Undescribed coral diversity at mesophotic depths: a phylogenomic assessment of *Agaricia* and *Leptoseris*

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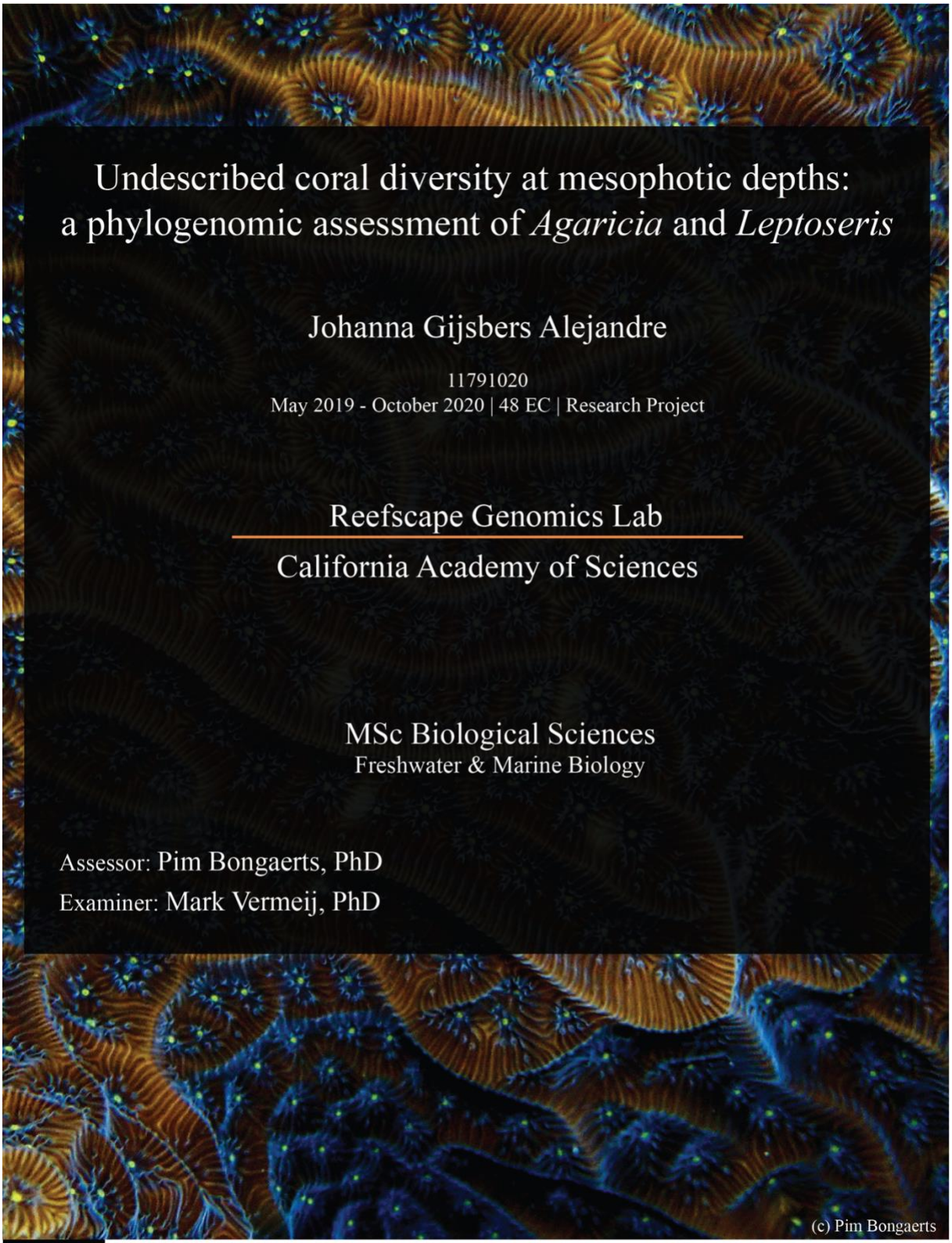

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Acknowledgments

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Abstract

Mesophotic coral ecosystems (MCEs) are known for the unique and distinct biological communities they can host, exemplified by the numerous species of unique fish that continue to be discovered and described in these ecosystems. In contrast, the number of zooxanthellate coral species (order Scleractinia) observed at mesophotic depths is generally low, and based on current taxonomy, many of these species exhibit opportunistic depth distributions rather than representing "deep-specialists". However, phylogenetic studies of corals have long suffered from pervasive issues with traditional sequence markers and have only rarely focused on mesophotic taxa, and it therefore remains unclear whether the reported low diversity at mesophotic depths is accurate. Here, we use a reduced-representation genome sequencing approach and provide a phylogenomic assessment of mesophotic taxa in the genera *Leptoseris* and *Agaricia*, as dominant members at mesophotic depths in respectively the Indo-Pacific and Caribbean. In contrast to sequence markers, we demonstrate that current taxonomic species could be successfully resolved as genetically distinct entities. However, overall phylogenetic relationships indicate substantial divergence within both the *Leptoseris* and *Agaricia* genera, with species delimitation analyses exposing undescribed biodiversity associated with many of the nominal species. Overall, our study sheds light on the diversity of these understudied keystone genera, confirms that the current taxonomy does not accurately reflect the actual species richness associated with mesophotic habitats, and highlights the need for this diversity to be further studied and documented.

Introduction

Mesophotic coral ecosystems (MCEs) occur in tropical waters below 30 - 40 m down to ~150 m in clear waters (Kahng et al. 2010; Lesser et al. 2009). Like their shallow-water counterparts, MCEs are characterized by the presence of zooxanthellate scleractinian corals; however, they remain poorly understood mainly due to the logistical difficulties of studying these habitats (Kahng et al. 2010; Englebert et al. 2017). Over the past decades, there has been an increasing interest in MCEs due to their potential to act as a refuge for shallow reefs against disturbances such as mass warm-water bleaching events and tropical storms, as well as their ability to host unique and distinct

biological communities (Glynn 1996; Hughes and Tanner 2000; Lesser et al. 2009; Bongaerts et al. 2010; Kahng et al. 2010; Bongaerts et al. 2015; Pochon et al. 2015; Muir et al. 2018; Rocha et al. 2018). Their status as unique biological communities is established due to the continuous discovery of new reef fish species restricted to mesophotic depths (Rocha et al. 2018; Pinheiro et al. 2019); however, in terms of scleractinian corals, the number of deep-specialist species is thought to be relatively low (Muir et al. 2018; Englebert et al. 2017; Muir and Pichon 2019).

