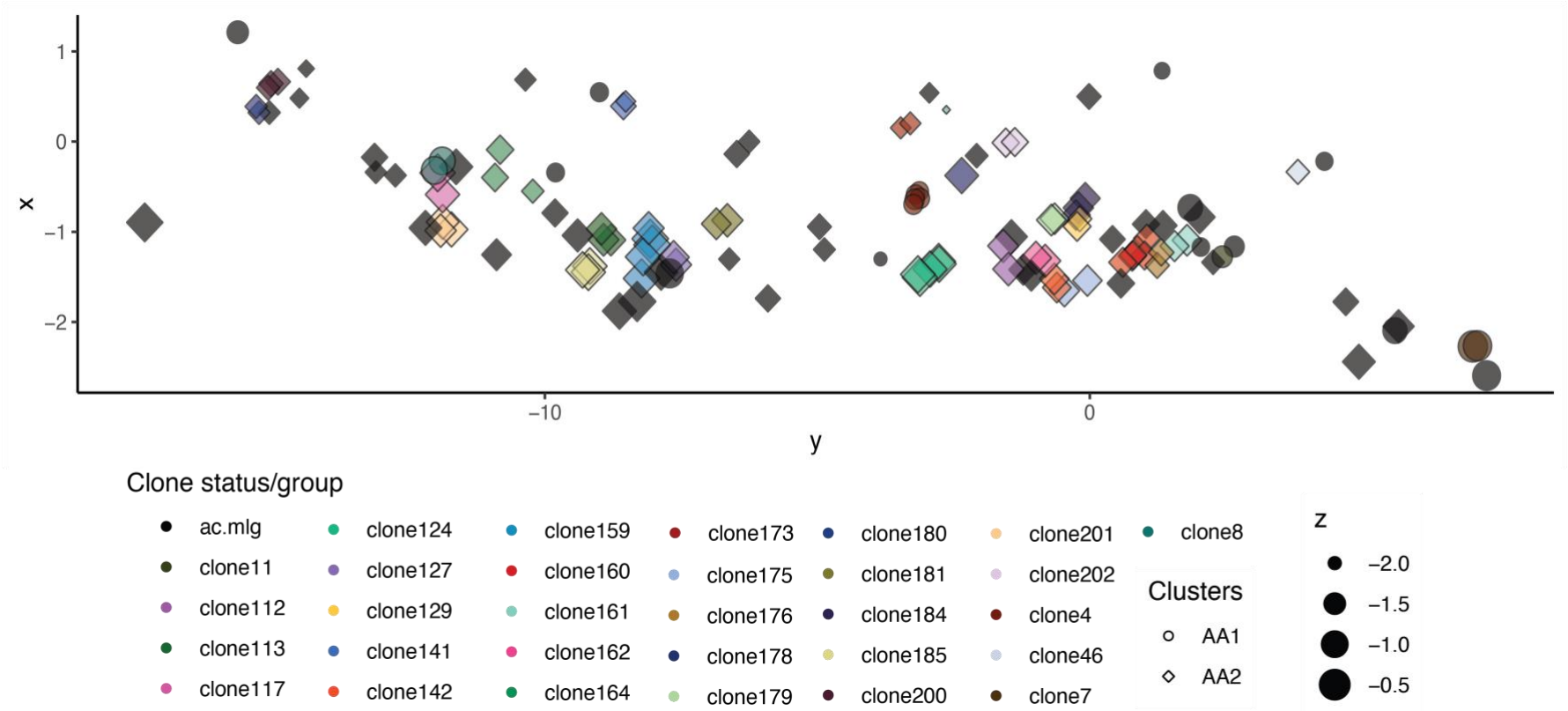
3.5.5 Benefits of measuring genetic variation across fine scales

Structure-from-motion photogrammetry enables highly accurate spatial mapping of samples *in silico* either before or after sampling. This facilitates substantial increases in sample sizes through allocation of more sampling time rather than mapping when SCUBA diving. This technology is leading to a new era of underwater and coral reef research, where previously SCUBA diving time limits have imposed restrictions in such data collections. For brooding corals, fine-scale mapping is relevant to their dispersal abilities and has been successful in determining dispersal variance for *A. agaricites* and *A. humilis* taxa (Table 3.6). The spatial distribution of clones is also expected to be highly important for corals, but few population genetics studies focus on this due to sampling at intervals for efficiency in capturing population-level genetic diversity. Goropse & Karl (2013) showed the utility of near exhaustive sampling in the complicated, mixed reproductive mode (*i.e.*, both brooding and spawning) coral, *Pocillopora damicornis* and found highly clonal populations dominated by a few genotypes and similarly to the present study found IBD within reefs (σ = 2.86 – 3.80 m), although in contrast found genetic homogeneity between reefs. Dubé et al (2020) was able to estimate fine-scale dispersal in a fire coral through parentage and spatial autocorrelation analyses (65% of dispersal within 300 m) and measured the spatial spread of clonality in an also highly clonal population. In the present study, I have expanded on this research and applied a novel mapping technique that is more efficient and accurate and applied higher resolution genomic markers. Comparatively studying multiple species is also important for direct comparison of their traits and repeated location and depth sampling enables both a replicated design and to assess whether depth acts as a dispersal barrier (as seen in Gorospe & Karl, 2015). Photogrammetry alleviates issues faced by underwater research and supports the study of reefscape genomics (*i.e.*, fine spatial scale genomic studies of benthic organisms on reefs).

3.5.6 Conclusions

Using fine spatial scales, I have demonstrated that valuable inferences into the life history of corals can be made, such as providing one of the few estimates of dispersal variance in corals (Gazulla et al., 2021; Gorospe & Karl, 2013; Ledoux et al., 2010). *Agaricia* corals are said to have 'weedy' life histories where they are able to easily colonise disturbed areas (Darling et al., 2012). But despite being congeners, all three taxonomic species of *Agaricia* corals exhibit varied combinations of dispersal capacity, clonal propagation, and inbreeding, which will have different consequences regarding demographic and evolutionary processes. For example, within *A. agaricites* and *A. humilis* taxa small dispersal distances will lead to local dominance but population recovery abilities from migrants will be more limited than for *A. lamarcki* taxa if populations are devastated. All three species harboured cryptic taxa and most demonstrated inbreeding, which means total abundances and their effective population sizes are lower than when considering census numbers of the three species, especially *A. humilis*, which is split into three taxa and has high inbreeding levels. However, there is potential for interspecific gene flow among *A. humilis* taxa to increase genetic diversity. This scenario may occur in other coral species with cryptic taxa where semi-permeable boundaries enable hybridisation when population sizes decrease. Clonal propagation mechanisms in *Agaricia* species are mostly low and localised and thus populations are able to maintain genetic diversity across fine scales through sexual reproduction. Further degradation of coral reefs within the Caribbean could lead to the dominance of 'weedy' species (see Roff, 2020) but there is much still unknown about how much life-history variation there is among 'weedy' species, and how many cryptic taxa there are within all other coral species. It remains challenging to estimate dispersal for species with long range dispersal as factors such as cryptic taxa, hydrological flows, disturbance events and other historical processes could affect accurate estimates. Regardless, self-recruitment is likely important for many reefs and measuring dispersal across fine scales is possible and could be used for predicting demographic recovery or adaptation outcomes on local scales. Considering the ubiquitous nature of cryptic coral taxa is required for measuring demographic processes and given the abundance and relative increases in brooding taxa in the Caribbean, implementing local scale protection is crucial for the health of their populations.

3.6 Supplementary materials



S-Figure 3.1. *Distribution of both clonal genotypes and multilocus genotypes within two taxa of Agaricia agaricites (AA1 and AA2) across 25 by 2 m plot at 20 m depth West Point, Curaçao. Each different clone group has a different number see key for clone group assignment, taxa assignment and depth found in plot. The x- and y-axes correspond to down slope and across slope respectively*

S-Table 3.1. Number of samples, SNPs and percentage of missing data used in each analysis.

Analysis	*# Samples*	*# SNPs*	*% Missing data*
PCA/Admixture – all individuals	557	762	20
PCA/Admixture/ F-stats – A. agaricites	335	1629	20
PCA/Admixture/ F-stats – A. humilis	121	1664	20
PCA/Admixture/ F-stats – A. lamarcki	98	954	20
Clonal threshold – A. agaricites	512	22274	20
Clonal threshold – A. humilis	105	25380	20
Clonal threshold – A. lamarcki	142	18374	20
Isolation-by-distance/RDA/F-stats – AA1	30 (35)[1]	465	20
Isolation-by-distance/RDA/F-stats – AA2	247 (300)	1461	20
Isolation-by-distance/RDA/F-stats – AH1	37 (61)	803	20
Isolation-by-distance/RDA/F-stats – AH1	21 (29)	565	20
Isolation-by-distance/RDA/F-stats – AH1	13 (15)	580	20
Isolation-by-distance/RDA/F-stats – AL1	20	896	20
Isolation-by-distance/RDA/F-stats – AL2	47	946	20
Kinship – AA1	35	487	20
Kinship – AA2 WP	106	912	20
Kinship – AA2 CA	48	874	20
Kinship – AA2 SB	64	990	20
Kinship – AA2 SQ	29	1052	20
Kinship – AH1	61	809	20
Kinship – AH2	29	602	20
Kinship – AH3	15	623	20
Kinship – AL1	20	510	20
Kinship – AL2	47	730	20

[1]Number of individuals included for *F*-statistics in brackets

Chapter 4: Subtle divergences in physical microhabitats between cryptic coral taxa using 3D-reefscapes

4.1 Abstract

Closely-related scleractinian species that harbour photo-endosymbionts often have disparate but overlapping depth distributions. Additionally, genomic studies are increasingly uncovering morphologically cryptic but genetically divergent coral taxa that are either incompletely depth partitioned or without obvious ecological patterns. Depth alone may not explain the environmental niches of such species and taxa. Within depths, the reefscape is highly structurally complex and thus species and taxa may partition their niches by occupying different microhabitats. Using structure-from-motion photogrammetry, I assessed whether three coral species within *Agaricia* (*A. humilis*, *A. agaricites* and *A. lamarcki*) and seven recently discovered taxa within these species occupy different microhabitats. I examined these distributions at three depths (5, 10 and 20 m) across the leeward reefs of Curaçao (Southern Caribbean) by quantifying microhabitat characteristics from the 3D point clouds surrounding annotated colonies. These microhabitat characterisations were compared amongst species and taxa at the depths where they co-occur using linear mixed-effect models. The parameters were also incorporated into a multivariate analysis to compare the multidimensional environmental niches of cryptic coral taxa. The deeper-occurring species, *A. lamarcki* generally inhabited microhabitats that were likely to have less light than the shallower-occurring species, *A. agaricites*. Focusing on cryptic coral taxa within species, no differences were found when assessing each microhabitat parameter individually, but subtle differences were found in their multidimensional niches (1 – 3%, $p < 0.05$). The comparisons for *A. agaricites* taxa followed that of the between species comparisons, where the taxon found predominantly at deeper depths (20 m) occupied microhabitats likely to have less light than the taxon that occurred at all depths (5 – 20 m). This study represents a novel approach for the quantitative assessment of physical microhabitats experienced by benthic organisms and suggests that species and cryptic taxa of corals are likely to have subtle divergences in their microhabitat usage when found at the same depth.

4.2 Introduction

Closely related taxa are generally expected to share similar niches due to phylogenetic inertia (Blomberg & Garland, 2002). Yet, no two divergent entities are expected to experience the exact same the ecological niche (Grinnell, 1917). Mostly, ecological (Grinnellian) niches, that focus on the habitat of organisms, are defined by broad-scale environmental measures over geographic ranges (Melo-Merino et al., 2020; Soberón, 2007). The prevalence of such comparisons can be attributed to advancements in remote sensing, where large, global datasets comprising environmental measures have been made available and applied to species distributions (*e.g.*, World-Clim, Hijmans et al., 2005) and claims that abiotic variables at coarser scales may be more important in determining physiological limits than finer scale variables (Pearson & Dawson, 2003). However, the environment that occurs at the scale of the individual is what organisms experience, and thus the species' fit to the local environment should determine their distribution on the fine scale (*i.e.*, environmental filtering). Yet, fewer studies explore species distributions at this scale in animals (but see Bennice et al., 2019; Ficetola et al., 2018; Kearney & Porter, 2017; Vries et al., 2021). Interestingly, comparisons between these spatial scales have found closely related taxa exhibiting similar niches at large geographic scales and yet divergent niches at fine scales (Ficetola et al., 2018). For sessile organisms (*e.g.*, benthic marine organisms and plants) we expect the local environment to be especially important for species niches because they are unable to escape local environment fluctuations. Indeed, in plants fine-scale filtering is more extensively studied (Ball et al., 2020; Bergholz et al., 2017; Graham et al., 2012; Musker et al., 2021; Papuga et al., 2018; Sedio et al., 2013). Thus, microhabitat characteristics could be more informative in determining habitat niches for a range of taxa where coarse scales have been unable to detect environmental distinctions.

Coral reefs provide habitat for a diverse range of taxa that coexist at fine spatial scales (Jackson, 1991). Across medium and large scales (kms – 10s of kms) coral reefs present mosaic patches and within reefs (<1 – 100s of metres) they generally exhibit high structural complexity (Pittman et al., 2009). Environmental differences between these spatial scales require separate considerations. But, also, important for marine organisms, there is the third spatial dimension (depth), which represents a steep environmental gradient similar to the altitude gradient in terrestrial systems but of greater magnitude. It is likely that coral reef habitat complexity affects taxonomic distributions at various spatial scales. Current genomic analyses are increasingly

revealing morphologically cryptic, but genetically divergent, scleractinian coral taxa that are sympatric within reefs (Bongaerts, Cooke, et al., 2021; Combosch & Vollmer, 2015; Cooke et al., 2020; Matias et al., 2022). Furthermore, such cryptic taxa often appear to be ecologically partitioned yet linked by some gene flow (Matias et al., 2022; Prada & Hellberg, 2020; Rippe et al., 2021; Chapter 2), suggesting recent and ecologically assisted divergence. These cryptic taxa could exhibit varied microhabitat usage within reefs due to niche diversification. Traditionally sampling regimes for genetics have been undertaken at the reef (or population) level and failed to note location and characterise the microhabitat to the level of the individual colony. Thus, the ecological processes that separate morphologically similar coral taxa but yet enable their co-occurrence has rarely been assessed.

Depth is the main environmental axis investigated that divides closely related species of marine taxa (Knowlton, 1993). Indeed, depth partitioning among taxa is apparent in hard corals (Bongaerts et al., 2011; Carlon & Budd, 2002; Johnston et al., 2022; Knowlton et al., 1997; Prada & Hellberg, 2013; Rippe et al., 2021). Many environmental parameters scale with depth such as temperature, nutrients, salinity, water flow, and disturbance level (Dollar, 1982; Lesser et al., 2009). However, perhaps the most important parameter for corals is irradiance (Veron, 1995), the spectra and intensity of which scale with depth. This is likely linked to certain photosynthetically activate radiation profiles and intensity required for dinoflagellate endosymbionts (Family: Symbiodiniaceae) (Goulet et al., 2019), which are essential for the holobiont's survival (*i.e.*, coral host, algal symbiont, and microbial community) (Muscatine, 1990). Indeed, depth partitioning of the algal symbiont is found across many species (Bongaerts, Carmichael, et al., 2015; Goulet et al., 2019), but also other species harbour specific strains/spp. and thus are more limited in their depth ranges (Bongaerts, Carmichael, et al., 2015; Bongaerts et al., 2013; Prada et al., 2014; Thornhill et al., 2014). Others forms of habitat partitioning in coral taxa occur across reef zones (although these are often related to depth), *e.g.*, lagoon, intertidal, reef crest, near slope, far slope, back reef, and front reef (Rippe et al., 2021; Suzuki et al., 2012; Tisthammer & Richmond, 2018; van Oppen et al., 2018). For example, a recent study aimed at teasing apart ecological differences between *Pocillopora* corals, found that they associated with different depths, light irradiance, and daily water temperature variance (Johnston et al., 2022). These same taxa also exhibited differences in bleaching mortality (Burgess et al., 2021) suggesting differences in sensitivity to warming climates.

In the heterogenous coral reef environment, very fine-scale (metres to centimetres) environmental differences within certain depth or habitat zones may provide ample variation within environmental space for habitat partitioning but has so far been mostly overlooked. Most apparent, light environments can change dramatically over a fine-scale through both the angle a coral colony is facing and whether colonies are shadowed by an "overhang" (Vermeij & Bak, 2002) or other obscuring structures over the course of the day. The angle a surface sits at in relation to the source of radiation determines the amount it receives, where the maximum would be a surface sitting perpendicular to the incoming direction of radiation, *i.e.*, the maximum irradiance angle being, $\theta \approx 0°$. Thus, coral colonies may inhabit suitable light environments via the angle(s) created through settlement choice on a particular substrate, post-settlement selection or adjustments in colony growth. An overhang shielding a coral colony prevents direct irradiance and thus the colony receives radiation through only scattered light. Both the angle(s) of the coral colony's surface in relation irradiance and the presence of an overhang or other shaded structure allow species that generally occur at deeper depths due to specific light adaptations to occur in lower-light situations at shallower depths, *i.e.*, corals that predominately occur at mesophotic depths (>30 m) occurring at shallow depths (<30 m) (Laverick et al., 2017; Muir & Wallace, 2016; Vermeij & Bak, 2002).

Light, however, is not the only important environmental factor for corals. Increased water flow provides numerous benefits for coral colonies including enhanced photosynthesis by symbiont algae and respiration rates for the coral host (Dennison & Barnes, 1988), increased host ingestion and uptake of dissolved nutrients (Kaandorp et al., 1996; Sebens et al., 1998, 2003) and sediment removal (Rogers, 1990). A coral colony's position within the reef and the physical structures around it may alter the water flow environment. For example, a colony on top of an outcrop may experience increased unidirectional flow from their exposed position, whereas a colony sitting within a crevice may experience a more stagnant water flow (Sebens et al., 2003). Increased reef rugosity, a measure of how convoluted a surface is, reduces unidirectional water flow and generates turbulence, and this turbulence helps to distribute the water flow across the reef and can improve reef-wide growth of coral and coralline algae (Graham & Nash, 2013). Additionally, the colony slope angle not only modulates the light environment, but a steep slope may aid sediment removal. Thus, localised structural complexity measures including slope, overhang, outcrop position and environmental rugosity may explain, in part, the micro-niches coral taxa experience and consequently their preferences.

While these microhabitat attributes have long been appreciated, it has not been feasible to rigorously quantify microhabitat at the fine scale experienced by individual colonies. The relatively new technology of structure-from-motion photogrammetry, however, can provide an accurate representation of the bathymetry of static features on the seafloor (see Bryson et al., 2017; Burns et al., 2015; Henderson et al., 2013; Pizarro et al., 2017; Storlazzi et al., 2016) and therefore new quantitative insights into microhabitat features (Bongaerts, Dubé, et al., 2021). Repeated overlapping photographs are taken of the seafloor, and these images are then stitched together through pattern-matching software to create point clouds, where each point occupies a 3D-coordinate, and its position and direction is confirmed by multiple camera angles. These point clouds can then be converted into 2D orthomosaics and 2.5D digital elevation models to get top views of the reefs or can be constructed into 3D meshes. Typically, structural complexity measures are either made from the whole plot or equally divided sections of the plot, and describe *e.g.*, the slope, surface rugosity, fractal dimension, or height variability (Torres-Pulliza et al., 2020). These measures are then applied to explain community-level patterns (Price et al., 2019; Pygas et al., 2020; Torres-Pulliza et al., 2020; Urbina-Barreto et al., 2022) or particular fish species abundances through estimating the distribution and abundance of different microhabitat types (González-Rivero et al., 2017). So far, none of these measures have been extracted to describe the microhabitats surrounding particular individuals (*e.g.*, static benthic organisms). Photogrammetry is particularly powerful in that it enables individual-based mapping and thus characterisation of the surrounding microhabitat as well as quantitative measures of the shape, size, and condition of such individuals, which that can be incorporated into genomic assessments (*i.e.*, reefscape genomics, Bongaerts, Dubé, et al., 2021). Here, I implement novel approaches to calculate structural complexity measures of microhabitats surrounding previously genotyped individual colonies, mostly, directly from the point clouds, in order to assess microhabitat preferences of particular species and cryptic taxa within the genus *Agaricia*.