Contrary to reef fish communities, species-level identification of mesophotic corals based on visual surveys can be extremely challenging given the significant variation in macromorphological traits within species over depth (Luck et al. 2013), and the fact that current-day reference collections are based almost exclusively on shallow material (Dinesen 1980). In an attempt to address this problem and resolve evolutionary relationships among mesophotic species, studies have integrated traditional sequence markers and morphological traits in phylogenomic assessments (Luck et al. 2013; Pochon et al. 2015). Nevertheless, mitochondrial sequence markers in corals often lack resolution or result in incomplete lineage sorting (van Oppen et al. 2001; Forsman et al. 2009; Bongaerts et al. 2015; Terraneo et al. 2016), mainly caused by the slow mitochondrial DNA mutation rate in corals. On the other hand, the high levels of intraspecific and interspecific variation of multi-copy nuclear rDNA regions (e.g., 18S) and transcribed spacers (e.g., ITS) and their complex evolution makes it often impossible to distinguish among recently diverged species (Vollmer and Palumbi 2004; McFadden et al. 2010). The development of next-generation sequencing (NGS) platforms has enabled access to a substantial amount of genomic data at a relatively low cost, providing a solution to these pervasive issues (Andrews et al. 2016). In particular, reduced-representation sequencing approaches, involving transcriptome sequencing, targeted capture, and restriction-site associated DNA sequencing (RAD-seq), have become essential cost-effective tools for phylogenetic and population genomic studies (e.g., Combosch and Vollmer 2015; Kitchen et al. 2015; Bongaerts et al. 2017; Quattrini et al. 2019; Arrigoni et al. 2020; Bongaerts et al. 2020; Erickson et al. 2020). Despite the advantages and drawbacks of each of these methods, RAD-seq remains one of the most popular approaches because it enables strong resolving power by sequencing tens of thousands of short fragments of DNA (restriction sites) across the entire genome of multiple individuals and does not require a reference genome (Andrews et al. 2016). In anthozoans with high phenotypic plasticity, wide distribution ranges, and complex evolutionary history, such as corals, reduced-representation sequencing has been

particularly promising to explore and understand processes shaping phylogenetic relationships between taxa, bringing attention to the occurrence of sympatric cryptic species in several taxa (e.g., Herrera and Shank 2016; Rosser et al. 2017; Quattrini et al. 2019; Cunha et al. 2019; Arrigoni et al. 2020).

MCEs around the world are dominated by zooxanthellate scleractinian corals with plating growth forms, which is beneficial in low light conditions because it maximizes photosynthesis (Kühlmann 1983; Kahng et al. 2010). Members of the Agariciidae family, specifically the genera *Agaricia* and *Leptoseris,* in the Western Atlantic and Indo-Pacific, respectively, are often observed covering large areas of substrate across a wide depth range and provide important habitat structure for reef-associated organisms (Bongaerts et al. 2013; Edinger & Risk 1995; Pochon et al. 2015). The Indo-Pacific genus, *Leptoseris,* occurs ubiquitously at mesophotic depths throughout this region (Kahng et al. 2010). Although *Leptoseris* spp. can also be found in shallow conditions, often associated with cryptic environments, such as crevices and shaded locations on the reef (Dinesen 1980); this genus is particularly dominant at lower mesophotic depths (>60 m). The deepest records of light-dependent corals for Eastern Australia (125 m; Englebert et al. 2014), Israel (145 m; Fricke and Knauer 1986), Hawaii (153 m; Kahng and Maragos 2006), and French Polynesia (172 m; Rouzé et al. in press), all represent *Leptoseris* communities. In Eastern Australian waters, four species (*Leptoseris scabra*, *L. glabra*, *L. mycetoseroides*, and *L. hawaiiensis*) were found to be dominant members of mesophotic habitats (Englebert et al. 2017; Muir et al. 2018). In the Red Sea, these four species have also been reported to be present (Terraneo et al. 2017; Eyal et al. 2019; Kramer et al. 2020), although only *L. glabra* was reported as highly abundant at mesophotic depths (Kramer et al. 2020). On the opposite side of the Indo-Pacific, *L. glabra* is absent on the reefs of the Hawaiian Archipelago, but *L. hawaiiensis* and *L. scabra* have been documented to be abundant at lower mesophotic depths while absent at shallower depths (Luck et al. 2013). Phylogenetic and symbiont diversity assessments have indicated depth-related specialization, specificity in Symbiodiniaceae associations, and polyphyletic patterns among different *Leptoseris* species (Luck et al. 2013; Pochon et al. 2015; Terraneo et al. 2017). Although based on traditional markers, these findings revealed limited support for the current taxonomic assignment and highlighted the necessity of further exploration using a phylogenomic approach.

The Western Atlantic genus *Agaricia* consists of seven species with distinct but overlapping depth ranges (Kühlmann 1983; van Moorsel 1983; Bongaerts et al. 2013; Gonzalez-Zapata et al. 2018a). Even though the phylogeny of this genus has remained unresolved using mitochondrial markers (Bongaerts et al. 2015), phylogenetic studies have shown a deep partitioning into two clades, corresponding to morphology (Stemann 1991) and general depth ranges (Bongaerts et al. 2013). *Agaricia humilis* and *A. tenuifolia* are usually found at shallow depths and are characterized by a bifacial colony morphology (van Moorsel 1983; Stemann 1991; Robbart et al. 2004). *A. fragilis*, *A. lamarcki*, and *A. agaricites* have a more extensive depth range and are known as depth-generalists, characterized by unifacial colonies, although bifacial colonies of the latter taxon are often found in shallower habitats as well. *A. grahamae* and *A. undata* (depth-specialists) are distinguished by unifacial colonies that occur almost exclusively in the mesophotic zone (>40 m; Bongaerts et al. 2013; Gonzalez-Zapata et al. 2018a; Prata et al. in preparation). The different *Agaricia* species have been shown to associate with different species-specific Symbiodiniaceae types (Bongaerts et al. 2013, 2015), although deep colonies of *A. lamarcki* share the same type as *A. grahamae*, reflecting the genetic similarity of the coral hosts. Nevertheless, sequence markers have not been able to distinguish between *A. lamarcki* and *A. grahamae* (Bongaerts et al. 2013, 2015) or *A. humilis* and *A. agaricites* (Bongaerts et al. 2013). Recent population genetic studies showed the resolving power of reduced-representation methods, revealing genetic structuring within *A. fragilis* (Bongaerts et al. 2017), *A. grahamae* (Prata et al. in preparation), *A. lamarcki* (Hammerman et al. 2018; Prata et al. in preparation), and *A. undata* (Gonzalez-Zapata et al. 2018a). Nonetheless, this genetic substructure has not been assessed in a phylogenomic or species delimitation framework, leaving the taxonomic status of this exposed diversity uncertain.