Members of the genus *Agaricia* extend the entire depth range available for photosynthetically dependent organisms and are known to be highly abundant at mesophotic depths. Each known taxonomic species has a unique depth distribution that overlaps with one or more congeners (Bongaerts et al., 2013). Two mesophotic occurring species, *A. lamarcki* and *A. grahamae* have been shown to harbour cryptic genetic groups (Hammerman et al., 2018; Chapter 2), with *A. lamarcki* exhibiting incomplete depth-partitioning between the shallow and mesophotic depths

and *A. grahamae* showing no habitat differentiation (Chapter 2). In Chapter 3, I found two additional cryptic taxa within *A. agaricites* and three taxa within *A. humilis* with some evidence of depth partitioning, as well as the same two found in *A. lamarcki*. Additionally, species of *Agaricia* appear to be coadapted with certain symbiont strains (Bongaerts et al., 2013; excepting *A. lamarcki* taxa, Chapter 2). Thus, this genus appears to be an excellent candidate for studying microhabitat differentiation, especially concerning light habitats. *A. agaricites* colonies exhibit diverse morphologies such as unifacial encrusting or plating or form bifacial fronds. *A. agaricites* can occur at ~5 m but are most abundant from 10 – 20 m and do occur in mesophotic (> 30 m) but at lower abundances. *A. humilis* colonies exhibit massive or encrusting morphologies and are mostly found at 5 m, can extend to 10 m, and occur rarely at 20 m. Lastly, *A. lamarcki* colonies which exhibit plating morphologies are found rarely at 10 m and more commonly at 20 m but occur most abundantly in the upper mesophotic (30 – 50 m) (all distributions related to leeward reefs of Curaçao from personal observations, Chapters 2 and 3 and from Bongaerts et al., 2013).

Here, I use novel geometric approaches to characterise the physical habitat surrounding genotyped colonies. Morphologically cryptic yet divergent genetic groups exist within each taxonomic species within the dataset. I test whether there are differences in microhabitat usage amongst all taxa. I first examine each environmental microhabitat parameter separately using univariate statistics in linear mixed-effects models to test differences between taxa as discrete groups in each depth they co-occur. Then all microhabitat parameters were incorporated into a multivariate framework to examine multidimensional niche differences among taxa. Predictions and rationale relating to the specific microhabitat parameters can be found in Table 4.1. Testing whether closely related taxa are associated with different ecological niches is a necessary first step towards understanding how species' ecological and genetic differentiation starts and is maintained.

Table 4.1. *Rationale of microhabitat predictions (related to the structural environment) among taxa of Agaricia corals. These predictions are under the assumption that taxa found shallower (regarding metres below sea level) will prefer microhabitats with more light, and that deep water taxa will prefer microhabitats with less light, when found at the same depths.*

Environmental variable	Shallow taxon	Deep taxon	Rationale
Substrate angle	Lower angle	Higher angle	At a higher angle, less light will be received from directly above suiting the deep taxa. Conversely colonies may be modulating their morphology to compensate for a high substrate angle by growing towards light, thus no difference between shallow and deep taxa might be observed.
Overhang proportion	Lower overhang proportion	Higher overhang proportion	At higher overhang proportion, less light will be received from directly above suiting the deep taxa.
Outcrop proportion	Higher outcrop proportion	Lower outcrop proportion	At higher outcrop, colonies are less likely to be shaded and thus receive more light than colonies at the bottom of an outcrop so that shallow taxa would be more frequent on high outcrops.
Local height	Higher local height	Lower local height	There is more light at a higher height (lower depth) and colonies are less likely to be shaded so that shallow taxa would be more frequent at high local heights.
Environment rugosity	Higher environment rugosity	Lower environment rugosity	At the bottom of outcrops (where they may be shaded), colonies are more likely to be next to sand patches, thus there is less rugosity in their immediate surrounds.

4.3 Methods

Methods for sampling protocols, photogrammetry reconstructions, and annotations are detailed in Chapter 3. Briefly, four locations across of the leeward side of Curaçao were used for the study (see Figure 3.1). Photogrammetry plots (25 m length x 4 m width) were reconstructed across the slope at 5, 10, and 20 m depths for three locations (West Point: WP, Cas Abao: CA, and Snake Bay: SB) and for one location plots were made at 12 and 20 m depths (Seaquarium: SQ). Within these plots all *Agaricia* spp. colonies were exhaustively sampled for genomic sequencing and were assigned a taxon status in Chapter 3. Two taxa were found in each of *A.*

agaricites (AA1 and AA2) and *A. lamarcki* (AL1 and AL2) and three taxa found in *A. humilis* (AH1, AH2 and AH3). Sample sizes per plot (*i.e.*, depth) and location for each cryptic taxon used in the analyses can be found in Figure 4.1.

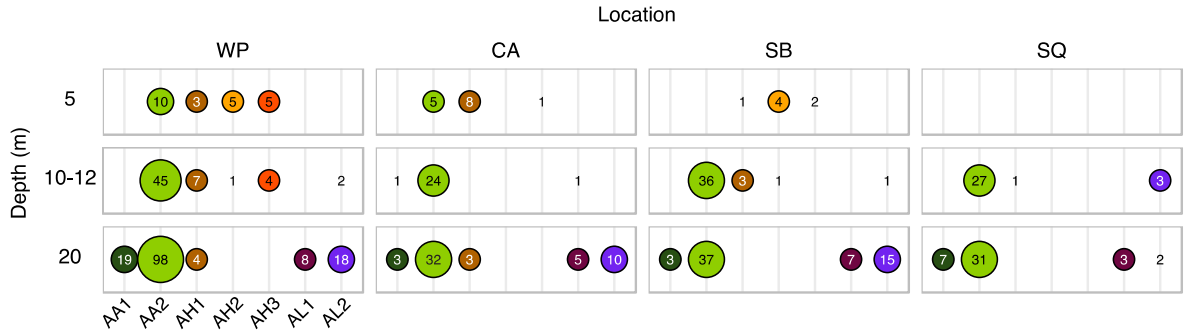


Figure 4.1. *Distribution and abundances of the sampled colonies available for analysis representing Agaricia spp. which were collected from 5, 10 and 20 m depths across four sites on the leeward side of Curaçao. AA1 and AA2 refer to cryptic taxa within A. agaricites, AH1, AH2, AH3 are taxa within A. humilis and AL1 and AL2 are taxa within A. lamarcki.*

The precise locations of the genotyped colonies were annotated on 3D virtual plots, that comprised XYZ points, RGB values, and point normals and stored in PLY files. Colonies were annotated in the pointcloud with the help of point-of-view video recordings of the sampling and that were then visually matched to the reconstructions using CloudCompare v.2.11 (*Cloud Compare*, 2021). For these annotations three points were taken per colony, one centroid point and two points marking the widest diameter of the colony. These three points were used to derive measures surrounding the colony and for isolating the colony from the point cloud.

4.3.1 Calculations of 3D-microhabitat measures

4.3.1.1 Point preparation: rotations, scaling, and subsets

Custom scripts were created for all 3D transformations and measures and can be found at www.github.com/kepra3/coralscape. For each plot, the point clouds were scaled using known distance markers and an "up-vector" was determined using depth markers in Viscore (Fox et al., 2019; Petrovic et al., 2014). The direction of the up-vector from the origin to this point is the water surface in which movements along this axis reflect changes in depth. To orient the model's origin along this up vector, angles between the origin and up vector were calculated using the tangent formula to define θ and ψ in radians (eq 1 and 2),

$$\theta = tan^{-1}\left(\frac{\Delta x}{\Delta z}\right) \quad (1)$$

$$\psi = tan^{-1}\left(\frac{\Delta y}{\Delta z}\right) \quad (2)$$

Where θ is angle of rotation about the y-axis and ψ is the angle of rotation about the x-axis. These two angles were then used in the function *get_rotation_matrix_from_xyz(psi, theta, 0)* from the Open3D package v.0.14.1 (Zhou et al., 2018) to obtain the rotation matrix with the Euler angle rotation theorem. The point cloud and annotations were rotated using this rotation matrix. Because we did not have georeferenced points, the φ angle, *i.e.*, rotation about the z-axis, was not calculated. Thus, for each plot, after the rotation, the Z-axis reflected changes in depth. The units for each axis were in metres.

Subset point clouds were used to calculate measures for each colony. First, a radius of points found around the annotated centroid of the colony was determined by one half of the distance between the two longest colony edge points. This radius was used to select a sphere of points around the centroid of the colony using the *search_radius_vector_3d()* function in Open3D. Thus, the radii selected for each colony varied due to different sized colonies. These groups of points, we name 'colony points'. For some analyses, the colony points were cleaned through meshing with the ball pivot function, *create_from_point_cloud_ball_pivoting()* in Open3D and then reducing down to the largest connected mesh cluster, which worked in isolating the colony from non-colony surrounds, removing floating points and creating a consistent surface area. Then, a second group of points was defined for each colony. For each colony, 0.2 m was added to the colony radius and this new radius was used to subset points in a sphere around the colony centroid and was named 'environment points'. Using information from these two sets of points, we calculated five microhabitat parameters: substrate slope, overhang proportion, outcrop proportion and environment rugosity. See Figure 4.2 for conceptual diagrams of each parameter.

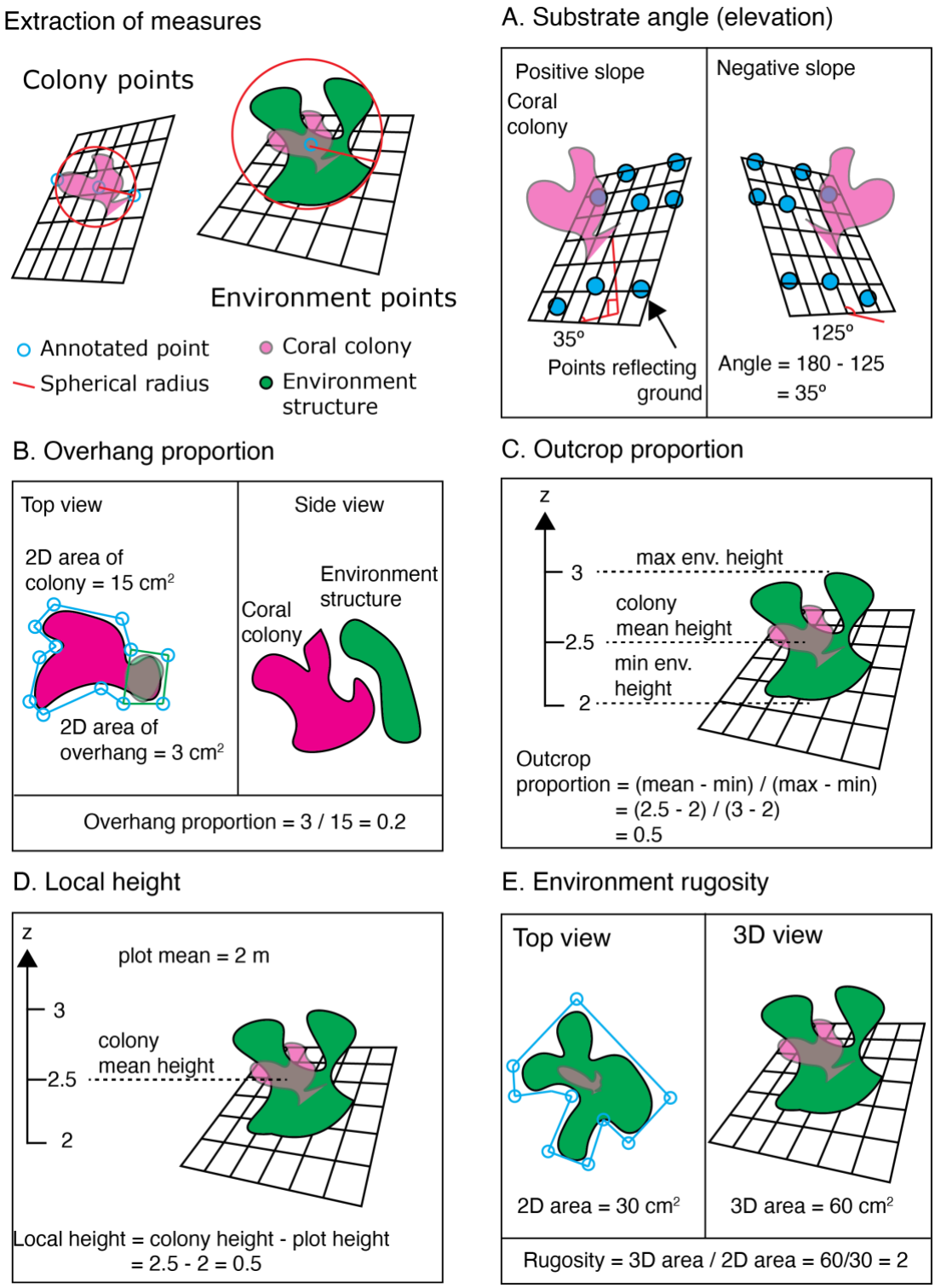


Figure 4.2. *Conceptual diagram of the extraction of measures and each of the five microhabitat parameters. Colony points were extracted by using three annotated points, one point within the centre of the colony and two on the longest edges. A radius was created by one half of the distance of the two longest edge points and then used to include all the points within that radius around the centroid. This radius was extended by 0.2 m to include the environment points. The five measures are defined: A) Substrate angle (elevation), B) Overhang proportion, C) Outcrop proportion, D) Local height and E) Environment rugosity.*

4.3.1.2 Substrate slope

Points surrounding the colony were first isolated by removing colony points from the environment points. A substrate slope was calculated by fitting a plane through the points using the RANSAC method with the function *segment_plane()* in Open3D. Then the angle of elevation of the best fit plane and the horizontal XY plane was calculated as the angle between the two normals of the planes (eq 3),

$$\cos(\theta) = \frac{\vec{p}\cdot\vec{z}}{\|\vec{p}\|\|\vec{z}\|} \quad (3)$$

This equation returns only positive angles thus cannot determine whether the orientation of the best fit plane is up or down. Because we were not interested in the Azimuth angle (due to the data not being oriented), any angle greater than 90°, then was flipped by subtracting it from 180°.

4.3.1.3 Overhang proportion

The overhang proportion was calculated through first identifying the points found directly above the colony points. This was achieved through subsetting the environment points with the same XY-coordinates $\pm$0.01 m of the colony points but with higher z-coordinates than the maximum Z-coordinate of the colony points. Then, if points within these dimensions existed then the colony points and overhang points were projected to 2D by using only the XY-coordinates and then the 2D areas of each polygon was calculated as the convex hull in the "alphashape" v.1.3.1 package. Overhang proportion was calculated as the 2D overhang area divided by 2D colony area (eq 4),

$$P_{overhang} = \frac{A_{overhang}}{A_{colony}} \quad (4)$$

4.3.1.4 Outcrop proportion

Within the environment points, the outcrop proportion was calculated as the mean Z-coordinate of the colony points divided by the range of Z-coordinate values of the environment points, where C and E refer to colony and environment points (eq 5),

$$P_{outcrop} = \frac{\mu_{Cz}}{z_{Emax} - z_{Emin}} \quad (5)$$

4.3.1.5 Local height

Within the colony points, the mean z-coordinate was used for local height. For each plot, this value was standardised across colonies.

4.3.1.6 Environment rugosity

To calculate surface rugosity of the environment points, the 3D surface area and 2D surface area were calculated. Using the ball and pivot method mentioned above to mesh the points, the 3D area was calculated as the summation of all triangles. To calculate the 2D area first each set of points had to be oriented flat to account for the slope confounding the measure. The points were oriented flat through an affine transformation where the normal of best fit plane of the environment points becomes the Z-axis and the X-axis and Y-axis are calculated as normal to the Z-axis (eq 6 - 11), where (eq 6) reflects the normal of the plane of best fit, (eq 7) is a random point used to find orthogonal axes to (eq 6), and (eq 10) is the resulting rotation matrix,

$$\vec{z} = \begin{bmatrix} a \\ b \\ c \end{bmatrix} \tag{6}$$

$$p = [x' \;\; y' \;\; z'] \tag{7}$$

$$\vec{x} = \frac{p \times \vec{z}}{\|p \times \vec{z}\|} \tag{8}$$

$$\vec{y} = \vec{z} \times \vec{x} \tag{9}$$

$$R = \begin{bmatrix} \vec{x} \\ \vec{y} \\ \vec{z} \end{bmatrix} \tag{10}$$

$$R \cdot P = P' \tag{11}$$

The 2D area was then calculated by finding the convex hull of the transformed points using only the oriented X- and Y-coordinates with the package “alphashape”. Then surface rugosity was calculated (eq 12),

$$SR_{env} = \frac{3D\ SA}{2D\ SA} \tag{12}$$

4.3.2 Microhabitat statistics

All statistics were conducted in R v.4.2.0. Individual colonies were assigned to each of the seven taxa. Individuals with mixed ancestry were removed and clonal colonies were included. Pearson’s Chi-squared test was used to examine differences in ratios of abundance of taxa among depths. Here, the abundance of each taxon was divided by its total abundance in order to account for differences in sample sizes among taxa due to sampling issues (*i.e.*, *A. humilis* taxa are less abundant in this dataset due to frequently being unable to locate colonies in the plots) and natural differences in abundances among taxa. All locations were pooled together for this analysis.

4.3.2.1 Univariate

To assess taxa niche differences (both within and between species) in each microhabitat parameter when taxa co-occur at certain depths, I used linear mixed-effects models with the package "lme4" v.1.1-29 (Bates, 2010) and the *lmer()* function estimated using REML and a nloptwrap optimiser. Here, microhabitats are assumed to be related to the environmental niche of each taxon. Separate models were performed at each depth (5, 10 and 20 m) for each microhabitat parameter (1. substrate slope, 2. overhang proportion, 3. outcrop proportion, 4. environmental rugosity and 5. local height). The microhabitat parameter was the response, taxa assignment was the categorical fixed effect with either four (at 5 and 10 m) or five (at 20 m) levels depending on the co-occurrence of taxa at particular depths, and location was treated as a random effect that allows different intercepts at each location (eq 8),

$$microhabitat \sim Taxa + (1|Location) \quad (8)$$

Each pair of microhabitat parameters were assessed for any correlations. The square-root of environmental rugosity (a microhabitat parameter) was used to create a more gaussian-like distribution. For the microhabitat parameter, overhang proportion, the data was zero-inflated due to real occurrences of zero within the data, *i.e.*, many colonies had no overhang present. In order to deal with the zero-inflation a generalised linear mixed-effects model was applied (function = *glmer()*) using a binomial distribution family with a logit link and was estimated using Maximum-Likelihood and a Nelder-Mead optimiser. Although, the distribution family was not ideal for the data requirements (*i.e.*, a distribution related to continuous proportions and can deal with zeros), it was the best available. Model diagnostics were assessed through confirming that the residual patterns were of equal variance and had random distributions above and below zero. To examine the pairwise differences among taxa a post-hoc Tukey test was applied with the "emmeans" package v.1.7.5 (Lenth, 2022).

4.3.2.2 Multivariate statistics

Multivariate statistics were used in order to compare niche differences amongst taxa when considering the combined effect of all the microhabitat parameters (*i.e.*, the multidimensional niche). Each microhabitat parameter was scaled and then for all pairs of individual colonies, the Euclidean environmental distances were calculated using all five microhabitat parameters. Then using the *adonis2()* function from the "vegan" v.2.6-3 package (Oksanen et al., 2018), I compared pairwise differences among taxa in their multidimensional environmental niche at

the depths that they co-occur using the location as the 'strata'. The *adonis2()* function uses a permutational MANOVA and with the strata option constrains the permutations within each location. This method was only applied on cryptic taxa within species, AA1 vs. AA2, AL1 vs. AL2 and all pairwise comparisons of the *A. humilis* taxa (AH1, AH2, and AH3). For visualisation, the individual environmental distances were plotted as points on two multidimensional axes and the vector scores for each microhabitat parameter were plotted as arrows.

4.4 Results

Microhabitat parameters were extracted for 502 colonies that were assigned to each of the seven taxa, two taxa for *A. agaricites*, three for *A. humilis* and two for *A. lamarcki*. There were differences in the abundances of taxa at certain depths (Figure 4.1) and taxa were found to vary among depths in proportion relative to their abundances ($\chi^2 = 562.48$, df = 12, $p < 0.001$, Figure 4.3). Comparisons of the microhabitat parameters were made between taxa at the depths where they co-occur. Microhabitat parameters for individual colonies within each taxon group were highly variable (S4.1 – 4.5) and for some comparisons the statistical power was low due to the low abundance of colonies at some sites (*i.e.*, for *A. humilis* taxa).

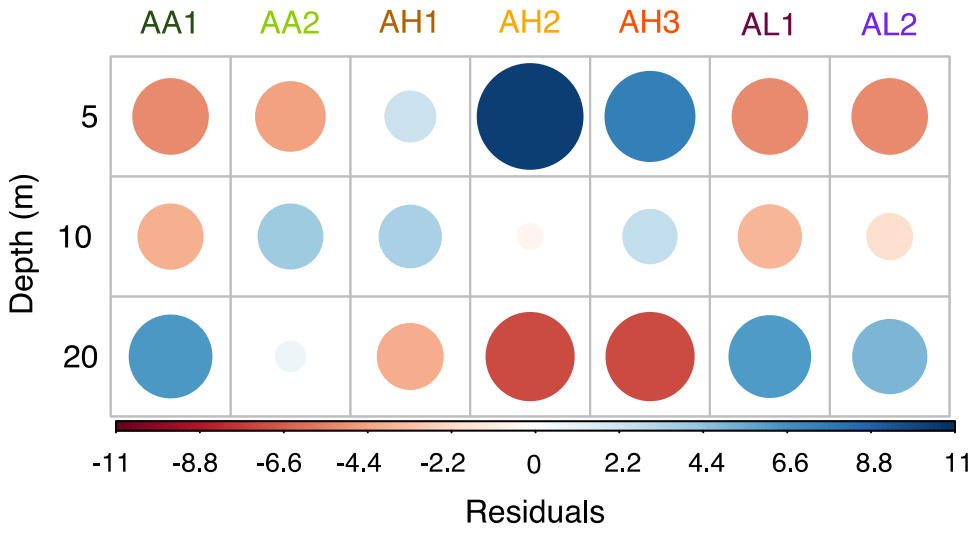


Figure 4.3. *Differences in the relative proportions of cryptic taxa of Agaricia spp. at each depth (5, 10-12, 20 m). Locations across Curaçao have been pooled together (WP, CA, SB and SQ).*

4.4.1 Univariate statistics

By examining each microhabitat parameter separately at each depth for taxa that co-occur, niche partitioning could be observed. Significant differences were found between species (Table 4.2, Figure 4.4), and four out of the five of the parameters followed the predictions (Table 4.1). *A. lamarcki* taxa (mostly AL2) more often occupied microhabitats that are likely to receive less light than *A. agaricites* taxa (mostly AA2) at 20 m. Substrate angle was found to be higher for AA2 than AL2, against the predictions that it should be higher for the deeper-occurring taxon (Figure 4.4). However, all other parameters followed the predictions, regarding comparisons between AL2 and AA2 for overhang proportion, outcrop proportion, local height, and environment rugosity. In outcrop proportion, comparisons between AL1 and AA2, and between AL2 and AA1 followed the predictions. Environment rugosity was the only parameter to also show differences at 10 m (all other comparisons were at 20 m), which was between AL2 and AH1, this parameter also showed differences between AL1 and AL2 vs. AA2 at 20 m.

Table 4.2. *Predictions for differences in microhabitat parameters (related to the structural environment) between taxa and species of Agaricia corals across three depth zones and collected from the leeward side of Curaçao. Predictions are made for each taxon pair at the depth zones they co-occur and then empirical results are reported.*

Environmental variable	***Taxon pair*** *(deep - shallow)*[a]	***Depth (m)***	***Prediction***	***Trend/Finding***
Substrate angle	AH1 – AH2/3	5	AH1 > AH2/3	✓✓
	AH1 – AH3	10	AH1 > AH3	✓
Within species	AA1 – AA2	20	AA1 > AA2	✗
	AL1 – AL2	20	AL1 > AL2	✓
	AA2 – AH	5	AA > AH	✗✗✗
	AA2 – AH1/3	10	AA > AH1/3	✗✓
	AL2 – AA2	10	AL2 > AA2	✗
	AL2 – AH1/3	10	AL2 > AH1/3	✗✓
Between species	AA – AH1	20	AA > AH1	✗✗
	AL – AA	20	AL > AA	✗✗✗✗ (AL2 < AA2**)
	AL – AH1	20	AL > AH1	✗✗
Overhang proportion	AH1 – AH2/3	5	AH1 > AH2/3	✓✗
	AH1 – AH3	10	AH1 > AH3	✓
Within species	AA1 – AA2	20	AA1 > AA2	✓
	AL1 – AL2	20	AL1 > AL2	✗
	AA2 – AH	5	AA > AH	✓✓✗
	AA2 – AH1/3	10	AA > AH	✗✗
	AL2 – AA2	10	AL2 > AA2	✓
	AL2 – AH1/3	10	AL2 > AH1/3	-
Between species	AA – AH1	20	AA > AH1	✗✗
	AL – AA	20	AL > AA	✓✓✓✓ (AL2 > AA2**)

	AL – AH1	20	AL > AH1	?
Outcrop proportion	AH1 – AH2/3	5	AH1 < AH2/3	✓✓
	AH1 – AH3	10	AH1 < AH3	✓
Within species	AA1 – AA2	20	AA1 < AA2	✓
	AL1 – AL2	20	AL1 < AL2	✗
	AA2 – AH	5	AA2 < AH	✗✓✗
	AA2 – AH1/3	10	AA2 < AH1/3	✗✗
	AL2 – AA2	10	AL2 < AA2	✓
	AL2 – AH1/3	10	AL2 < AH1/3	✓✓
Between species	AA – AH1	20	AA < AH1	✗✗
	AL – AA	20	AL < AA	✓✓✓✓ (AL2 < AA1***) (AL1 < AA2***) (AL2 < AA2***)
	AL – AH1	20	AL < AH1	✓✓
Local height	AH1 – AH2/3	5	AH1 < AH2/3	✓✓
	AH1 – AH3	10	AH1 < AH3	✓
Within species	AA1 – AA2	20	AA1 < AA2	✓
	AL1 – AL2	20	AL1 < AL2	✗
	AA2 - AH	5	AA2 < AH	✗✗✓
	AA2 – AH1/3	10	AA2 < AH1/3	✓✓
	AL2 – AA2	10	AL2 < AA2	✓
	AL2 – AH1/3	10	AL2 < AH1/3	✓✓
Between species	AA – AH1	20	AA < AH1	✓
	AL – AA	20	AL < AA	✓✓✓✓ (AL2 < AA2***)
	AL – AH1	20	AL < AH1	✓✓
Environment rugosity	AH1 – AH2/3	5	AH1 < AH2/3	✗✗
	AH1 – AH3	10	AH1 < AH3	✓
Within species	AA1 – AA2	20	AA1 < AA2	✓
	AL1 – AL2	20	AL1 < AL2	✓
	AA2 - AH	5	AA2 < AH	✗✗✗
	AA2 – AH1/3	10	AA2 < AH1/3	✓✓
	AL2 – AA2	10	AL2 < AA2	✓
	AL2 – AH1/3	10	AL2 < AH1/3	✓✓ (AL2 < AH1*)
Between species	AA – AH1	20	AA1/2 < AH1	✓✗
	AL – AA	20	AL1/2 < AA1/2	✓✓✓✓ (AL1 < AA2**) (AL2 < AA2*)
	AL1/2 – AH1	20	AL1/2 < AH1	✓✓

[a] deeper taxon is always on the left

[b] Ticks (✓) indicate alignment to predictions and crosses (✗) indicate that results are opposite to predictions, where the number of symbols equals the number of plots where comparisons between the taxa is possible. Occasionally taxa are combined in comparisons due to the predictions being the same. Significant comparisons are indicated in brackets. *P < 0.05; ** P < 0.01; *** P < 0.001

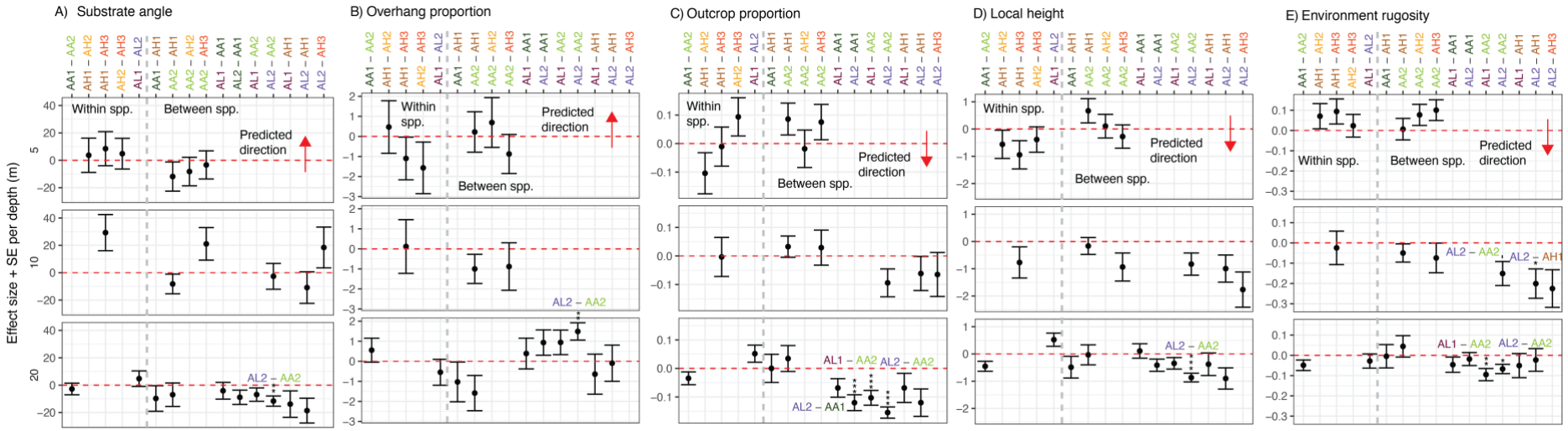


Figure 4.4. *Effect sizes between each pair of taxa from the linear mixed-effects models for each microhabitat parameter. Arrows indicate the predicted direction of the relationship (see Table 4.1) A) Substrate angle, B) Overhang proportion, C) Outcrop proportion, D) Local height and E) Environment rugosity*

No significant differences were found for univariate comparisons between taxa within species, however consistent trends following predictions were seen for comparisons between *A. agaricites* taxa for four out five of the microhabitat parameters (the same differences in parameters for the comparisons between *A. lamarcki* and *A. agaricites* taxa) (Table 4.2, Figure 4.4). Trends were opposite of the predictions for *A. lamarcki* taxa for the same four microhabitat parameters (Table 4.2, Figure 4.4). Regarding comparisons between *A. humilis* taxa, trends were quite variable and due to the low sample sizes, no interpretations of these results were made (Table 4.2, Figure 4.4).

4.4.2 Multivariate statistics

Through incorporating all microhabitat parameters into a multivariate analysis, differences in the multidimensional niche were tested amongst taxa within species. Significant differences were found between both *A. agaricites* taxa (AA1 vs. AA2) and *A. lamarcki* taxa (AL1 vs AL2) but no differences were found among *A. humilis* taxa (multiple pairwise tests amongst, AH1, AH2 and AH3) (Table 4.3). Despite detecting differences between *A. agaricites* taxa and *A. lamarcki* taxa ($p < 0.05$), these differences only explained 1 and 3% of the variation respectively. Most of the directions of the parameters followed our predictions for *A. agaricites* taxa yet not for *A. lamarcki* taxa (Table 4.1, Figure 4.5).

Table 4.3. *Permutational MANOVA results for differences in microhabitat parameters (related to the structural environment) among taxa (genetically divergent groups within species) of Agaricia corals at two depth zones where they co-occur (5 and 20 m).*

Taxa comparisons	*Depth*	*d.f.*	*R^2*	*F*	*P*
AH1 - AH2 - AH3	5	2, 27	0.085	1.203	0.526
AH1 – AH2	5	1, 19	0.038	0.758	0.752
AH1 – AH2	5	1, 18	0.110	2.233	0.127
AH2 – AH3	5	1, 27	0.012	1.203	0.537
AA1 – AA2	20	1, 228	0.012	2.738	0.029*
AL1 – AL2	20	1, 66	0.033	2.234	0.041*

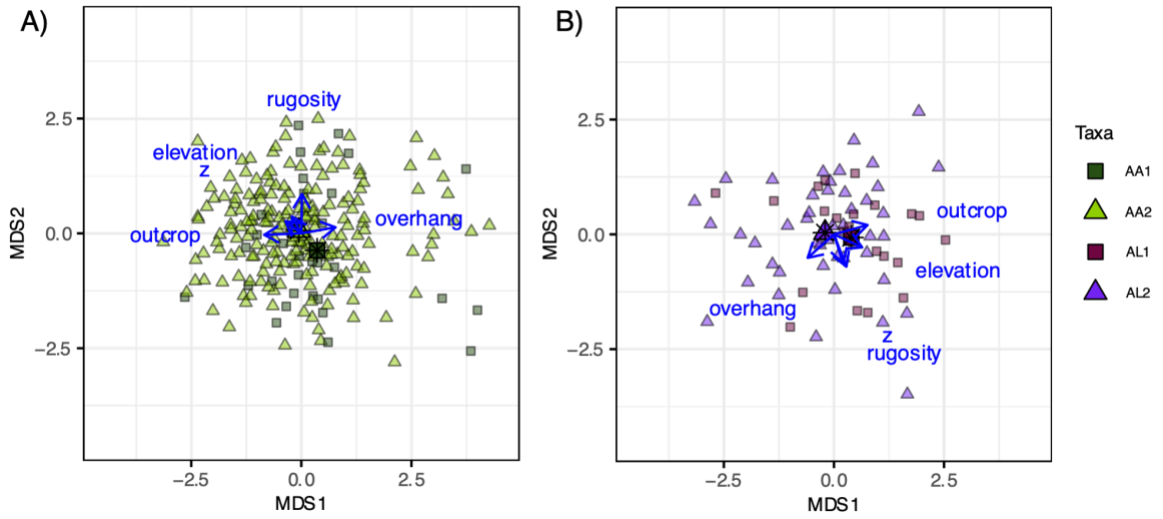


Figure 4.5. *Differences among the multidimensional environmental niches of cryptic taxa. A) A. agaricites and B) A. lamarcki*

4.5 Discussion

Genetically divergent groups that are morphologically similar and occur sympatrically are commonly observed in population genomic studies of corals (Bongaerts, Cooke, et al., 2021; Combosch & Vollmer, 2015; Cooke et al., 2020; Matias et al., 2022). Often, these groups are somewhat partitioned by depth but simultaneously exhibit large depth overlaps. At depths where these cryptic taxa co-occur, they may partition across microhabitats due to competition or local-scale environmental filtering, but this remains largely untested. In the present study, I assessed differences between the physical microhabitats occupied by species and taxa of *Agaricia*. The *A. lamarcki* vs. *A. agaricites* taxa often followed predictions, where the deeper-occurring species (*A. lamarcki*) was more commonly found in microhabitats that are presumed to have less light than the shallower species (*A. agaricites*). Small differences in the multivariate microhabitat space were found between cryptic taxa within *A. agaricites* and *A. lamarcki* where the differences among *A. agaricites* taxa followed our predictions (similar to the comparisons of *A. agaricites* vs. *A. lamarcki*). These results demonstrate the utility of structure-from-motion photogrammetry in extracting physical attributes of the reef and tying them to biologically relevant processes, such as the microhabitats preferences of species and cryptic taxa. When considering the multidimensional environmental niche, I show that cryptic coral taxa occupy slightly divergent microhabitats where they co-occur, and thus environmental heterogeneity across fine scales in coral reefs may promote divergence and niche diversification in coral reef organisms.

4.5.1 Depth partitioning among taxa

Depth represents a steep environmental gradient and many marine organisms including corals harbour closely-related species, or sister taxa that are incompletely partitioned by depth (Bongaerts, Cooke, et al., 2021; Bongaerts et al., 2011; Carlon & Budd, 2002; Johnston et al., 2022; Knowlton, 1993; Prada & Hellberg, 2013, 2020; Rippe et al., 2021). But most important for the basic energy requirements of photosynthetically-dependent corals is the light environment (Veron, 1995), and light intensity can vary dramatically at a particular depth within reefs due to shading (Vermeij & Bak, 2002). The three species within the current study, *A. humilis*, *A. agaricites* and *A. lamarcki* exhibit unique but overlapping depth ranges: with *A. humilis* found at the shallowest depths (2 – 10 m), *A. agaricites* found at moderate depths (5 – 50 m) and *A. lamarcki* found at the deepest depths (10 - 50 m) (Figures 3, 4; Bongaerts et al., 2013).

Such depth partitioning may be also occurring between the cryptic taxa within species. The AA1 taxon was most abundant at 20 m (although still less abundant than AA2 at this depth) and very rarely occurred at 10 m despite AA2 being abundant ($\chi^2 = 562.48$, df = 12, $p < 0.001$, Figures 3, 4). The depth range of *A. agaricites* extends much deeper than the depths sampled (~50 m, Bongaerts et al., 2013), and thus AA1 could be near the edge of their range at 20 m. Consequently, AA1 possibly occurs more abundantly at deeper depths, where then AA2 becomes less common. Several subspecies related to different growth forms of *A. agaricites* have been suggested, one of these previously including *A. humilis* (previously *A. agaricites humilis*) (van Moorsel, 1983) but none of these growth forms associated with the two taxa. Within the present study AA1 (the deeper taxon) had green mouths and AA2 had mostly orange mouths, a colour difference that was also observed by van Moorsel (1983). van Moorsel (1983) also noted that green-mouthed colonies were more abundant at 30 m, which corresponds with our prediction that AA1 would be more common at deeper depths. Such colour differences may relate to particular fluorescent proteins which are known for modulating light, either acting as photoprotectants to prevent damages or as amplifiers for photosynthesis (Vermeij et al., 2002), and thus could provide further evidence that taxa are differentially adapted to particular light environments. Further work could look into this character to test whether it does sort these taxa. However, caution should be applied, because gene families of this character show polyphyly among *Agaricia* spp. (*e.g.*, *A. agaricites*, *A. fragilis* and *A. lamarcki*) (Meyers et al.,

2013) and particular fluorescence profiles are not successful in determining phylogenetic relationships among *Madracis* spp. which also partition across the depth range (Vermeij et al., 2002).

Regarding the depth partitioning among *A. lamarcki* taxa, AL1 was previously found to be more common at mesophotic depths compared to AL2, with also AL2 being more common than AL1 at shallow depths (Chapter 2). Following this pattern and within this study, AL1 was less common than AL2 at shallow depths at both 10 and 20 m (Figures 3, 4). In *A. humilis*, one taxon AH1 was found at 20 m, whereas the other two were not, however no obvious depth partitioning was found among AH2 and AH3 (Figures 3, 4). Thus, due to the observed depth partitioning amongst species and cryptic taxa, physiological differences related to adaptations for particular depth environments may guide divergent microhabitat preferences where they co-occur, and this may be related to particular environments representative of the depths where they are most abundant, such as light or water flow environments.