Here, we present a phylogenomic assessment to shed light on mesophotic species diversity within the Agariciidae family in both the Indo-Pacific and Caribbean. Focusing on eight dominant mesophotic taxa from the *Leptoseris* and *Agaricia* genus, we evaluate whether the current classification of these species illustrates an accurate representation of the genera from a phylogenetic perspective and assess whether undescribed diversity is present among the dominant species associated with MCEs. Using a reduced-representation genome sequencing (nextRAD) method, we genotyped 204 individuals from shallow and mesophotic depths (ranging from 2 – 127 m depth), focusing primarily on Eastern Australia and the Southern Caribbean, but with samples

from ten different regions in total (Figure 1). Using species delimitation methods, we assess species diversity within the four dominant *Leptoseris* species at mesophotic depths of the Western Coral Sea and Great Barrier Reef, and the four dominant *Agaricia* species in the Western Atlantic. Overall, we argue that there may be substantial undescribed species diversity within these genera and that both the diversity and level of specialization of scleractinian corals at these depths may be higher than currently acknowledged.

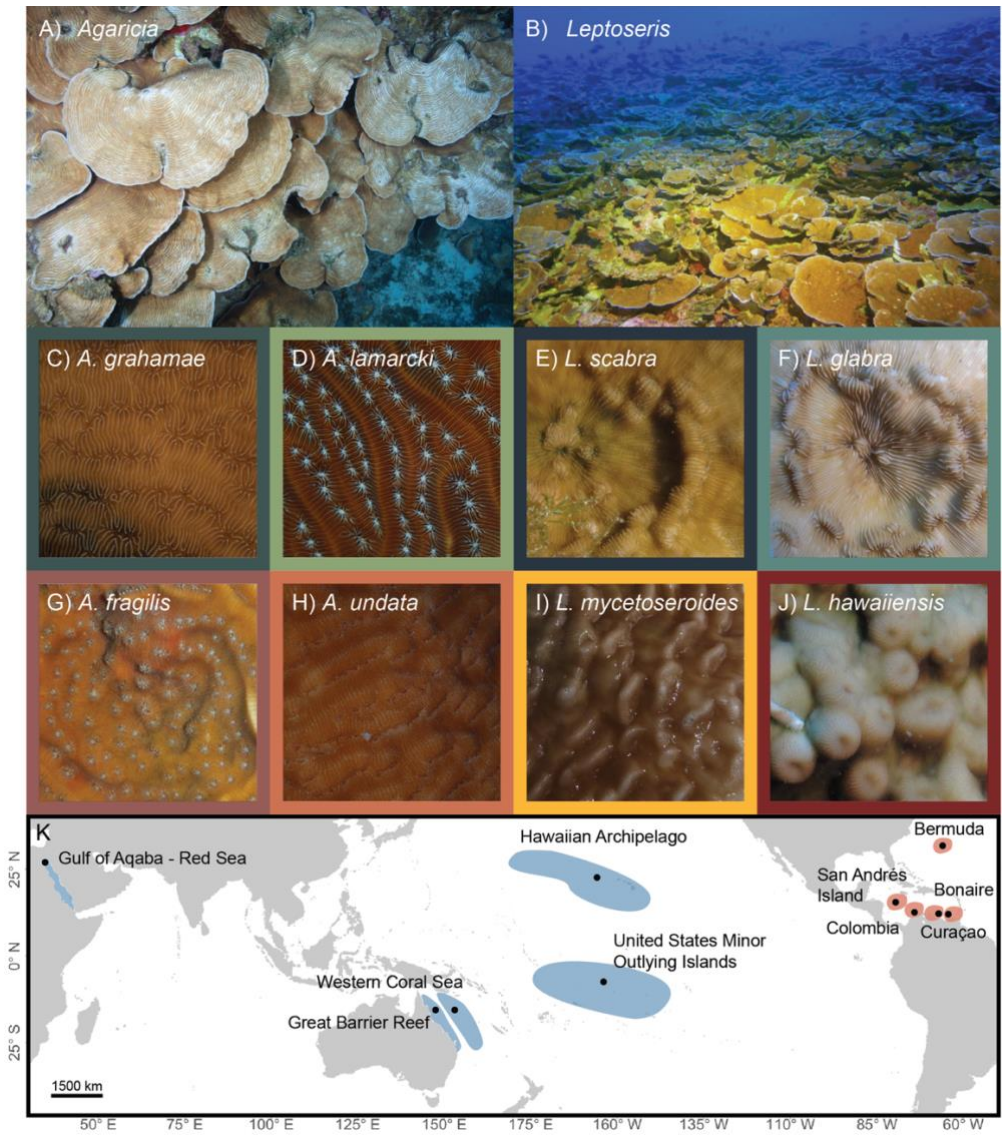

Figure 1. Overview of the focal species examined in this study. (A) *Agaricia*-dominated mesophotic reef at 40 m in Curaçao, Southern Caribbean. (B) *Leptoseris*-dominated mesophotic reef at 90 m in the Hawaiian Archipelago. (C) *A. grahamae*. (D) *A. lamarcki*. (E) *L. scabra*. (F) *L. glabra*. (G) *A. fragilis*. (H) *A. undata*. (I) *L. mycetoseroides*. (J) *L. hawaiiensis*. (K) Map of the sampling regions in the Indo-Pacific (blue) and Western-Atlantic and Caribbean (orange). Photo credits: P. Bongaerts (A); N. Englebert (E- F, I -J); Hawai'i Undersea Research Laboratory Archive (B); T. Phelps (H); K. Prata (C, D).