4.5.2 Physical microhabitat differences among taxa

The microhabitat parameters within the study were chosen to reflect potential relationships with light, flow, and sedimentation environments, which have all shown to be important to corals (Goulet et al., 2019; Kaandorp et al., 1996; Rogers, 1990). Light is likely the most important environmental parameter for photosynthetic corals due to its attenuation with depth (and observed differences in depth distributions of taxa) and variations of it between more exposed and shaded environments within the same depth can be easily predicted. However, water flow and sedimentation may have more complex relationships with physical structure of particular reefs depending on local hydrological and geological conditions which may not be easily predicted. Thus, most of the interpretations of the results relate to the light environment, but it is important to note that other environmental or biological factors may be responsible for the microhabitat differences found.

Coral species that are highly abundant at mesophotic depths are often found at shallow depths (Laverick et al., 2017; Muir & Wallace, 2016) and when they are found in shallow locations, they appear to prefer shaded microhabitats (Dinesen, 1983; Kramer et al., 2019; Muir et al., 2018). At 20 m, *A. agaricites* and *A. lamarcki* co-occur and inhabit varied microhabitats and these microhabitats are likely related to different light environments (See Tables 4.1, 4.2,

Figure 4.4). Taxa of *A. lamarcki* (mostly AL2) are found at a lower local height, lower on an outcrop, in an environment with less rugosity, and have a higher overhang proportion than *A. agaricites* taxa (mostly AA2). This pattern was the same for the cryptic taxa within *A. agaricites*, (*i.e.*, the deeper-occurring taxon vs. the taxon occurring at all depths sampled followed the same directional differences as between *A. lamarcki* vs. *A. agaricites*), although only statistically significant when comparing multidimensional environmental differences (Table 4.3, Figure 4.5). The environment at the bottom of an outcrop within the present study is a local low point, the surrounding environment has more sand (*i.e.*, lower rugosity) and this environment is more likely to be overshaded by surrounding structure and thus will likely constitute a lower light environment. Larval choice for different surfaces has been demonstrated between *A. lamarcki* (prefers underside of tiles) and other unidentifiable *Agaricia* sp. and *A. agaricites* (prefer topside of tiles) (Vermeij, 2006), demonstrating adaptive larval habitat selection (also likely related to light preferences). Such behaviour reduces capacity for direct competition amongst these species during recruitment and thus habitat preferences could be in part driven by competition. The predictable differences in microhabitat parameters among species and partially among cryptic taxa are intriguing and pave the way for more accurate quantification of particular habitat preferences created by the physical structure of reefs.

4.5.3 Other patterns and considerations

Regarding the parameters that did not follow the predictions and the finding of no significant microhabitat differences among *A. humilis* taxa, there are a few *post hoc* explanations to consider. The parameter that did not follow the predictions within the species comparisons and between the *A. agaricites* taxa was the substrate angle, which was higher for shallower taxa (*A. agaricites* taxa and AA2) than for deeper taxa (*A. lamarcki* taxa and AA1), but this may be due to the high complexity of structure surrounding *A. agaricites* colonies at the top of the outcrops (likely other *A. agaricites* colonies and other benthic organisms) which deviations may not reflect the light environment. Especially since *A. agaricites* often forms bi-facial plates that grow vertically, the angle of the substrate may be of less relevance to the light environment it experiences. Additional to light being important, bifacial forms of *A. agaricites* colonies (at 20 m) have been found to face the dominant direction of flow (Helmuth & Sebens, 1993) and thus, the flow environment must be important for *A. agaricites*, and so relatively high points on the reef may provide better exposure to flow.

Regarding comparisons among *A. lamarcki* taxa, the differences in their multidimensional niche did not follow our predictions, for all parameters except one, the substrate angle. The substrate angle is likely more important for *A. lamarcki* taxa than *A. agaricites* taxa, as *A. lamarcki* colonies form unifacial plates and thus mostly receive light from one direction. However, this parameter made only a small contribution to the multivariate differences (Table 3, Figure 6). Differences within the microhabitat parameters tested may have been difficult to observe, given that *A. lamarcki* taxa were already occupying "lower light" microhabitats compared to *A. agaricites* taxa. Physiological changes of *A. lamarcki* have been found to scale with depth at one location but at a different location, colonies at deeper depths had similar physiologies to those at shallow depths (Laverick et al., 2019). This study suggests that colonies of *A. lamarcki* may be able to either undergo physiological changes (*i.e.*, switch to less heterotrophy) or occupy particular microhabitats to live at shallow depths while expressing the same physiology as it would at mesophotic depths. However, the cryptic taxon status was not incorporated into this study and thus these differences could be physiological differences among taxa (*i.e.*, one taxon harbouring a physiology adapted for mesophotic depths and the other preferring higher light). Potentially, a more vertical surface on the side of an outcrop may constitute a lower light microhabitat than lower on an outcrop. Incorporating light measurements of these microhabitats would help further verify such interpretations.

Low sample size for *A. humilis* taxa resulted in low statistical power to detect differences among taxa. This was due to a few reasons, (1) *A. humilis* colonies had a higher total abundance to *A. lamarcki* colonies but was split into three taxa (Appendix 1), (2) there were 16 individuals that were admixed between AH1 and AH3 that were not included in the analysis (Appendix 1), and (3) detection of *A. humilis* colonies within the photogrammetry plots was difficult due to their small size, or because the turnover of colonies was high. Regarding reason (3), because the plots were annotated one year after sampling, some of the colonies may have died. Since *A. humilis* has been described as an opportunistic species, able to reproduce all year long, and that it resides in a highly disturbed habitat and only grows to small sizes (van Moorsel, 1983), the turnover of colonies may be higher than for *A. agaricites* and *A. lamarcki* taxa. Observing this substantial loss in *A. humilis* colonies after one year and the high inbreeding levels (Chapter 3) corresponds with other observations of its decline across the Caribbean, predictions of susceptibility to further decline and the resulting critically endangered status on the IUCN threatened species list.

4.5.4 Co-occurrence of species and cryptic taxa

Whether the observed microhabitat differences amongst species and taxa are mostly driven by microhabitat partitioning in order to avoid competition (Blanchet, 2000), or by their physiological requirements is unknown. Competition during juvenile recruitment has been specifically observed to be high for these species within Curaçao, indirectly from generally competitive sponges or algae (Vermeij, 2006). But, before these competing explanations regarding niche differentiation can be evaluated, a pattern of niche differentiation needs to be established, as I have endeavoured to test here.

The differences in measurable niche space that we detected were relatively small. It is possible that our quantifications of species' microhabitats, while more comprehensive than previous attempts, failed to capture some important aspects of species' niches, such as other habitat parameters (*e.g.*, a shade parameter), trophic differences, or biotic interactions. In plants, variations at the microscale in hydrological conditions, pH, or altitudinal relief provide habitat partitioning (Ball et al., 2020; Bergholz et al., 2017; Musker et al., 2021) among other biotic components such as soil microbes (Luo et al., 2018). Frequent disturbances may also obscure observable niche differences or enable partitioning through varied responses (Shmida & Ellner, 1984). Disturbances allow chance colonisations of less competitive recruits and once a colony is established it may experience weaker competition (Vermeij & Sandin, 2008). Mass effects, where large stocks of nearby populations from one habitat are able supplement new recruits into other habitats via dispersal (Shmida & Wilson, 1985) may be strong in this system given the importance of the depth gradient. Specifically, the co-occurrences of different taxa may be promoted by a constant supply of new recruits from nearby populations at different depths. This effect results in local diversity being somewhat inflated along habitat transitions (*e.g.*, field edges in terrestrial systems, Metcalfe et al., 2019), especially when dispersal is high between habitats, and it implies that not every colony is perfectly adapted to where they are found. Whether co-occurrence is driven by reductions in competition or abiotic processes is difficult to decipher. However, such cryptic taxa are likely share similar ecosystem function, and their existence may promote functional redundancy in the face of disturbances (see Burgess et al., 2021). Understanding physiological differences among cryptic taxa is important, as it may drive different patterns in their responses to climate change.

4.5.5 The structural environment of coral reefs

Structure-from-motion photogrammetry allows measurements of the physical structure of reefs at a spatial scale that is relevant to community-level patterns (Price et al., 2019), fish species (Eggertsen et al., 2020; González-Rivero et al., 2017; Urbina-Barreto et al., 2022), other coral reef invertebrates (Montalbetti et al., 2022; Prado et al., 2020) and as shown in the present study, the microhabitats of corals. Coral reefs spout highly diverse and productive communities and one of the reasons suggested for such high biodiversity is the availability of microhabitats (Birkeland, 2015). The parameters used within this study aimed to describe some of these microhabitats that are suggested to promote species diversity. Extracting aspects of the structural environment that are important to coral colonies is difficult for many reasons, outlined here: (1) knowing the spatial scale of the microhabitat that is important to the colony, (2) knowing which parameters are relevant or which are noise, (3) whether the parameter is sufficiently quantifying the physical structure as intended and (4) defining the multidimensional world effectively and efficiently (*i.e.*, avoiding under and over parameterisation). Of course, this is true in all systems, but this study is the first that I am aware of to attempt to use photogrammetric 3D reconstructions for defining individual microhabitats for corals and thus there are no standards to follow. Whether colonies are on horizontal or vertical surfaces explained the different niches of *Madracis* spp./eco-morphs (Vermeij et al., 2007), and thus measures of the substrate angle are important for those *Madracis* spp. colonies. Here, substrate angle was defined by points surrounding the colony, but this measure may contain some noise, as it was not possible to determine whether some of these points were other benthic organisms or the substrate to which the colony was attached. It is also likely that the radius surrounding the colony chosen here (0.2 m) may be too small to capture outcrops. During sampling and annotating the colonies within the plots, it was obvious that *A. lamarcki* colonies were generally at the bottom of outcrops and in shaded locations. The overhang proportion was sufficient for finding any structure that was directly above a colony, but other structures may also shade colonies when the sun is not directly overhead. Developing a "shade" parameter that incorporates the proportion of objects that shade the colony would be useful. Future work will increase this radius size to compare microhabitats at different spatial scales and add a shade parameter.

4.5.6 Conclusions and future directions

My results potentially provide the first quantitative insight into the structural environment surrounding coral colonies using photogrammetry and verify subtle differences in the microhabitat niches of species and cryptic taxa. Light is an important environmental parameter for coral holobionts and understanding the specific light requirements for different species will inform their physiological tolerances. Adding light measurements across the photogrammetry plots are necessary to verify that differences in microhabitats were because of light and substantiate the hypotheses that light environments enable co-occurrence of depth differentiated taxa at the same depths presented here. Given the prevalence of observations of thermally bleached colonies belonging to the same species and being spatially proximate (Burgess et al., 2021; Cunning et al., 2016; Durante et al., 2019; Forsman et al., 2020; Gómez-Corrales & Prada, 2020; Rose et al., 2021; Yee et al., 2008), it is important to understand whether this is due to (1) differences in tolerance levels among individuals in the same population (essential for predicting the adaptive capacities of populations), (2) different cryptic species or (3) microhabitat differences. Here I show that cryptic taxa can differ in microhabitat niches, supporting the idea that bleaching outcomes among colonies may be affected by taxon identity or small-scale environmental differences. Furthermore, understanding how divergent groups segregate by microhabitat, can ultimately help active restoration efforts by guiding selection of genotypes to outplant and where exactly to plant them. Transplant and larval studies should be conducted to verify whether selection is acting at the microhabitat scale (*e.g.*, opposing performance levels between taxa in different microhabitats and non-random selection of microhabitats by larvae). Improving these microhabitat parameters and understanding the effects of microhabitats is of great relevance to coral reef researchers interested in understanding niche partitioning, but also to researchers and managers wanting to quantify physiological requirements, adaptation (*in situ* by ruling out environmental effects) and guiding effective restoration.

4.6 Supplementary materials

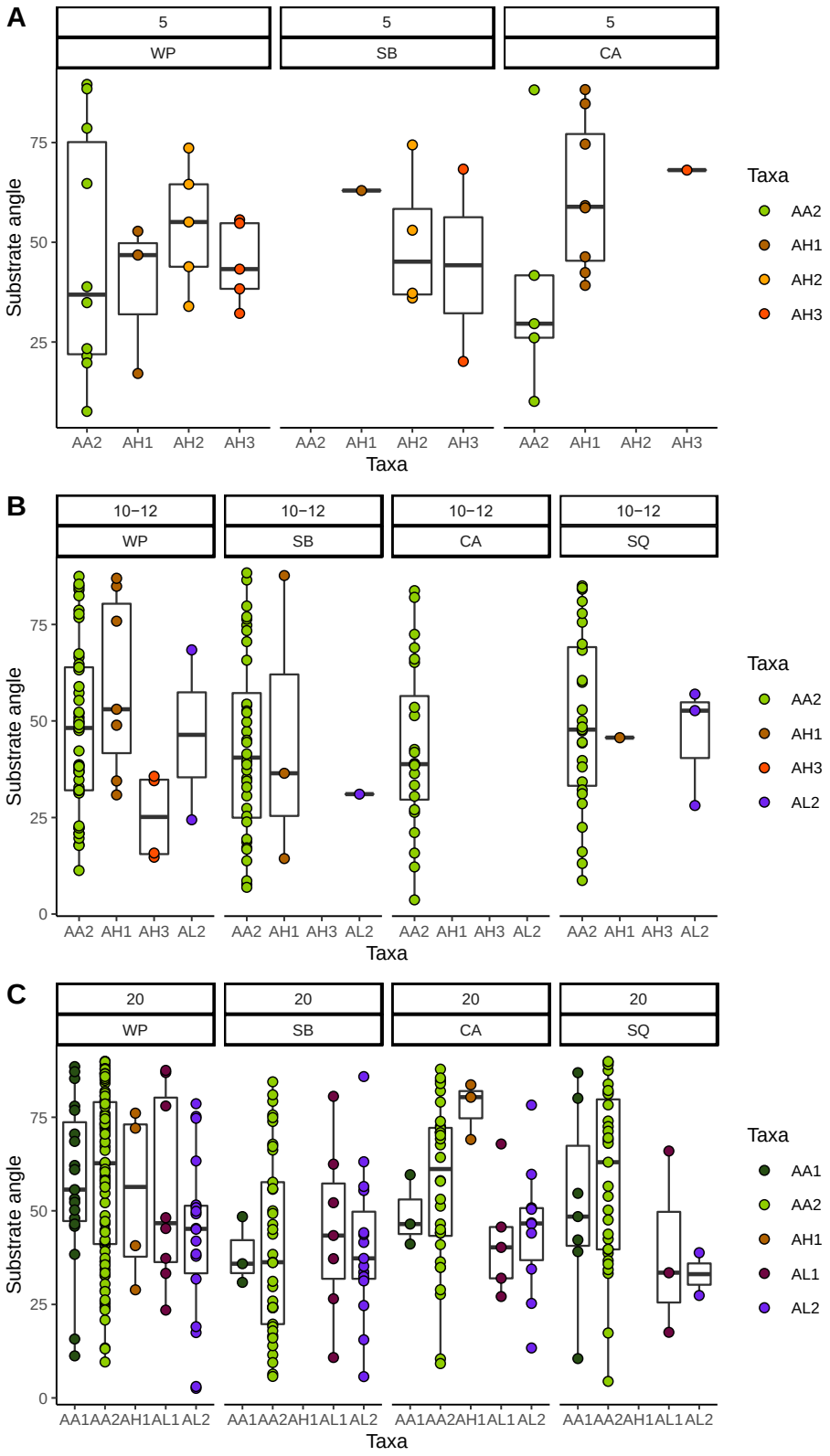


Figure S4.1 *Individual colony microhabitat measures of the substrate angle for all Agaricia taxa at 5, 10-12 and 20 m at the four locations in Curaçao (WP, SB, CA and SQ)*

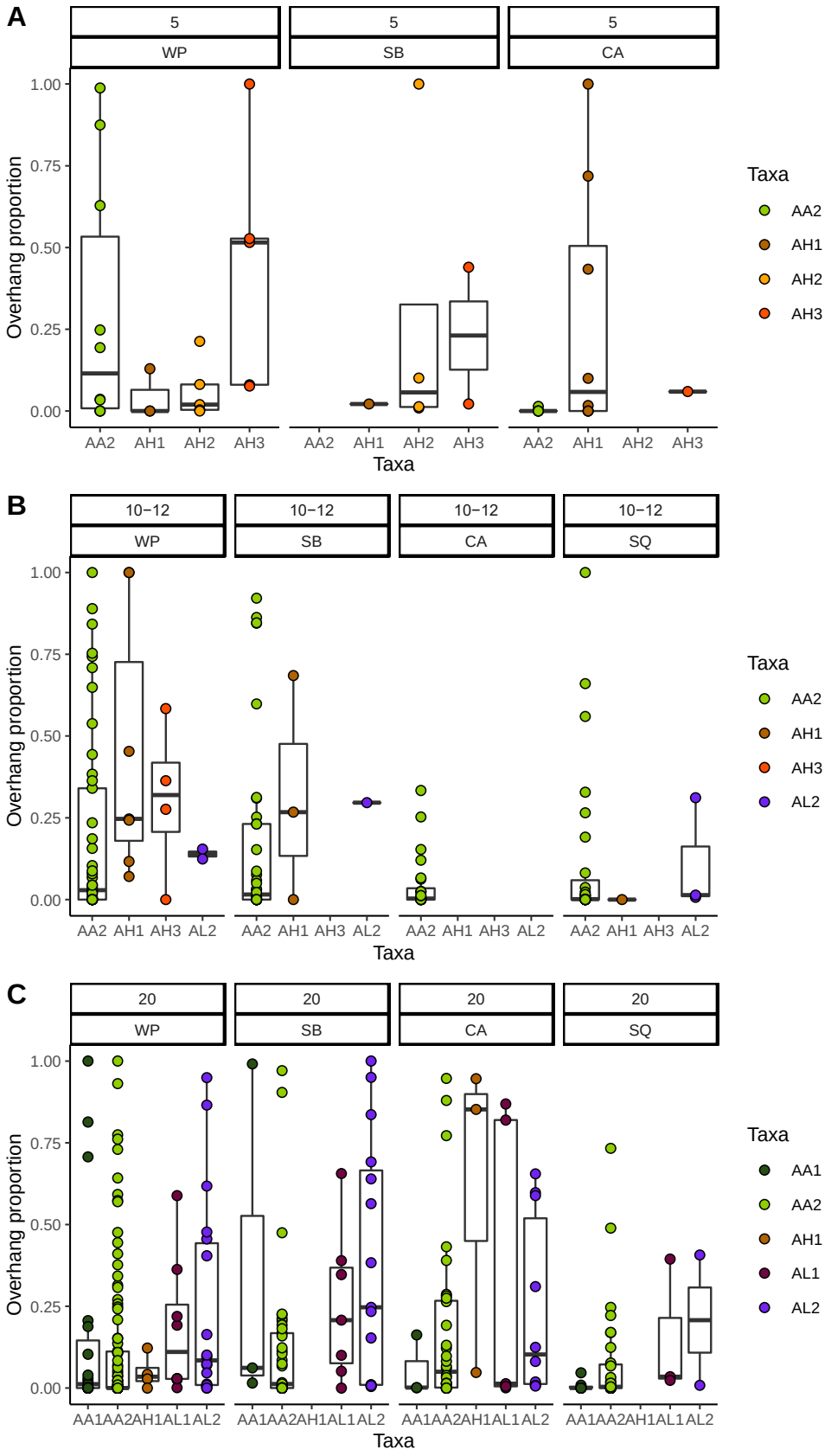


Figure S4.2 *Individual colony microhabitat measures of the overhang proportion for all Agaricia taxa at 5, 10-12 and 20 m at the four locations in Curaçao (WP, SB, CA and SQ)*