Methods

Sample collection and preparation

Coral specimens from the genera *Leptoseris* and *Agaricia* were collected as part of the "XL Catlin Seaview Survey", focused respectively on Eastern Australia (Western Coral Sea and Great Barrier Reef) and the Southern Caribbean (Curacao and Bonaire). Sample collections in Eastern Australia are documented in Englebert et al. (2017) and Muir et al. (2018) and were performed using a Seabotix vLBV300 (with a parallel jaw manipulator) supplemented with SCUBA at shallower depths. Collection efforts in the Southern Caribbean were performed as documented in Bongaerts et al. (2015), using the "Curasub" submersible operated by "Substation Curacao" supplemented with technical SCUBA. Small fragments of the specimens were stored in ethanol or a salt-saturated solution of 20% DMSO and 0.5M EDTA for genomic DNA extraction. Skeletal specimens were preserved for all *Leptoseris* and a subset of the *Agaricia* through cleaning in bleach solution (4% hypochlorite for 36–72 h), followed by freshwater rinsing and drying, and deposited at the Queensland Museum Collection or the Invertebrate Zoology collection at the California Academy of Sciences (Table S1).

For comparative purposes, additional tissue samples from *Leptoseris* were acquired from the Hawaiian Archipelago and US Minor Outlying Islands (collected using a manned submersible), and from the Gulf of Aqaba in the Red Sea (collected using closed-circuit rebreathers). Similarly, additional *Agaricia* and *Helioseris* tissue samples were acquired from San Andrés Island, Cartagena, and Santa Marta in Colombia (collected using closed-circuit rebreathers). Genomic DNA was extracted as described in Bongaerts et al. (2017, 2020), using the additional centrifugation steps to reduce endosymbiont contamination when sufficiently high gDNA yield could be obtained. We also include/reanalyzed existing nextRAD sequence data of *A. fragilis* (n = 6) and *Stephanocoenia intersepta* (n = 3) from Bermuda (Bongaerts et al. 2017; PRJNA361144), *A. undata* from (n = 6) from San Andrés and Cartagena in Colombia (Gonzalez-Zapata et al. 2018a; PRJNA385083), and *Pachyseris speciosa* (n = 3) from Eastern Australia (Bongaerts et al. 2020; PRJEB23386).

Library preparation, sequencing, and nextRAD clustering

Extracted gDNA was used to create nextRAD DNA libraries (SNPsaurus, LLC). This method uses selective PCR primers to genotype genomic loci consistently between the entire set of samples (Russello et al., 2015). Using Nextera reagent (Illumina Inc), the gDNA was fragmented and then ligated with Nextera adapters (Illumina Inc). Once ligated, fragmented DNA was PCR-amplified (26 cycles at 73°C) using a matching primer for the adapter that extended into the gDNA with a 9-base pair (bp) selective sequence ("GTGTAGAGG"). The final libraries were sequenced on the Illumina HiSeq 2500 platform to generate 100 bp single-end reads.

Nextera adapters and low-quality ends (with a PHRED-quality score below 20) were trimmed using TrimGalore v 0.6.4 (https://github.com/FelixKrueger/TrimGalore), to discard reads shorter than 30-bp and trim sequences up to 100 bp. IpyRAD v 0.7.30 (Eaton & Overcast 2020) was then used for locus clustering and variant calling. After initial runs at different thresholds, we decided on a clustering threshold of 85%, a minimum coverage of 10, and a minimum of 4 samples in a final locus, with most other settings run at default recommended values (Supplementary material).

Identification of symbiont contamination was made through a BLASTN comparison between each RAD locus against our Symbiodiniaceae RAD database (Bongaerts et al. 2017), and two culture-based Symbiodiniaceae genomes: *Cladocopium goreaui* (Liu et al. 2018), and *Durusdinium trenchii* from Dougan et al. (unpublished data). Positive matches with a maximum E-value of 10-15 were extracted from the coral RAD loci, and potential microbial contamination was identified by an additional BLASTN comparison, this time, against the National Center for Biotechnology Information (NCBI) non-redundant database. The taxonomic IDs of positive matches (maximum E-value of 10^{-4}) were classified to phylum according to the NCBI Taxonomy database, and the non-cnidarian taxa were extracted from our dataset. Once contamination was filtered, monomorphic SNPs (Single Nucleotide Polymorphisms) were removed to ensure only sites with two or more alleles were included, and then, the RAD loci were trimmed to 90-bp, to avoid overrepresentation of SNPs. To assess the sample performance of each individual, a modified version of the vcf_gdmatrix.py script was used (Python v 3.4.5; available through https://github.com/pimbongaerts/radseq), which allowed us to filter the samples with poor-performing levels (<1000 SNPs), from both the SNPs Variant Call File (VCF) and the RAD locus dataset. During this step, three individuals were removed: DC1946, GEFC49, and GE0103.

Additionally, SK0124 was also removed due to the low genotyped percentage, despite the number of SNPs (>1000).

Phylogenetic assessment using sequence-based analyses

Three supermatrices were created with the remaining individuals after filtering. The first one containing all individuals and four outgroups (n = 200), referred hereafter as the "*Agalepto*" dataset, and two datasets consisting of the individuals of *Leptoseris* and *Helioseris cucullata* as outgroup (n = 132), and *Agaricia* and *S. intersepta* as outgroup (n = 64), which are referred hereafter as the "*Leptoseris*" and "*Agaricia*" datasets, respectively. The resulting datasets were additionally filtered to only retain loci genotyped for at least ten samples ("*Agalepto*": 17,642 loci; "*Leptoseris*": 9,770 loci; "*Agaricia*": 10,424 loci). The remaining loci were used to generate independent concatenated-loci alignments, which are required to infer maximum likelihood (ML) phylogenetic relationships as well as for Bayesian Inference (BI) analyses. ML relationships for each dataset were assessed using RAxML-ng v. 0.9.0 (Kozlov et al. 2019) under the GTR + I + G4 nucleotide substitution model as suggested by ModelTest-ng v x.y.z (AIC= 27,262,957.1; Darriba et al. 2019). Independent searches and bootstrap replicates were performed on each alignment until convergence was reached, which varied among the three datasets ("*Agalepto*": 20 ML searches and 1150 bootstrap replicates; "*Leptoseris*": 20 ML searches and 950 bootstrap replicates; "*Agaricia*": 20 ML searches and 450 bootstrap replicates). BI analyses were implemented using MrBayes 3.2.7 under the GTR model with gamma-distributed rate, with a burn-in fraction of 25% and a sampling frequency of 1000. Based on convergence, the number of Markov chain Monte Carlo (MCMC) generations run varied per dataset ("*Agalepto*": $10e^6$; "*Leptoseris*": 3.4e6; "*Agaricia*": $4.8e^6$). The convergence of the analysis was assessed using Tracer v.1.7 (Rambaut et al. 2018).