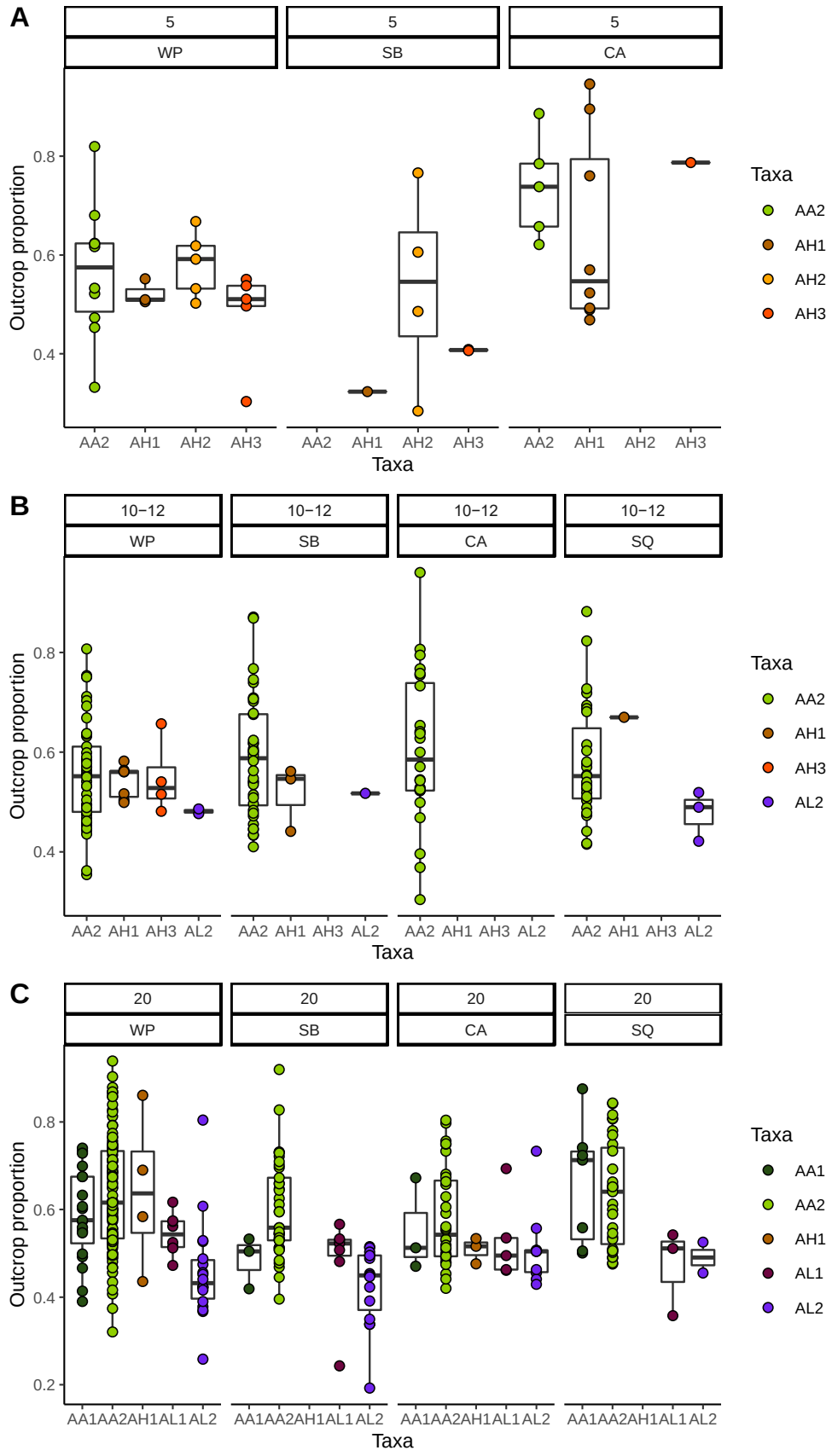


Figure S4.3 *Individual colony microhabitat measures of the outcrop proportion for all Agaricia taxa at 5, 10-12 and 20 m at the four locations in Curaçao (WP, SB, CA and SQ)*

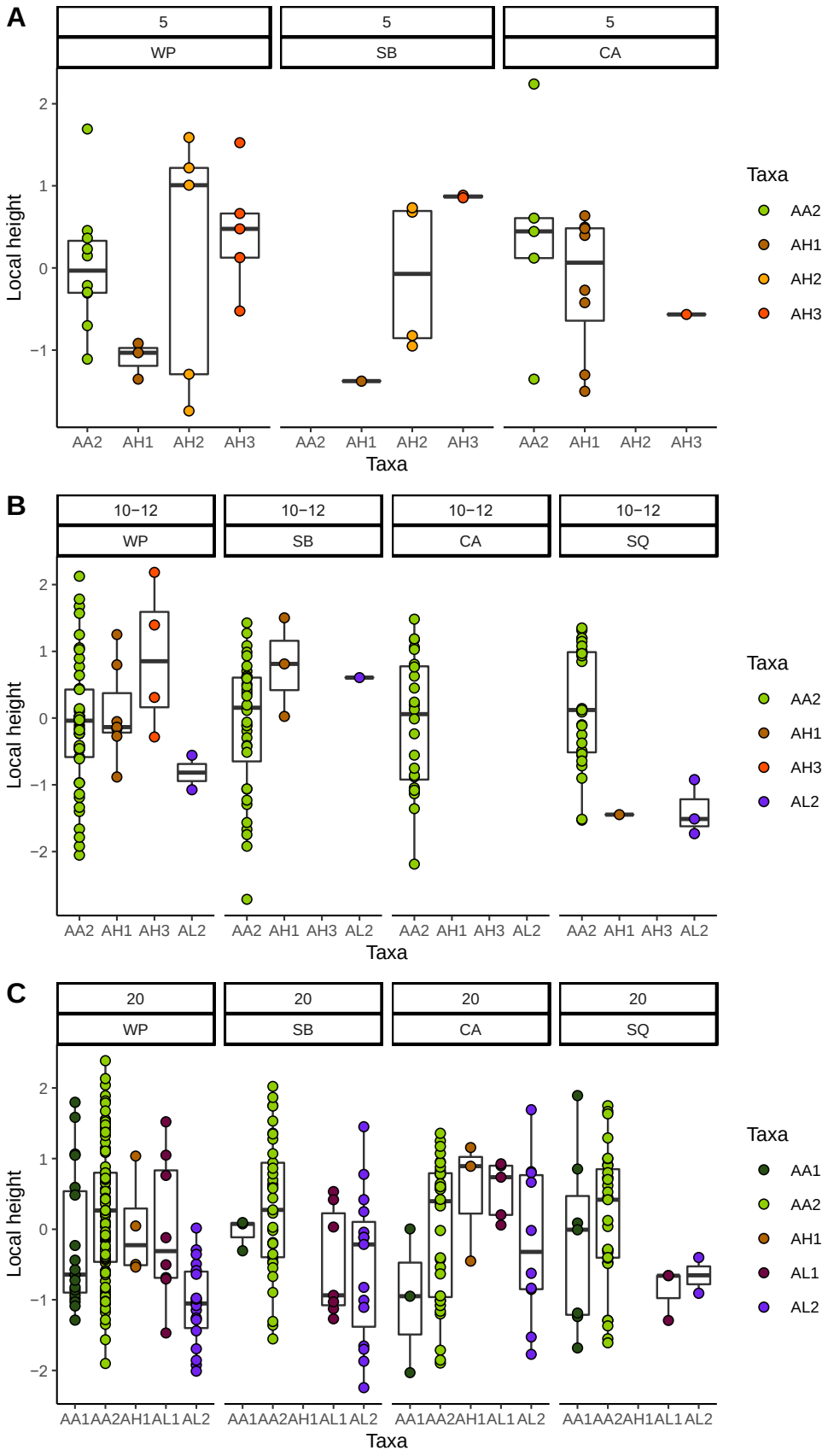


Figure S4.4 *Individual colony microhabitat measures of the local height for all Agaricia taxa at 5, 10-12 and 20 m at the four locations in Curaçao (WP, SB, CA and SQ)*

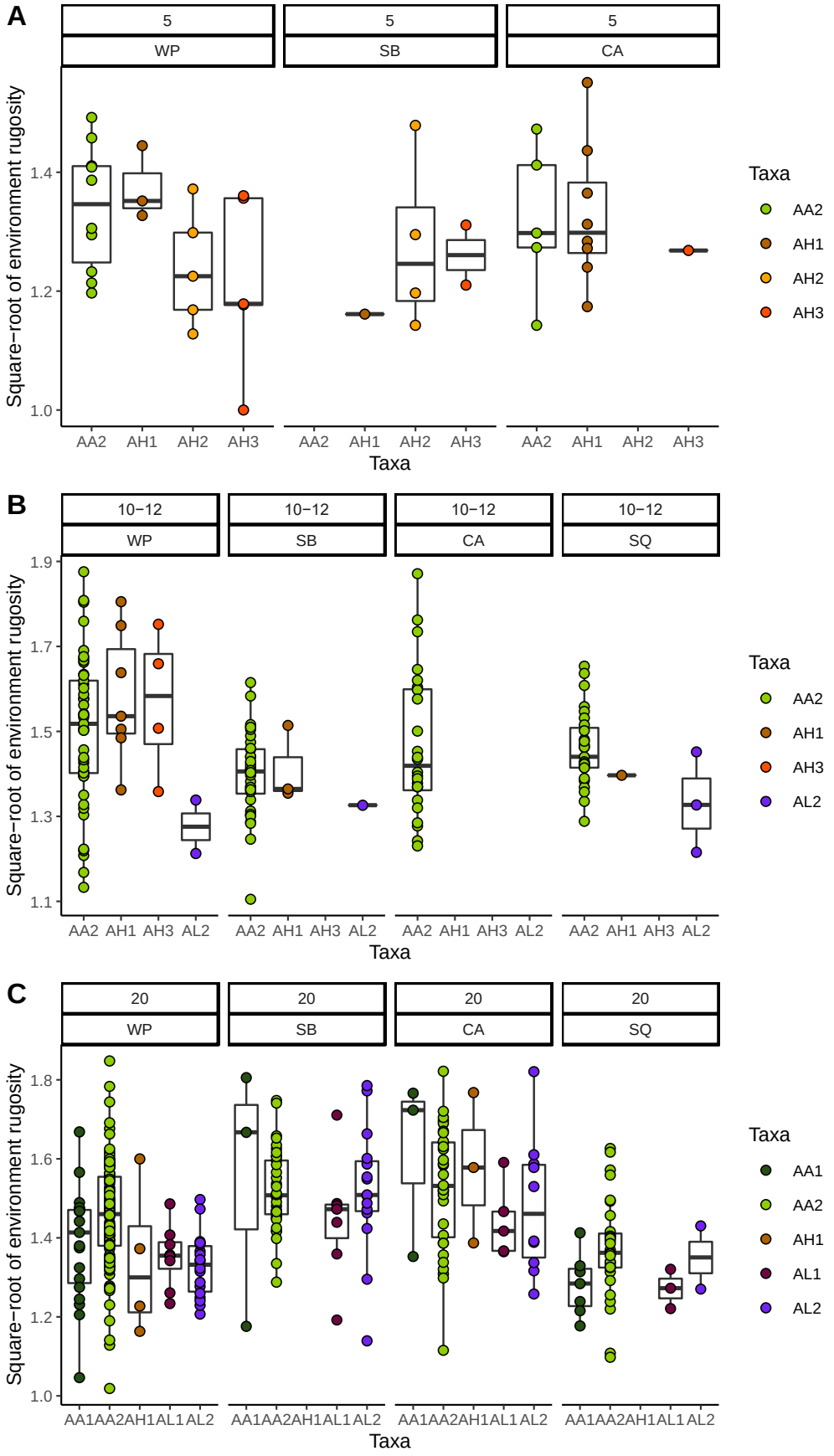


Figure S4.5 *Individual colony microhabitat measures of the environment rugosity for all Agaricia taxa at 5, 10-12 and 20 m at the four locations in Curaçao (WP, SB, CA and SQ)*

Chapter 5: General discussion

Scleractinian corals are the architects of tropical reefs. They provide habitat for potentially one million known species across most phyla (Fisher et al., 2015) and with close to one billion people now living within 100 km of coral reefs (Sing Wong et al., 2022), their persistence is important. Sadly, coral populations continue to decline at disturbing rates due to local and global scale impacts (Eddy et al., 2021). With new technological tools, we can bring the study of coral reef organisms into the modern era. Next generation sequencing, once inaccessibly expensive is now giving us unprecedented insights into the genomes of wild populations, exposing hidden diversity. Complementing DNA technology advances, structure-from-motion photogrammetry generates time unlimited access to coral reefs. Through creating virtual representations of the reef, we can map individuals onto reefs and explore their three-dimensional world. Very few people across the globe get to experience the fleeting sensations of diving on coral reefs and those who do wish for more time, especially those of us that perform underwater research. More time is required to understand how assemblages change across the reef slope, to observe which individuals live where and who they live next to. This information can help answer longstanding and important questions in coral research, such as, what are the processes that create or maintain cryptic taxa? And how far do coral larvae disperse?

While there is still a long road ahead in harnessing the true power of photogrammetry, my thesis provides unique examples on how it can be coupled with genomics through linking high resolution spatial, environmental, and genetic data to infer demographic and evolutionary patterns within coral populations. In my thesis, I used these two technologies for comparative study of species in the genus, *Agaricia*. My thesis adds to the body of literature documenting an abundance of morphologically cryptic taxa hidden within known species; that gene flow occurs interspecifically; that depth or perhaps environmental parameters associated with depth are likely to partition taxa; that dispersal in some coral taxa is highly limited (across metres); and lastly, that within reefs, microhabitats could provide niches for taxa to co-occur.

5.1 Analyses and inferences enabled by reefscape genomics

Combining reduced representation genomic sequencing methods and structure-from-motion photogrammetry enabled me to make inferences into cryptic taxa, hybridisation, historical gene flow, dispersal, and depth- and microhabitat- niche partitioning in *Agaricia* corals. Reduced representation sequencing and other genomic-level methods provide both sufficient intraspecific and interspecific diversity to undertake comparative population studies on species. Whereas microsatellites demonstrate high intraspecific genetic diversity for elucidation of spatial genetic patterns, due to homoplasy, null alleles, and the few loci available, they add ambiguity into datasets with complex or subtle patterns (*e.g.*, cryptic taxa, hybridisation, recent isolation). In contrast, genomics offers greater resolution, and through implementing reduced representation sequencing in both Chapters 2 and 3, I was able to delineate cryptic taxa and discover backcrossed hybrids (Chapter 2) or highly admixed individuals (Chapter 3). Cryptic taxa were treated separately, and potential hybrids were removed, to discover continuous spatial patterns (Chapters 2 and 3) and subtle differences in microhabitat niches (Chapter 4). Questions about the nature of divergence between sister taxa are widespread in the coral literature (*e.g.*, whether gene flow occurred initially, Prada & Hellberg, 2020). Harnessing genome-level variation in Chapter 2, I was able to characterise the allele frequency spectrum and perform demographic analysis (*e.g.*, dadi, Gutenkunst et al., 2009) to assess gene flow scenarios amongst taxa and discovered divergence histories with periods of gene flow across all taxon pairs (*i.e.*, both between taxa within species and across species). My thesis has demonstrated that genomic methods have incredible potential to resolve once pervasive issues within corals (*e.g.*, cryptic taxa and relationships amongst closely-related groups) and determine gene flow across space and between taxa at both ecologically and evolutionary relevant scales.

Implementing spatially-explicit individual-based analyses can be achieved through the pairing of reduced representation sequencing and photogrammetry. Dispersal is a highly important parameter for determining the connectivity of populations. In corals (and other marine taxa), because we cannot directly track their larvae, indirect genetic methods are used to infer dispersal: population structure (*e.g.*, F_{ST} or Assignment methods), assigning new recruits to adult populations (*e.g.*, Assignment methods), spatial autocorrelation methods (*e.g.*, Moran's *I*), isolation-by-distance, or demographic modelling (*e.g.*, coalescent or diffusion methods). Both genetic structure-based analyses and demographic modelling are for measuring gene flow

over longer timescales than is what is relevant to contemporary dispersal regimes. However, spatial autocorrelation and isolation-by-distance can measure more recent dispersal (across ~10 generations) and estimate the variance of dispersal (σ^2). In Chapter 3, through exhaustively sampling colonies within a given area and mapping these virtually, I demonstrated fine spatial scale dispersal could be estimated (with dispersal variance, σ^2 = 3 – 25 m). Estimation of dispersal variances required an independent individual density measure that can either be calculated through census numbers (enabled by exhaustive sampling of areas) or more appropriately estimates of the effective population size (N_e). Kinship was a great complementary analysis as it provided estimates of N_e and confirmed dispersal estimates through finding localised kin (including siblings). Discovering three parent-offspring pairs in Chapter 3 is an exciting result because few studies have been able to directly estimate dispersal (but see Dubé et al., 2020), and sampling was only conducted across small areas (50 m^2) per depth x location. Potentially, exhaustive sampling of larger areas could detect more cases of direct dispersal, like the incredible attempts by Jones et al., (1999) where tagging large numbers of fish larvae enabled insights into self-recruitment and the relative composition of migrants and locals, even in species with highly dispersive larvae. In some coral populations, asexual reproduction can be high (see Baums, 2008), and thus it is important to assess genotypic diversity and what the spatial scales of clonality are for many coral species. Particularly since in both broadcast spawners and brooders, the time window of fertilisation is small due to dilution and so the distance that sperm (or both gametes) is able to travel is limited. Thus, large areas of low genotypic diversity could reduce sexual reproduction rates. In Chapter 3, clonal analysis enabled by the sampling design confirmed the maintenance of genotypic diversity across fine scales despite low genotypic richness in *A. agaricites,* and thus measuring genotypic richness should be conducted at the scale of gamete dispersal to predict the effects that clones have on sexual reproduction. In summary, in this thesis, I show that spatially-explicit study designs enabled by photogrammetry can provide inferences into the understudied fine spatial scale patterns of coral populations, including ecologically relevant dispersal of sexual and asexual propagules.

Photogrammetry uniquely enables quantification of the physical environment. In Chapter 4, I applied this technology in a novel way to describe the microhabitats surrounding individuals and found subtle differences amongst species and cryptic taxa. Microhabitats have been described before, such as the particular preferences of *Madracis* spp. with regards to surface

orientation at different depths (likely related to light preferences) (Vermeij & Bak, 2002). However, photogrammetry greatly improves our ability to accurately describe these microhabitats in three dimensions and provides many possibilities in quantifying different parameters. In Chapter 4, differences in microhabitats were predicted to be related to different light environments; but, only subtly differentiated cryptic taxa when considering the multidimensional environment. Potentially only small differences in niches are required for co-existence due to intermediate disturbance levels of coral reefs and large sources of the less competitive taxon nearby; or other parameters may better explain their niches. So little is known about the environmental niches of different coral species (especially cryptic taxa) or other benthic organisms in coral reefs and my thesis explores this using a unique method. Tying structural complexity measures extracted from photogrammetry to environmental variables (*i.e.*, light, water movement, temperature, nutrients) will bring greater understanding of how the physical environment is proximally affecting organisms. Additionally, assessing whether biotic interactions predict species distributions is possible using photogrammetry. Thus, further application of this technology could help elucidate the processes responsible for the coexistence of the high numbers of species across fine spatial scales in coral reefs and as well as the physiological limits of taxa *in situ*, which is important for their conservation given environmental changes.

5.2 Cryptic taxa delimitation using the genotypic cluster definition

There are many differing opinions on species concepts, which all agree when 'good' species are found as they exhibit full reproductive isolation and have markedly different phenotypes and ecology. But, when examining sister groups that have recently diverged and have incomplete reproductive barriers, there is disagreement.

Throughout this thesis, I have employed the logic of the 'genotypic cluster' definition (Mallet, 1995) in order to study relevant demographic processes occurring within groups and assess whether these groups are ecologically divergent to elucidate a reason for their divergence. Taxa are defined as individuals that form genetically distinct groups using standard population genomic analyses (*i.e.*, PCA and Admixture) and are differentiated across the genome yet are sympatric at multiple locations (*e.g.*, axis of differentiation between divergent and sympatric groups is greater than any geographic axis of differentiation) (see Appendix 1). Separating the

taxa this way, for *A. agaricites* and *A. humilis*, I detected signals of isolation-by-distance within all taxa (Chapter 3) and thus this organisation appears relevant for measuring neutral demographic processes occurring within groups (*e.g.*, dispersal). Not only was this assignment relevant for measuring demographic patterns but most taxa pairs within species (except AH2 and AH3) partition by depth (Chapters 2 and 4) and potentially microhabitats (unclear for *A. humilis* taxa, Chapter 4), suggesting that they are ecologically divergent, and thus there is evidence for selective mechanisms driving or maintaining divergence. Taxa are described in this thesis as morphologically cryptic and at least at the macromorphological scale, there do not appear to be any differences among the taxa and so it might be difficult to identify these taxa without genetic analysis. However, further investigation of the micromorphological characters may reveal differences.

The term ecotype, first coined 100 years ago in 1922 by Gröte Turesson, describes ecologically distinct populations of the same species. The taxa described in my thesis could be considered ecotypes due to their ecological divergence but given they are divergent across the genome (not at a few select loci like other ecotype examples, *e.g.*, sunflowers, Andrew & Rieseberg, 2013), they may be further along the speciation road than is typical for "ecotypes". Thus, the question arises, when should these groups be considered different species? Perhaps, a more compelling case for species assignment would be if these same taxa were found across each species' range. In Chapter 2, *A. grahamae* taxa were found at locations >1,000 km (where spatial decay within groups was present) and a phylogenetic assessment by Gijsbers et al (2022) has revealed further cryptic diversity within *A. grahamae* and but for *A. agaricites, A. humilis* and *A. lamarcki* the sampled geographic and depth ranges were limited. Future studies should analyse more samples of these species across the Caribbean (and across relevant depth gradients) to assess their geographic distributions. Ultimately, for the interests of local preservation of these taxa and their populations, they require separate consideration (regardless of species status) for predicting demographic responses, as hybridisation does not occur frequently enough to provide demographic support.

5.3 Why are there so many cryptic taxa and what does it mean?

Finding cryptic taxa in population genetic studies of coral is common. This means that coral species diversity could be severely underestimated. In Chapters 2 and 3, two or three cryptic taxa were found in each taxonomic species. In some studies, up to five cryptic taxa have been found (Ladner & Palumbi, 2012). Especially at mesophotic depths, many species have not been targeted for genetic analysis and there appears to be undescribed genetic diversity within dominant mesophotic species (*i.e.*, *Agaricia* and *Leptoseris*, Gijsbers et al., 2022). Not only does this imply that some spatial genetic structure estimates could be wrong (*i.e.*, less structure among reefs and more structure among regions for studies that failed to account for cryptic taxa, Sheets et al., 2018), but that census numbers are smaller than they appear, and that taxa are more ecologically limited.

Depth is suggested as the main speciation driver in coral taxa (Prada & Hellberg, 2020), but depth is not an environmental parameter in and of itself but rather represents several (*e.g.*, light intensity and spectrum, temperature, nutrients, disturbance level etc.). Taxa that are less abundant at shallow depths may inhabit sheltered locations and be adapted to the environmental parameters that generally scale with depth (*e.g.*, *A. lamarcki* taxa compared to *A. agaricites* and *A. humilis* taxa and AA1 compared to AA2 in Chapter 4). Given that few studies incorporate mesophotic or different depth sampling in population genetic studies, nor do they record characteristics of the particular habitat that samples were taken from, there is potential that depth or microhabitat partitioning may be more common. The consequences of such, may mean that deep populations, while likely to escape shallow reef bleaching, may not be able to repopulate shallow populations (*i.e.*, refuting the Deep Reef Refuge Hypothesis for some taxa, Baird et al., 2018; Bongaerts & Smith, 2019) and if they do, they could be maladapted and prefer certain microhabitats. Importantly, there are many cryptic taxa where we do not know whether or how they are ecologically partitioned. In Chapter 2, two *A. grahamae* taxa were found at mesophotic depths with no apparent depth partitioning but greater sampling across the depth range is needed for confirmation. Yet in Chapters 3 and 4, there were two *A. humilis* taxa without depth separation and were unlikely to occur at deeper depths than sampled; thus, these taxa could be ecologically differentiated in different ways. Conducting transplant experiments for the differences in microhabitats observed within Chapter 4 (or if other differences are observed with improved quantification of microhabitats) would help to support the hypothesis

that these taxa are adapted to specific microhabitats. My thesis confirms that factors that scale with depth are the main drivers for differentiation in the genus *Agaricia*.

A few studies have demonstrated differing responses of cryptic taxa to bleaching (Burgess et al., 2021; Gómez-Corrales & Prada, 2020; Rose et al., 2021) suggesting that they may be ecologically divergent. If cryptic taxa may be assumed to be ecologically equivalent, generalised trait-based approaches for assessing populations and communities may not account for them (*e.g.*, Madin et al., 2016). Also, if certain cryptic taxa harbour thermally adapted alleles, this begs the question of whether they could share these alleles easily among other taxa. Chapter 2 indicated ongoing but low levels of gene flow among *A. lamarcki*, and *A. grahamae* cryptic taxa and Chapter 3 found many admixed individuals between two *A. humilis* taxa and thus hybridisation amongst taxa could enable adaptation if one taxon harbours adaptive alleles. While cryptic taxa are often assumed to be ecologically similar, my research supports others that they are likely not and this could have important consequences regarding adaptation.

Another consequence of cryptic taxa is in estimating their individual abundances and risk of extinction from population declines and inbreeding depression. In both Chapters 2 and 3, inbreeding was detected in all taxa but one. Although, it appears that marine invertebrates like some plants frequently inbreed (see Olsen et al., 2020) and therefore the low levels of inbreeding detected in *A. agaricites* and *A. lamarcki* taxa are likely not an issue, but the extreme values seen in *A. humilis* taxa are of concern given their populations are sharply declining and the status of *A. humilis* was recently updated on the IUCN Red List as being critically endangered. The occurrence of cryptic taxa will make census numbers appear higher and thus obscure the severity of population declines. Thus, regarding ecological niches (*e.g.*, depth or microhabitat specialisation), adaptation (*e.g.*, the nature of reproductive boundaries between differentially adapted groups), and demographic processes (*e.g.*, measuring important populations metrics: census numbers and inbreeding levels) considering cryptic taxa in studies and their impact for the future persistence of species is highly important.

5.4 Dispersal, the need for more data across species

Dispersal is a central process for both the study of populations and communities. Dispersal can provide demographic and evolutionary resilience to populations through the provision of individuals and adaptive genes from neighbouring populations (or a chain of linked populations). The ability to disperse far distances should form more resilient populations as more individuals are connected over large distances (greater N_E and spread of individuals across different environments). Dispersal across short scales supports local population replenishment and promotes local adaptation, thus local dispersal could support metapopulations of differentially adapted groups across a reefscape providing high adaptive diversity and local support (propagating significant cover over a local area and being able to support local recovery after a disturbance). However, when global environmental changes occur, the scale of disturbance is likely larger than the dispersal (or gene flow) ability of certain species. Populations connected by gene flow across large scales may have a better chance of adapting to new climates, for example the thermally tolerant alleles moving towards higher latitudes. Thus, species with capabilities of dispersal that can escape threatened environments and disturbances appear more likely to survive in the near future given the current climatic changes.

The extent of dispersal is unknown in many coral species. Many studies will report population genetic structure and estimates of $F_{ST,}$ but these measures could represent historical patterns, and when spatial autocorrelation or isolation-by-distance are used, σ^2 is rarely estimated (but see Gazulla et al., 2021; Gorospe & Karl, 2013). Measuring genetic correlations across relevant spatial scales (*i.e.*, 10σ to 50σ) are best practice, thus studies should implement continuous individual-level sampling at such scales or if unknown then to sample at multiple scales. Changes in effective population sizes due to demographic or ecological processes (*e.g.*, local extinction, unequal offspring numbers, breeding sex ratio, and fluctuations in size of breeding groups) affect the genetic structuring among individuals inconsistently across spatial scales as well as with dispersal dynamics and thus the dispersal variance estimate varies itself. However, estimating general scales of effective dispersal (*i.e.*, is it across metres or kilometres?) are important for understanding marine connectivity, and these scales are likely shorter than inferred from population-level statistics.

In Chapter 3, I was able to measure dispersal variance for *A. agaricites* and *A. humilis* cryptic taxa. However, I was not able to for *A. lamarcki* due to no spatial signatures (confirmed in both Chapters 2 and 3). Mesophotic-specialist, *A. grahamae* also presented no spatial signatures across a slightly larger scale (between islands) and thus both *A. lamarcki* and *A. grahamae* dispersal capacities are larger than the congeners. Such disparate dispersal patterns for species that are all considered brooders (although not specifically tested in *A. lamarcki* and *A. grahamae*), shows the need for considering each species dispersal capacities individually and comparatively. Expanding the spatial distance classes sampled for *A. lamarcki* and *A. grahamae* taxa could allow estimation of their dispersal variance. With climate changing across ecological timescales, migrants from different populations may provide replenishment and adaptive alleles for others and isolated reefs may need to be supplemented to prevent population collapse, especially with the demonstrated limited effective dispersal discovered in *A. agaricites* and *A. humilis* taxa (across metres). Measuring ecologically relevant dispersal is critical for predicting the responses of coral populations and thus should be measured across a variety of different species found on coral reefs.

5.5 The future of reefscape genomics

Structure-from-motion photogrammetry paired with genomic data has great potential to perform other kinds of analyses than what I have demonstrated. For example, repeated snapshots of the areas over time enable temporal studies (*e.g.*, Underwood et al., 2018) to examine changes in allele frequencies due to death following bleaching or disturbance events. Additionally, through capturing phenotypes (*e.g.*, morphology or bleaching responses), we can disentangle environmental and genetic influences on particular traits. In another advance, reference genomes, including chromosomal level, are being created for many coral species at an incredible rate, and thus applying whole genome sequencing across many individuals is possible. Using this resource, recent studies have looked at the polygenic nature of heat tolerance (Fuller et al., 2019). Combining photogrammetry with whole genome sequence could further our understanding of the genomic basis of adaptation and introgression by incorporating phenotypic, environmental, and spatial components extracted from the photogrammetry in standard genomic analyses. Application of reefscape genomics can help us understand crucial processes that impact the survival of coral populations.

5.6 Conclusions

The genus *Agaricia* harbours species that are abundant across the reef slope (*i.e.*, from shallow to mesophotic) and thus are ecologically important in the Caribbean. The occurrence of nine cryptic taxa within four recognised species more than doubles the estimated diversity within this group and suggests that perhaps many other unstudied coral species harbour undescribed taxonomic diversity. My research proposes that cryptic taxa are likely partitioned by depth and different microhabitats may enable the co-occurrence of cryptic taxa. Additionally, some cryptic taxa have incomplete boundaries to gene flow, which could be important sources of genetic diversity for declining or maladapted taxa. It is important to understand the consequences of cryptic taxa on groups of species and overall ecosystem resilience and consider them when observing declines. The shallow and mesophotic species within *Agaricia* appear to possess disparate dispersal capacities which will lead to varied responses, where taxa with only metre-scale generational dispersal are likely vulnerable to extinction given global climate change and thus these findings should encourage researchers to obtain more estimates of ecologically relevant dispersal across many species. My thesis has demonstrated the application of reefscape genomics for investigating fine spatial scale processes of coral taxa and elucidates key factors such as dispersal and the partitioning of niches amongst cryptic taxa.

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Appendix 1 – Species and taxa evaluation

This appendix presents methods used to delineate taxonomic species and cryptic genetic groupings within species for Chapters 3 and 4 where coral colonies of *Agaricia* spp. were sampled from fringing reefs of Curaçao (Southern Caribbean). Colonies were exhaustively sampled from plots of 25 m across slope by 2 m along slope situated within three depths: 5, 10-12 and 20 m at four sites from North – South of the leeward side of the island: West Point (Playa Kalki), Cas Abao, Snake Bay and Seaquarium (sampled at 12 and 20 m) corresponding to a total of 11 plots, each with 50 m^2 area. For clarity, current taxonomic species will be referred to as "species" and potential cryptic species will be referred to as "taxa". Taxa are defined as distinct groups of samples that are genetically distinct across the genome (not at just a few loci), contain non-admixed samples (based on assignment methods) and are sympatric (*i.e.*, do not associate with specific geographic locations, either allopatric or parapatric). As defined, these taxa can have a depth profile preference (*i.e.*, an environmental association) but this association must be consistent across sites to rule out spatial association over depth. If groups are associated with a spatial pattern, then the pattern is likely caused by dispersal limitation rather than intrinsic (genomic incompatibilities) or extrinsic (environmentally induced incompatibilities) barriers to gene flow. Thus, we take the conservative approach and say spatially associated groups belong to the same taxa. If groups are sympatric then they have the chance to reproduce (no dispersal limitation), and intrinsic or extrinsic barriers to gene flow are likely to exist. If there are no fully assigned samples, then the divergence between the two groups may not be sufficient to avoid homogenisation through mixing or groupings may be a statistical artefact. Although, statistical artefacts should be avoided by choosing an optimal K (explained below). It is possible that sympatrically occurring divergent samples are recent migrants from an unsampled population that have not yet amalgamated into the population. In this thesis I do not define taxa as biological species, but I use these taxa as groupings to account for confounding structure in neutral patterns for spatial analyses and genetic diversity statistics (Chapter 3), and to examine environmental associations with taxa for elucidation of ecological processes that may have helped to create or that maintain genetic divergence (Chapter 4).

Population genetic structure methods without a priori spatial or depth predictions were used to evaluate genetic groupings: Principal Components Analysis (PCA) and Admixture. I first examined all samples before separating datasets into species. I used morphology as a general guide for preliminary species assignment but relied on the genetic clusters to officially delineate species and taxa within. Morphological distinctions are obvious between *A. lamarcki* and *A. agaricites/A. humilis* and between some growth forms of *A. agaricites* (bifacial) and *A. humilis*, but small colonies of *A. agaricites* and average size colonies of *A. humilis* are difficult to distinguish (see Figure 1.1).

Three types of datasets were utilised for multiple analyses: "all", "unlinked" and "neutral and unlinked" SNPs. Here, "unlinked" refers to sampling SNPs at one per RAD locus, noting that linked SNPs may still be present due to physical linkage among short loci (100 – 300 bp). Linked loci from the same locus are not statistically independent and may be too short for recombination to break up. The R package, 'pcadapt' was used to identify SNP-loci that are highly correlated with certain PC axes and were outliers. These "outliers" were deemed putatively selective and thus may drive strong patterns not representative of the genome. Results presented here are of the unlinked and neutral datasets, but results were consistent for species and taxa assignments regardless of how loci were treated.

A1 Species assessment

The dataset containing all samples with a 20% missing data threshold was first assessed with PCA with all SNPs. For all PCAs, each PC axis was examined for clusters, outliers, or a linear spread of samples, until any structure dissipated. The eigenvalues were examined for each PC axes and PC axes that reached an equilibrium in explaining variation were not considered as important as the first few. Datasets including clones may create small groups of clones corresponding to a K in Admixture analyses, thus the presence of clones was assessed separately within each species dataset (see Figure A9 for clonal threshold). These clones were then removed from all datasets, and subsequent 'no clone' datasets were reassessed with Admixture. For all Admixture analyses, 10 different subsets of loci were used for consensus. These subsets corresponded to a different draw of one random SNP per RAD locus. Each of these subsets were run with cross-validation of 100. To determine the informative Ks and the K that delimited species or taxa, I considered: log-likelihood differences between Ks, cross-validation errors, biological relevance and whether samples presented full assignment to a certain K.

A1.1 Principal Components Analysis with all samples

Four potential species may have been sampled, these include: *Agaricia agaricites*, *A. humilis*, *A. lamarcki* and *A. grahamae*. Six samples from *A. lamarcki* and two samples from *A. grahamae* were supplied from Chapter 2. The three species show clear separation in PC1-2 (Figure A1), where *A. lamarcki* is found low PC1 and PC2 values, *A. agaricites* has high PC1 and low PC2 and *A. humilis* has mid PC1 and high PC2 values. One distinct *A. lamarcki* outlier found between the *A. lamarcki* and *A. agaricites* groups on PC1 and apart from *A. humilis* group on PC2. The supplemented *A. grahamae* samples from are shown to separate away from the *A. lamarcki* samples on PC5-6. Further genetic substructure within species shown from PC3 – 6. Species predictions based off rough morphological classifications corresponded to the three genetic groupings. Most of the misclassifications were named *A. agaricites* but grouped with *A. humilis* and a few samples were named *A. humilis* but grouped with *A. agaricites*.