Genetic structure and species delimitation using SNP-based analyses

Based on the clusters observed in both concatenated trees, the Bayes Factor Delimitation (BFD*) for SNP data was used to evaluate and compare alternative species delimitation models on the SNAPP package of BEAST v.2.6.1 (Bouckaert et al. 2014). Therefore, marginal likelihood estimations (MLE) for each scenario were calculated and ranked to determine the best possible model. In order to increase the convergence probability of the analysis, only the two individuals

with the most genotyping percentage per species were sampled for each model to test using VCFtools v.0.1.16 (Danecek et al. 2011). Since these analyses do not allow large amounts of missing data, the vcf_minrep_filter_abs.py script (https://github.com/pimbongaerts/radseq.git) was applied to make sure SNPs of all clades to test were included. Then, unlinked-SNPs datasets (~200 SNPs for each model) were generated using the vcf_single_snp.py script (available through https://github.com/pimbongaerts/radseq.git) and converted to nexus-binary files using the vcf2phylip script (https://github.com/edgardomortiz/vcf2phylip.git). The analysis was run following a path sampling method of 48 steps, each one consisting of the following parameters: MCMC length = 100000, pre-burnin = 1000, samplefreq = 1000, as suggested by Leaché et al. (2014). The species delimitation models were compared and ranked by their MLE value, then the Bayes Factor (BF) of each model was calculated as (2 * [modelA - modelB]) (Kass and Raftery 1995). The best-supported model was then used to generate a species tree with the following parameters: MCMC length = $10e^6$, pre-burnin = 1000, samplefreq = 10000, as suggested by Leaché et al. (2014). In addition, Discriminant Analysis of Principal Component (DAPC) from adegenet, which seeks to determine the genetic structure between clusters based on their similarity without considering a model of evolution (Pante et al. 2015), was employed to visualize and confirm the best-supported Bayes model. 19 principal components (PC) and 10 discriminant functions were retained for *Leptoseris* (K = 5, BIC = 215.34). 8 PC's and 6 discriminant functions for *Agaricia* (K= 5, BIC = 204.25).

Comparison with traditional sequence markers

To compare the resolution of RAD-seq with traditional mitochondrial markers, *cox*1-1-rRNA intron (COX1) sequence data of 101 individuals of the genus *Leptoseris* and one individual of the genus *Pavona* were amplified using the AGAH/AGAL primer pairs (Terraneo et al. 2014). The PCR amplifications were performed using the protocol reported in Bongaerts et al. (2013). Agarose gels were used to assess the quality of the PCR products, then cleaned using ExoSAP-IT and sequences in forward and reverse directions (ABI BigDye Terminator chemistry, Australian Genome Research Facility). Finally, Codoncode Aligner was used to analyze the sequences resulting. ML phylogenies were inferred using RAxML-ng v. 0.6.0 BETA on the concatenated alignment, under the GTR + I + G4 model, and with a calculation of 20 trees and bootstrap support values based on 52000 replicates.

Results

Species delimitation patterns in the genus *Leptoseris*

For the *Leptoseris* dataset, we obtained a total of 9,770 RADseq loci. Both phylogenetic approaches (RAxML and MrBayes) separated most unambiguously identified samples of our four *Leptoseris* focal species into distinct clades. The two approaches recovered similar topologies and arranged all taxa in well-supported clades. For three of the focal species, *L. scabr*a, *L. mycetoseroides*, and *L. hawaiiensis*, we observed separation of the taxonomically identified samples into highly divergent clades. Five of the *Leptoseris* specimens from the Red Sea were extremely divergent from the other samples, and as they likely represented a distinct genus, they were excluded from the *Leptoseris* phylogeny.