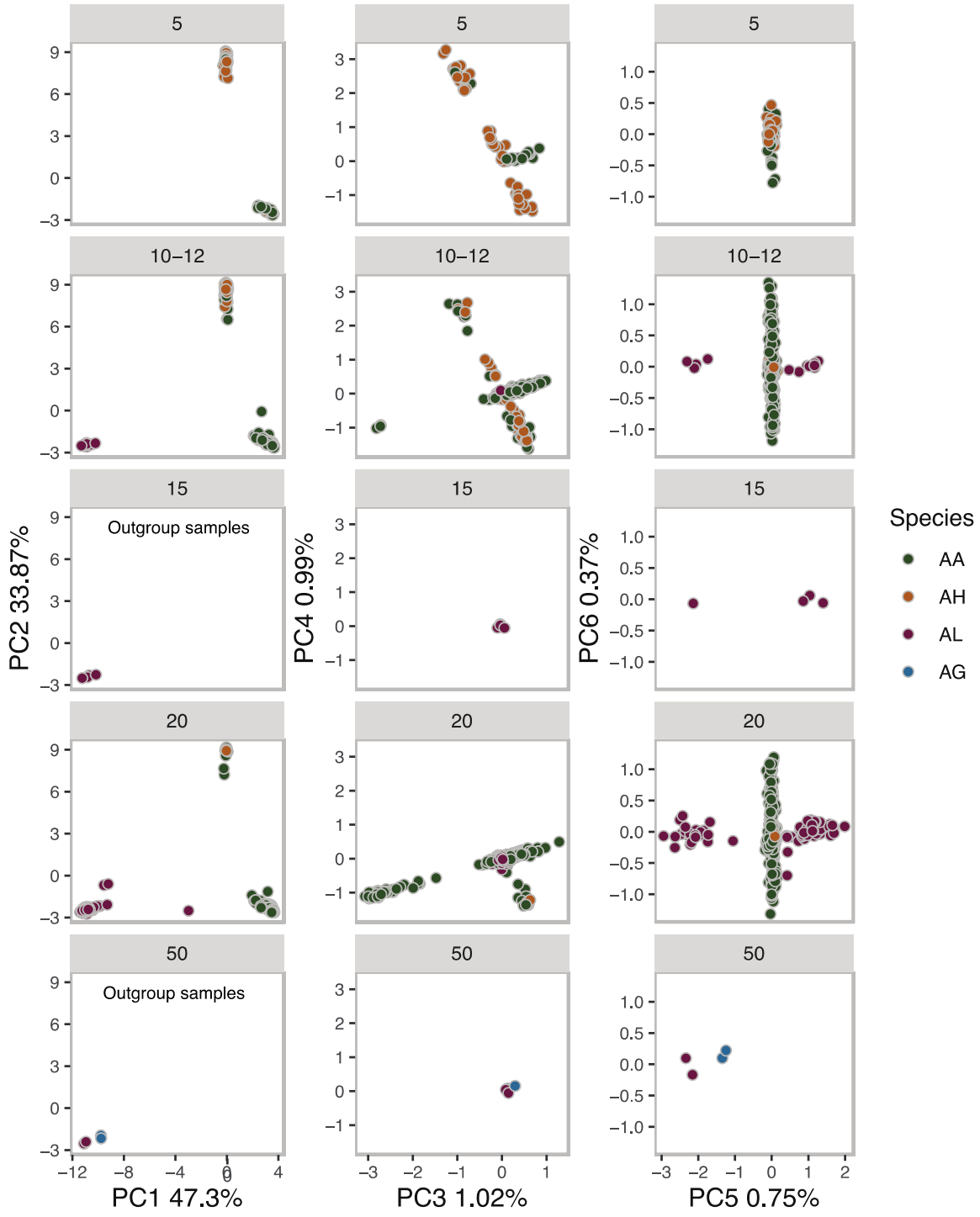

Figure A1. *Principal components analysis among all samples (557 MLG with 762 unlinked SNP loci). Samples were collected in Curaçao within plots at 5, 10-12 and 20 m depth from four locations (WP, CA, SB and SQ) spanning the North – South leeward side of the island. Colours represent morphologically identified species, Agaricia agaricites "AA", A. humilis "AH", A. lamarcki "AL" and A. grahamae "AG". Outgroup samples of AL and AG from 15 and 50 m are used for comparison. Morphological identified samples correspond to genetic groupings and are split into three groups on PC1 – 2.*

A1.2 All samples Admixture analysis

At K=3, CV error and log-likelihood results mostly reached equilibrium, but higher Ks may better explain more variation (*e.g.*, 4 or 5, Figure A2A). The three major groupings corresponded to the three taxonomic species at K=3 (Figure A2B), thus K=3 was used for taxonomic species assignment at a threshold of 0.8 (Figure A2C). The largest cluster representing *A. agaricites* which mostly were identified (through rough morphological classification) as *A. agaricites* with some *A. humilis* identified samples, vice versa for *A. humilis* samples assigning to *A. agaricites* (Figure A2B). All *A. lamarcki* appearing samples were assigned the *A. lamarcki* group, although one sample had ~0.5 assignment to *A. agaricites* group and *A. lamarcki* group (Figure A2B). At K=4, further structure within *A. lamarcki* is shown, which corresponds to the *A. lamarcki* taxa within Chapter 2. The *A. grahamae* samples showed admixed assignment to groups within *A. lamarcki* (Figure A2A). Within the PCA results no other samples clustered closely to the *A. grahamae* samples across PC axes (Figure A1). For these reasons it appears that no other samples assigned to *A. grahamae* within the current dataset. The all-samples dataset was subsequently split into three separate datasets based upon consensus between Admixture assignments at K=3 with 0.8 assignment threshold to a specific K and consensus with PC scores (section A1.1) and named hereafter as the three taxonomic species.

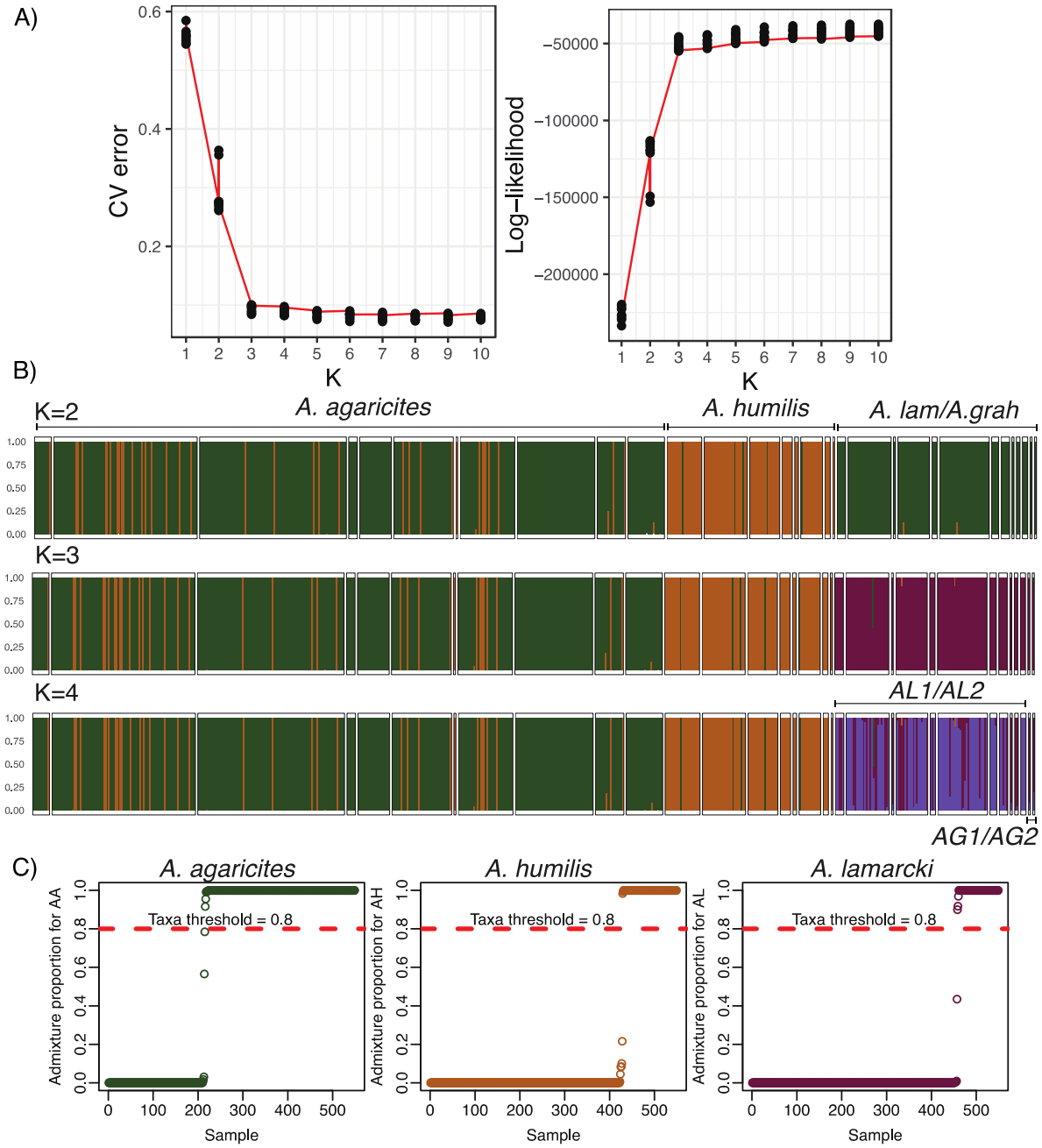

Figure A2. *Admixture analysis results among all samples (557 MLG with 762 unlinked SNP loci). A) Cross-validation error and log-likelihood values among 10 random draws on 1 SNP per contig run 100 times for K=1-10. B) Assignment plot where each bar is a sample, and the colours represent the proportion of assignment to a particular genetic group. C) Distributions of the proportion of assignment to a group. At K=3, the green cluster corresponds to predominantly morphologically identified Agaricia agaricites samples and thus samples with >0.8 assignment to this group are named A. agaricites, as with the yellow cluster for A. humilis and the maroon samples for A. lamarcki. K=3 sufficiently explains major genetic groupings due to mostly equalising of likelihood values, CV error, and equivalence with the taxonomic species groupings.*

A2 Taxa assessment

Samples assigned to species based on PC and Admixture results were separated from an earlier dataset before the strict 20% missing data threshold was applied in other to recover more species-specific loci that may have been lost (see Methods). Both the PC and Admixture results on the without clones, unlinked and neutral datasets were compared for consensus which samples to designate to taxa.

For consistency and consensus of reasonable breaks among genotypic clusters, the Admixture threshold (Q, proportions of assignment) was chosen as 0.8 amongst all groups. This threshold was chosen because for the most continuously differentiated groups (AH1 and AH3) it represented a break between higher and moderate assignments of AH3 samples with the break in assignment jumping from 0.79 – 0.92. Although, such a break does not exist for more continuous for samples with predominant assignment to AH1 and thus samples assigned to AH1 will still have some admixed ancestry.

A2.1 *A. agaricites*

The PCA for *A. agaricites* showed two highly divergent, distinct taxa that occur sympatrically but with one taxon occurring predominantly at 20 m (three of these samples occur at 10 m) separated on PC1 (Figure A3). Samples within the largest taxon presented clear spatial genetic structure among all sites on PC2 – 4.

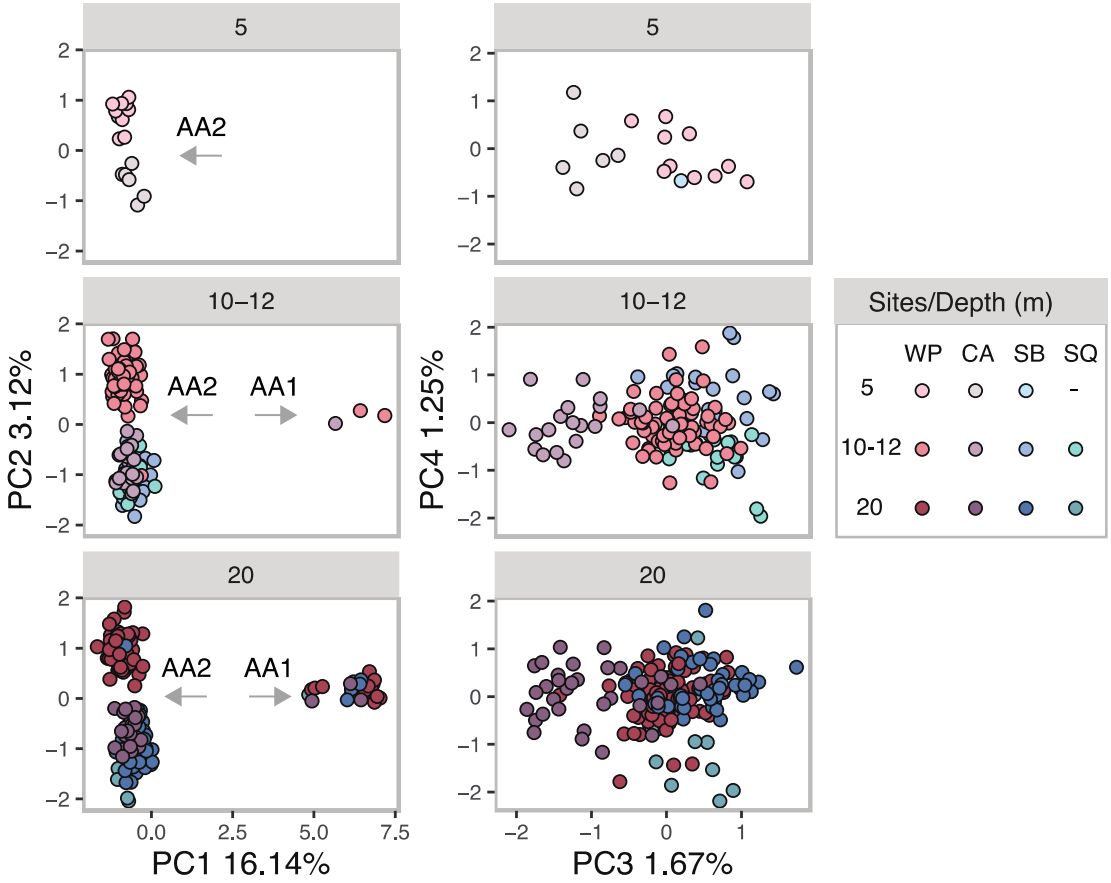

Figure A3. *Principal components analysis results of Agaricia agaricites (using 335 MLGs and 1,629 unlinked, neutral, SNP loci). Samples were collected in Curaçao within plots at 5, 10-12 and 20 m depth from four locations (WP, CA, SB and SQ) spanning the North – South leeward side of the island. PC1 separates samples into two groups and are named "AA1" and "AA2". AA1 predominant occurs at 20 m (excepting three samples at 10 m) and is found at all sites. PC2-4 spatially separates samples. Colours correspond to sites and depth and panels correspond to depths.*

The same two cryptic taxa found in the PCA were found within *A. agaricites* at K=2 within the Admixture results. These presented samples with full assignment, samples within the taxa sympatrically occur and there was no spatial pattern related to the taxa, these taxa were named "AA1" and "AA2" (Figure A4). A threshold of 0.8 assignment separates the two taxa. Spatial genetic structure is seen within the "AA2" taxon, at K=3 and K=4. At K=3 WP is separated from the other sites and then at K=4, CA is separated and SQ and SB form one cluster.

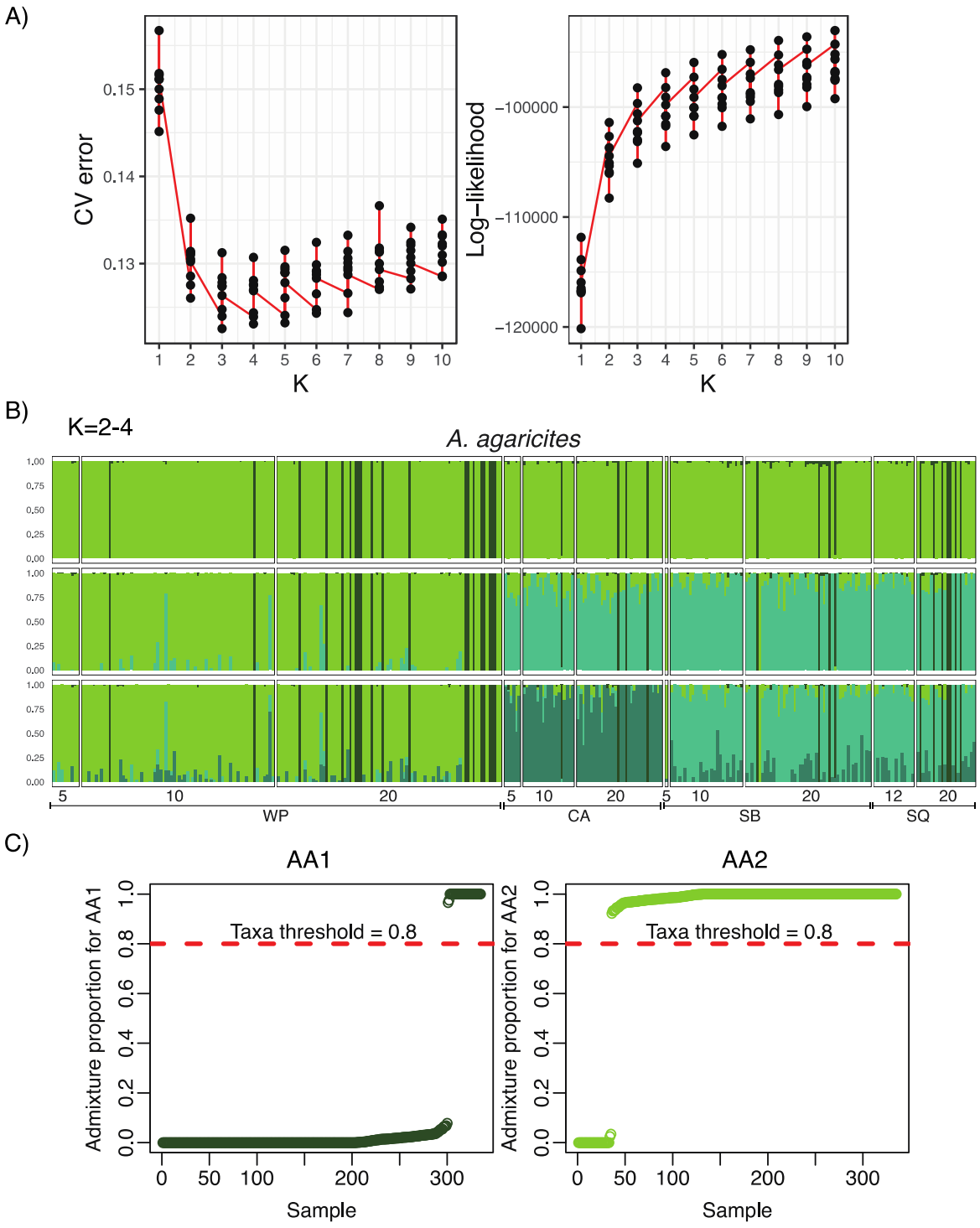

Figure A4. *Admixture analysis results for Agaricia agaricites (using 335 MLGs and 1,629 unlinked, neutral, SNP loci). A) Cross-validation error and log-likelihood values among 10 random draws on 1 SNP per contig run 100 times for K=1-10. B) Assignment plot where each bar is a sample, and the colours represent the proportion of assignment to a particular genetic group. C) Distributions of the proportion of assignment to a group, threshold for splitting samples in groups shown for K=2.*

A2.2 *A. humilis*

The PCA for *A. humilis* showed one highly divergent group, and two partially separated groups on PC1 (Figure A5). The partially separated groups showed further separation at PC2, with a few intermediate samples between them on both PC1 and 2. PC3 and 4 presented some spatial structure among sites with samples from CA separate from samples from other sites on PC3.

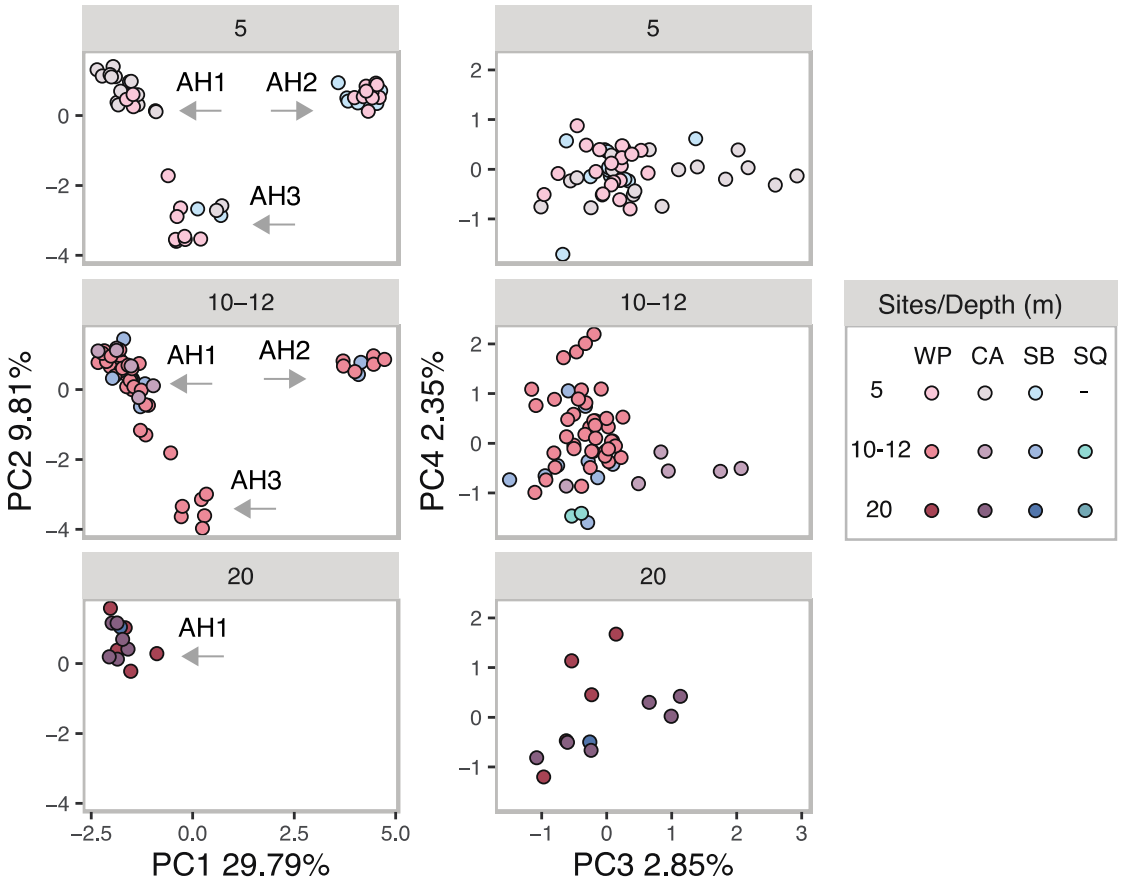

Figure A5. *Principal Components Analysis results of Agaricia humilis (using 121 MLGs and 1,664 unlinked, neutral SNP loci). Samples were collected in Curaçao within plots at 5, 10-12 and 20 m depth from four locations (WP, CA, SB and SQ) spanning the North – South leeward side of the island. Colours correspond to location and depth and panels correspond the depths.*

Admixture analysis also shows a high likelihood for three taxa (Figure A6). At K=3, groups present samples with full assignments and do not follow a spatial pattern. The largest group, named "AH1" is found at all depths but the other two named "AH2" (smaller group at K=2) and "AH3" are found mostly at 5 m and occasionally at 10 m. There are many mixed ancestry samples between "AH1" and "AH3" and the taxa threshold is split conservatively at a small break at 0.8 (see AH3). At K=4, spatial structure is found with the "AH1" group, separating samples from WP with samples from CA/SB/SQ.