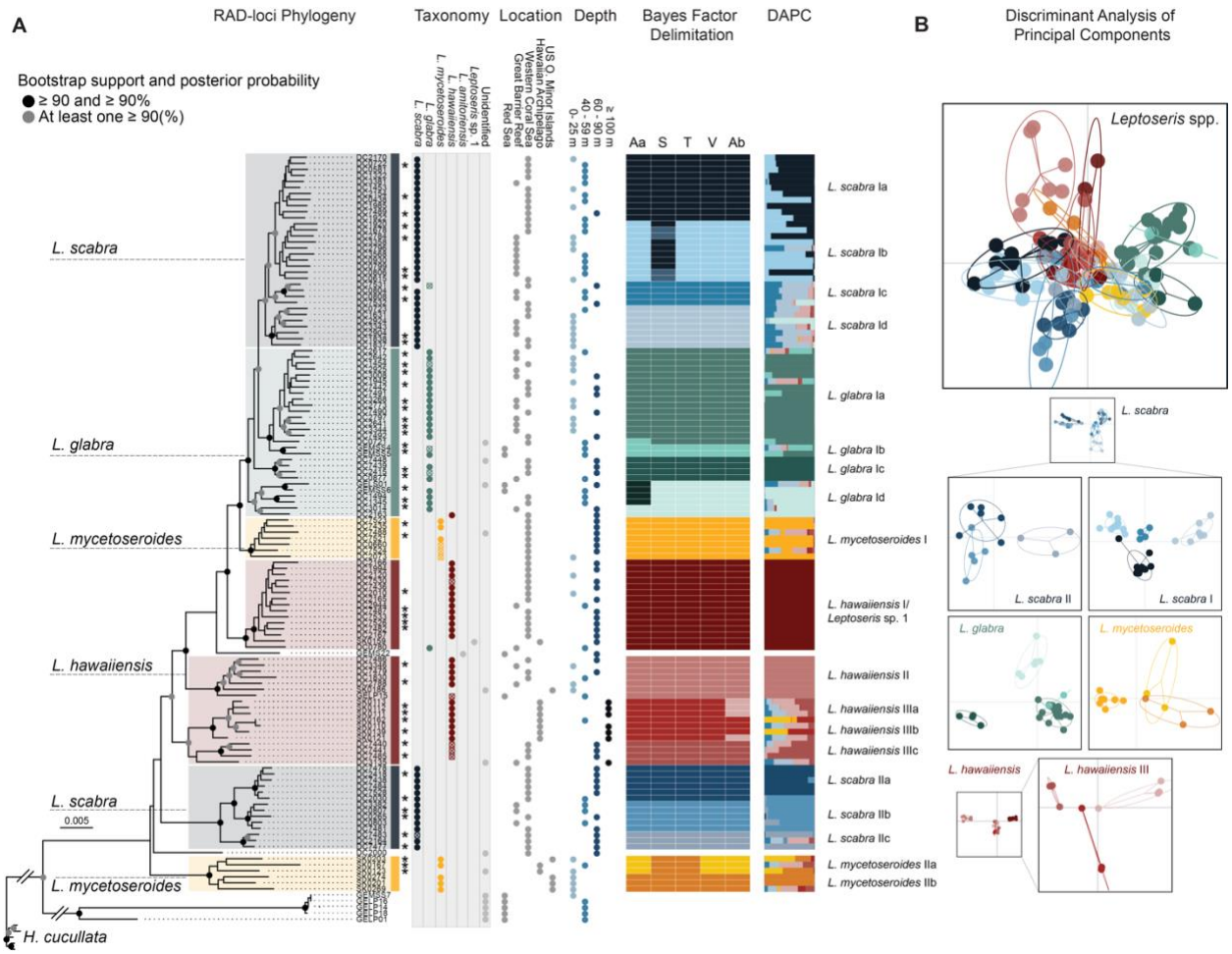

Figure 2. Phylogeny of the genus *Leptoseris* using reduced-representation sequencing. (A) Phylogenetic RAxML tree using 9,770 loci, with bootstrap values based on Maximum Likelihood (ML) and Bayesian Inference (BI), where only values ≥ 90 and posterior probabilities ≥ 90% are shown. Colored dots in the "Taxonomy" column indicate the taxonomic identifications of the specimens used for this study. The "Location" and "Depth" columns indicate the region and depth of our sampling. The "Bayes factor Delimitation" columns correspond to the five best-supported BFD* models, where (*) indicates the samples with the least amount of missing data used for the BFD* analysis. The "DAPC" column indicates the posterior membership probabilities of the DAPC in section (B). (B) DAPC scatter plot, illustrating *Leptoseris* spp. in the top, and independent runs for the four focal species on the bottom.

Despite the broad-scale correspondence of taxonomic identification and genetic clusters, we observed one mismatch (not taking into account specimens identified as "cf") for *L. scabra, L. glabra,* and *L. hawaiiensis* and eight samples that could not be taxonomically identified across the different clades of *L. glabra, L. mycetoseroides*, and *L. hawaiiensis,* with the exception of one (DC2000) that did not group in any clade. The only *Leptoseris* sp. 1 specimen from Hawaii used in this study grouped in a clade with *L. hawaiiensis* from Australasia. The *L. amitoriensis* specimen from the Red Sea was most related to two *L. hawaiiensis* clades, however, it appears to be divergent from both clades.

In addition to the deep divergence observed for *L. scabra, L. mycetoseroides*, and *L. hawaiiensis*, we observed further genetic differentiation within all four focal species. For *L. scabra*, up to seven distinct subclades (all in Australia) could be discerned across the two highly divergent clades (respectively *L. scabra* I and II), of which two only contained samples from a single region, two contained samples mostly from a single region, whereas the remaining three had representatives from both the Great Barrier Reef (GBR) and Western Coral Sea (WCS). In addition, some of the differentiation appeared to correspond to depth. *L. scabra* I was mostly observed at shallow and upper-mesophotic depths, with only three samples retrieved from the lower mesophotic zone; while *L. scabra* II was only present at upper and lower-mesophotic depths.

The taxonomic species *L. glabra* was split into three distinct clades, with two of the clades only containing specimens from mesophotic depths, whereas the third also contained shallow specimens. Two of the clades appear widespread as they contained representatives from both Australia (GBR and WCS) and the Red Sea. The two *L. mycetoseroides* clades corresponded to distinct sampling regions, the WCS (*L. mycetoseroides* I), and the Hawaiian Archipelago and US Minor Outlying Islands (UMO; *L. mycetoseroides* II), with further separation of the latter into two

sympatric clades (with one of the clades including an unidentified sample, that had similar characteristics as *L. incrustans*, and the other three specimens that were identified as "cf").

For *L. hawaiiensis*, we observed three highly divergent clades, with the first two clades mostly with representatives from Australia (but the first clade also contained a sample from Hawaii, and the second a sample from the UMO and the Red Sea), and the third clade containing an additional split corresponding to Australian and Hawaiian samples. The Australian samples in this third clade were identified as "cf" and also contained an unidentified sample, retrieved from 124 m. The Hawaiian specimens were almost exclusively from below 110 m, with only one sample retrieved from 55 m.

We evaluated the observed separation within taxonomic species through DAPC, and BFD* analyses (Figure 2). The best-supported model (Ab; MLE = -30.52, BF = -2,059.96) divided the genus in all of the above-mentioned splits, plus an additional split in *L. hawaiiensis,* and *L. glabra* (seven subclades in *L. scabra*, four in *L. glabra*, three in *L. mycetoseroides*, and five in *L. hawaiiensis*). DAPC analyses were partially congruent with the best-supported delimitation model. It confirmed the separation of *L. scabra* in two divergent clusters, and further subdivision into the 3-4 additional clades each (but with some overlap). Similarly, for *L. glabra* the three related clusters were separated. For *L. mycetoseroides*, the two divergent clades from Australia, and Hawaii and the OMU were separated, but the observed split in Hawaiian samples was not clear (although each group only contained 3 samples). Lastly, for *L. hawaiiensis,* the major split into three clusters was corroborated by the DAPC, as well as the further separation of the last cluster (although again with few samples each).

In contrast, the RAxML phylogenetic relationships retrieved using the *cox*1-1-rRNA mitochondrial marker of *Leptoseris* spp. individuals (n = 101) from Australia (GBR and WCS) were mostly incongruent with the taxonomic identifications and with the different RAD clades (Figure 3), failing to distinguish among clades of *L. scabra*, *L. glabra,* and *L. hawaiiensis*. Samples of the different clades of *L. scabra* I, *L. glabra*, and *L. hawaiiensis* were distributed across five different mitochondrial haplotypes regardless of their location or depth. *L. mycetoseroides* specimens formed a single haplotype, with the exception of one specimen that grouped with samples of *L. scabra* I. Moreover, the more distant *L. scabra* II was observed across two haplotypes, and they did not share a haplotype with any other taxon.

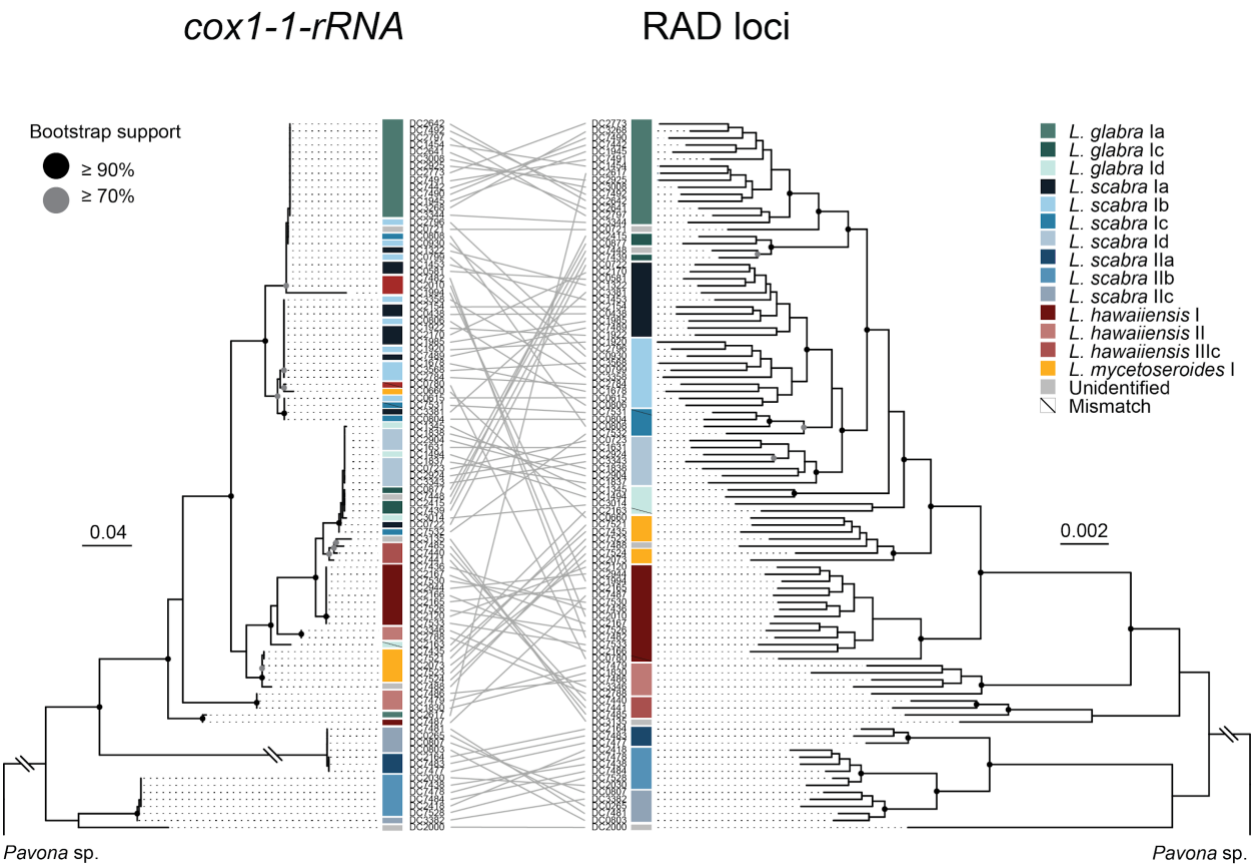

Figure 3. Comparison of Maximum Likelihood phylogenies of Australian *Leptoseris* using the mitochondrial *cox*1-1-rRNA marker and reduced-representation sequencing. RAxML phylogenies where the colors indicate the RAD clade and only bootstrap values ≥ 90% and ≥ 70% are shown.

Species delimitation patterns in the genus *Agaricia*

We genotyped a total of 10,424 RAD loci across the different *Agaricia* species. All four focal species formed well-supported monophyletic clades in the phylogenies reconstructed using maximum likelihood (RAxML) and Bayesian (MrBayes) approaches. The two approaches recovered highly similar topologies and placed all taxa in clades with strong node support. The phylogenies supported a major division of the genus into three major clades, one containing *A. grahamae*, *A. lamarcki*, *A. fragilis*, a second containing *A. undata*, and a third containing *A. agaricites*, *A. humilis*, and *A. tenuifolia* (Figure 4). For the latter "outgroup" species, it should be noted that *A. agaricites* and *A. tenuifolia* are confounded by the sampling region.