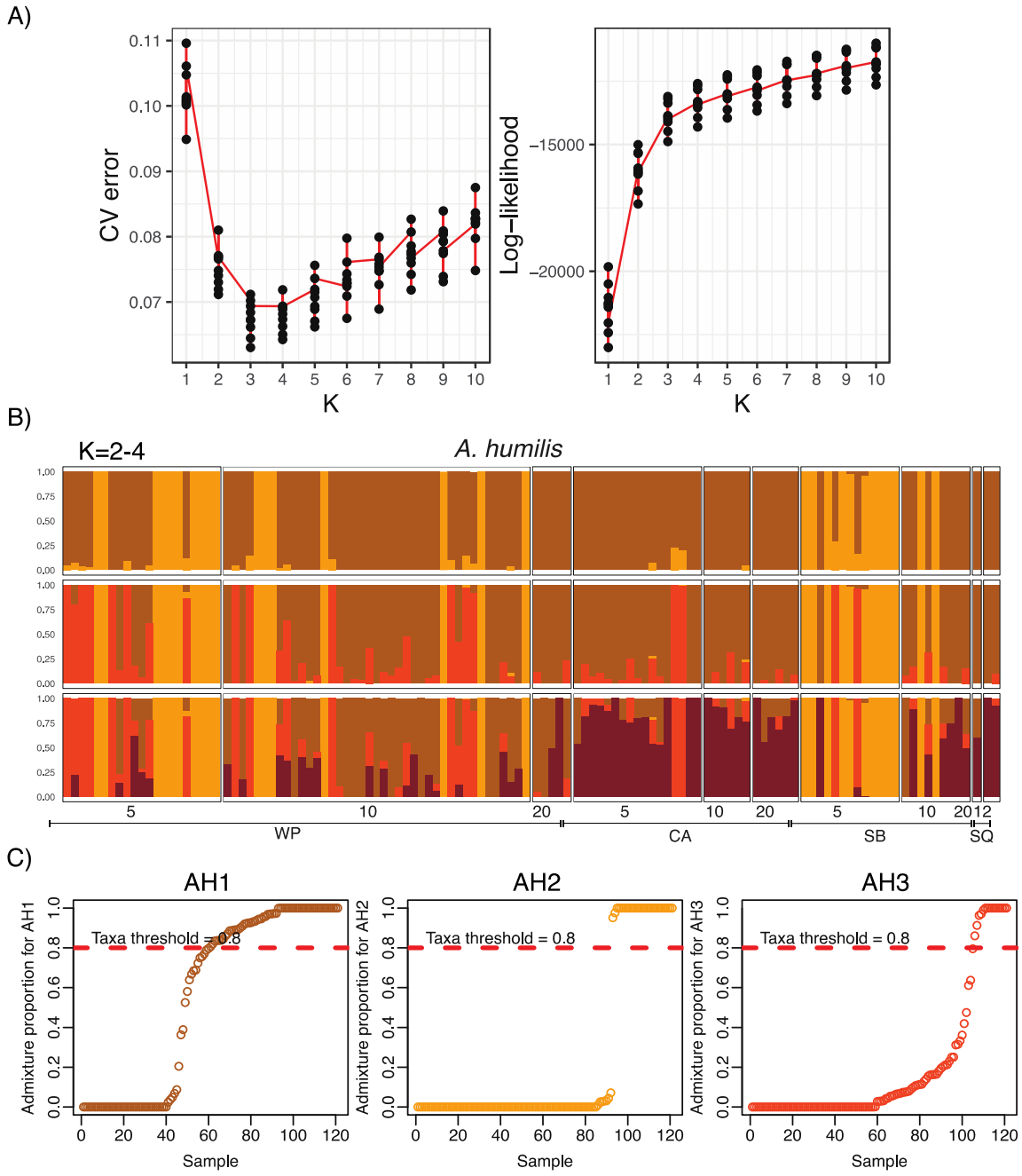

Figure A6. *Admixture results for Agaricia humilis (using 121 MLGs and 1,664 unlinked, neutral SNP loci). A) Cross-validation error and log-likelihood values among 10 random draws on 1 SNP per contig run 100 times for K=1-10. B) Assignment plot where each bar is a sample, and the colours represent the proportion of assignment to a particular genetic group. C) Distributions of the proportion of assignment to a group at K=3, the proportion of assignment for the separation of taxa is shown.*

A2.3 *A. lamarcki*

The PCA for *A. lamarcki* showed two divergent groups that corresponding to the same divergent groups in Chapter 2, "AL1" and "AL2" split at PC1 (Figure A7, outgroup samples at 15 and 50 m). No further spatial or depth structure was found in subsequent PC axes.

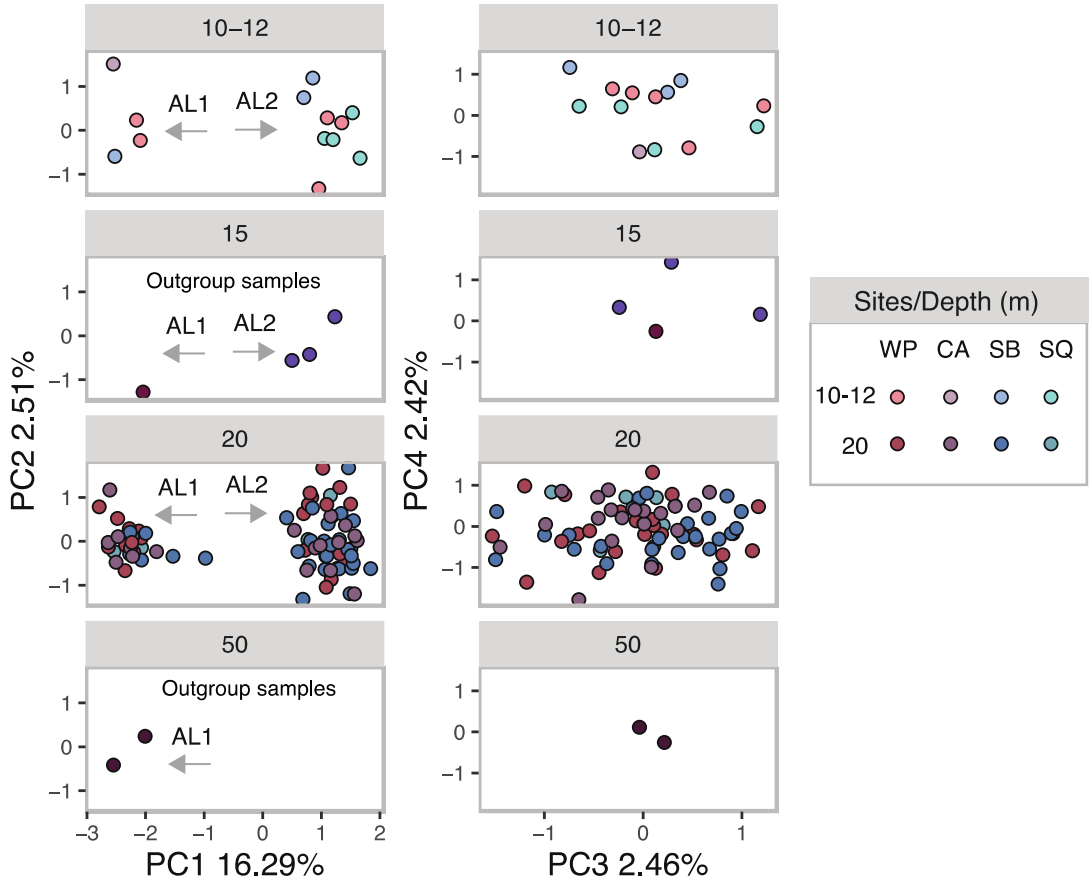

Figure A7. *Principal components analysis for Agaricia lamarcki (98 MLGs and 954 unlinked, neutral SNP loci). Samples were collected in Curaçao within plots at 5, 10-12 and 20 m depth from four locations (WP, CA, SB and SQ) spanning the North – South leeward side of the island and six additional samples of two taxa found within A. lamarcki: "AL1" and "AL2" taxa from 15 - 50 m depth and within Curaçao (WP, SQ and CARMABI). A. lamarcki separates in two groups "AL1" and "AL2", with no further spatial structure. Colours correspond to location and panels represent plot depths.*

Admixture assignments for *A. lamarcki* reflected the presence of two taxa and no spatial genetic structure (Figure A8) as seen in the PCA. These groups corresponded to the two taxa found in Chapter 2, AL1 samples represent the group with a smaller number of samples in the current dataset, AL1 was found more commonly at 50 m whereas AL2 was found more commonly at 15 m in Chapter 2. Ks higher than 2 increased in CV error and did not reflect much higher likelihood, where similar changes in likelihood between 2 – 3 were found for each increasing K (Figure A8A).

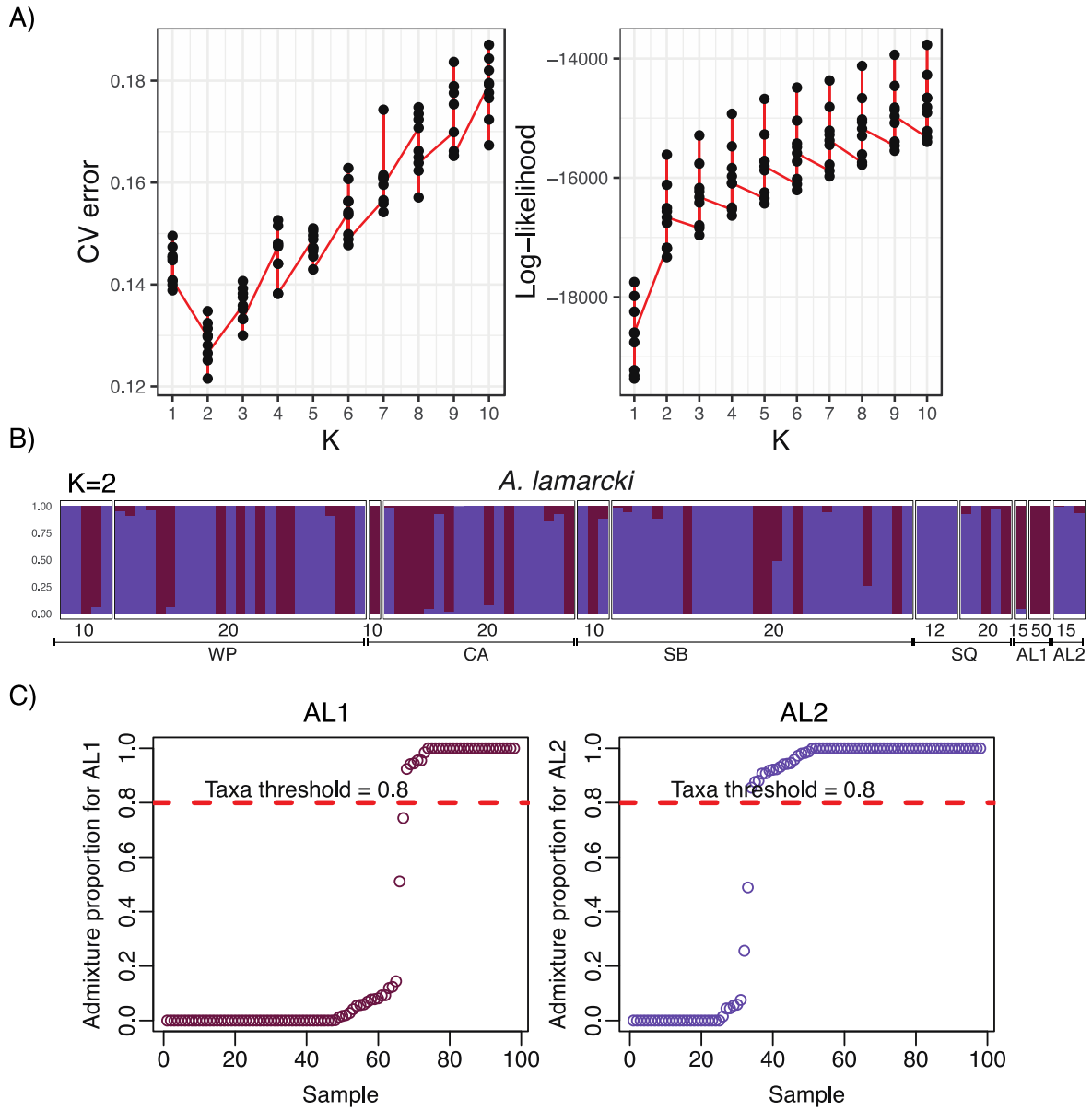

Figure A8. *Admixture results for Agaricia lamarcki (98 MLGs and 954 unlinked, neutral SNP loci). A) Cross-validation error and log-likelihood values among 10 random draws on 1 SNP per contig run 100 times for K=1-10. B) Assignment plot where each bar is a sample and the colours represent the proportion of assignment to a particular genetic group. C) Distributions of the proportion of assignment to a group at K=2, proportion threshold for separating taxa is shown.*

A3 Pairwise Genetic Distances (Hamming's distance)

Genetic distances using all SNPs were used to determine clonal thresholds (Figure A9). Distances were not used to determine taxa thresholds but show consistency with PCA/Admixture results (*i.e.*, two within *A. agaricites*/*A. lamarcki* and three within *A. humilis*).

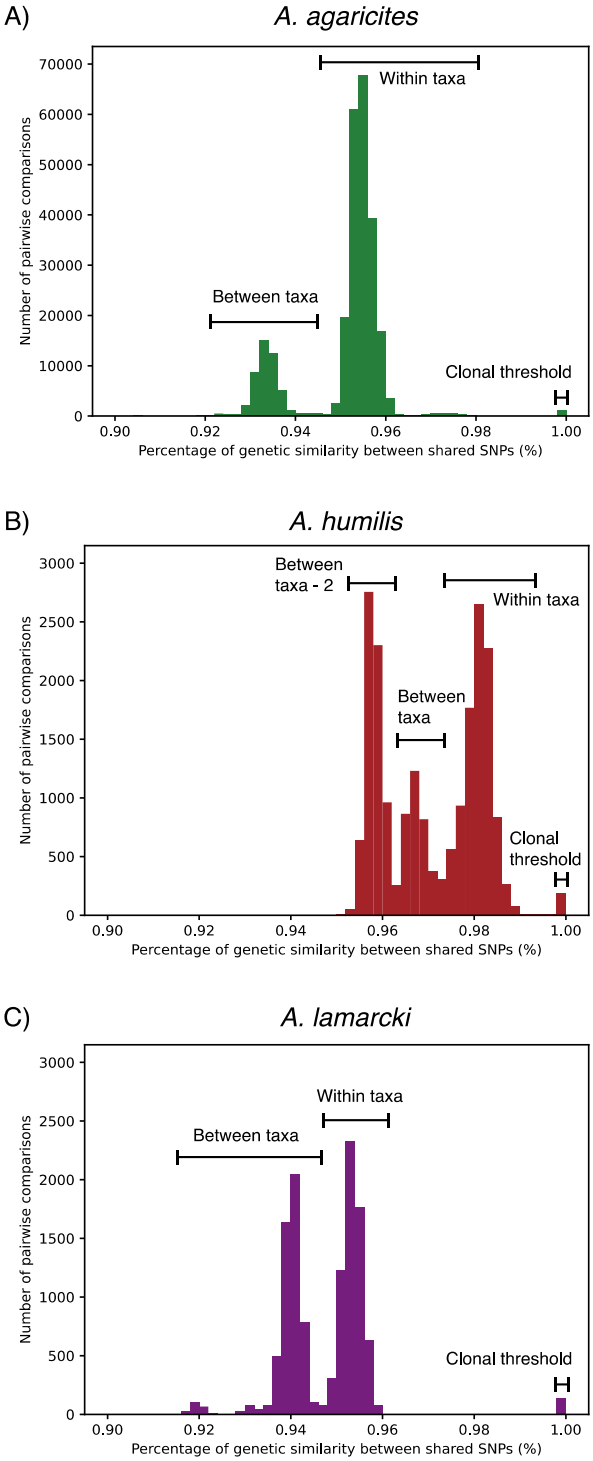

Figure A9. *Pairwise genetic distances (Hamming's distance) between all samples within each species (A – C). Clonal thresholds, within taxa and between taxa distances are shown.*

A4 Summary

Each taxonomic species contained taxa within and followed the general pattern of one more abundant taxon and one or two less abundant taxa (Table A1). Both *A. agaricites* and *A. humilis* showed spatial patterns within taxa and depth patterns between taxa (AA1 was found predominately at 20 m whereas AA2 was found abundantly at all depths, and only AH1 was found at 20 m while AH2 and AH2 were not). For *A. lamarcki*, samples were mostly found at 20 m (never at 5 m and occasionally at 10 m), there were no depth differences among taxa, only AL1 was less abundant than AL2. There were no admixed samples among. *A. agaricites* taxa, but there were 16 samples admixed among AH1 and AH3 taxa and *A. lamarcki* contained 2 admixed samples. Samples were considered admixed if they didn't assign >0.8 to either taxon. Admixed samples were not included in spatial or microhabitat analyses in Chapters 3 and 4.

Table A1. *The number of species and cryptic genetic taxa within species (coral colonies, Agaricia spp.) of multi-locus genotypes found at each location and depth sampled on the leeward side of Curaçao, Southern Caribbean.*

Species:		*A. agaricites*		*A. humilis*				*A. lamarcki*		
Depth (m)	**Location**	**AA1**	**AA2**	**AH1**	**AH2**	**AH3**	**admixed**	**AL1**	**AL2**	**admixed**
5	WP	0	10	3	10	7	3	0	0	0
	CA	0	6	13	0	2	2	0	0	0
	SB	0	1	1	10	2	0	0	0	0
	Total	**0***	17	17	20	11	5	**0***	**0***	**0***
10	WP	2	70	21	7	6	7	2	3	0
	CA	1	18	4	0	0	2	1	0	0
	SB	0	27	5	2	0	2	1	2	0
12	SQ	0	15	2	0	0	0	0	4	0
	Total	3	130	32	9	6	11	4	9	**0***
20	WP	18	66	5	0	0	0	9	16	0
	CA	3	29	6	0	0	0	8	11	0
	SB	4	43	1	0	0	0	5	23	2
	SQ	7	15	0	0	0	0	2	3	0
	Total	32	153	12	**0***	**0***	**0***	24	53	2
Within taxa total		35	300	61	29	15	16	28	62	2
With species total		335		121				92		
Overall total		548 (+1 admixed AA/AL)								