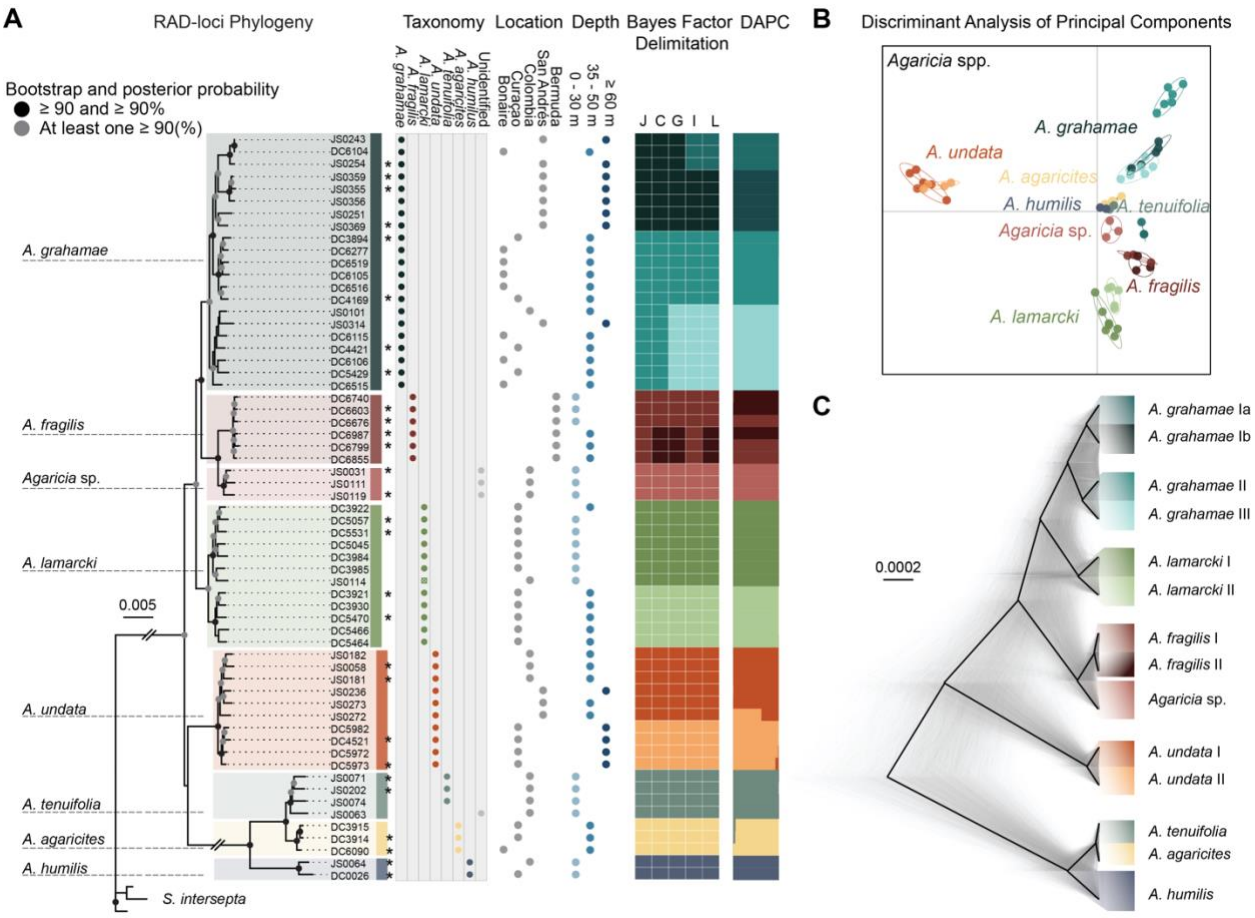

Figure 2. Phylogeny of the genus *Agaricia* using reduced-representation sequencing. (A) Phylogenetic RAxML tree using 10,424 loci, with bootstrap values based on Maximum Likelihood (ML) and Bayesian Inference (BI), where only values ≥ 90 and posterior probabilities ≥ 90% are shown. Colored dots in the "Taxonomy" column indicate the taxonomic identifications of the specimens used for this study. The "Location" and "Depth" columns indicate the region and depth of our sampling. The "Bayes factor Delimitation" columns correspond to the five best-supported BFD* models, where (*) indicates the samples with the least amount of missing data used for the BFD* analysis. The "DAPC" column indicates the posterior membership probabilities of the DAPC in section (B). (C) SNAPP species tree illustrates the best supported BFD* model.

The phylogenetic tree corroborated the taxonomic identifications but also confirmed further genetic differentiation within all four focal species dominant at mesophotic depths (*A. grahamae*, *A. lamarcki*, *A. fragilis*, and *A. undata*). Both the *A. grahamae* and *A. lamarcki* samples are split into two clades that occur sympatrically on Southern Caribbean reefs, with an additional split of one of the *A. grahamae* clades into three subclades, mostly represented by samples from San Andrés (South-western Caribbean; no samples from *A. lamarcki* were included for this location). The *A. undata* species is split into two clades that correspond with the Southern Caribbean (Curaçao) and South-western Caribbean (Colombia and San Andrés) sampling regions.

Representative samples of shallow and deep populations of *A. fragilis* from Bermuda separated in the tree but with minimal divergence. Of the four samples from Colombia that could not be reliably identified down to species, one grouped with *A. tenuifolia* samples from that region, whereas three grouped a separate clade most closely related to *A. fragilis* from Bermuda, indicating the presence of that clade in the Southern Caribbean.

We evaluated the observed separation within taxonomic species through DAPC, maximum clade credibility species trees (using SNAPP), and species delimitation model testing (using Bayes Factor Delimitation). For the coalescent BFD* framework, eleven species delimitation models were tested, with the model consisting of all of the above-mentioned splits (four clades in *A. grahamae*, two in *A. lamarcki*, two in *A. undata*, and three in *A. fragilis*) receiving the highest support (L, MLE: -1,938.54, BF: -2,337.46). For *A. grahamae* and *A. lamarcki*, this split was further confirmed by the SNAPP trees and the separation of clusters using DAPC (Figure 4, despite some overlap in *A. grahamae*). For *A. undata*, the split was confirmed by the SNAPP tree; however, the DAPC clusters showed substantial overlap. For the *"A. fragilis* clade", both SNAPP and DAPC showed clear separation of the *A. fragilis* from Bermuda and the unidentified samples from Colombia, although no clear separation was observed for the shallow and deep *A. fragilis* from Bermuda. The "*A. fragilis* clade" in the maximum clade credibility tree (MCC) appears to be ancestral to the *A. lamarcki* clade, as opposed to the concatenated trees. The *A. agaricites* and *A. tenuifolia* from respectively Curaçao and Colombia also showed no clear separation using both approaches.

Genetic structure across the Agariciidae family

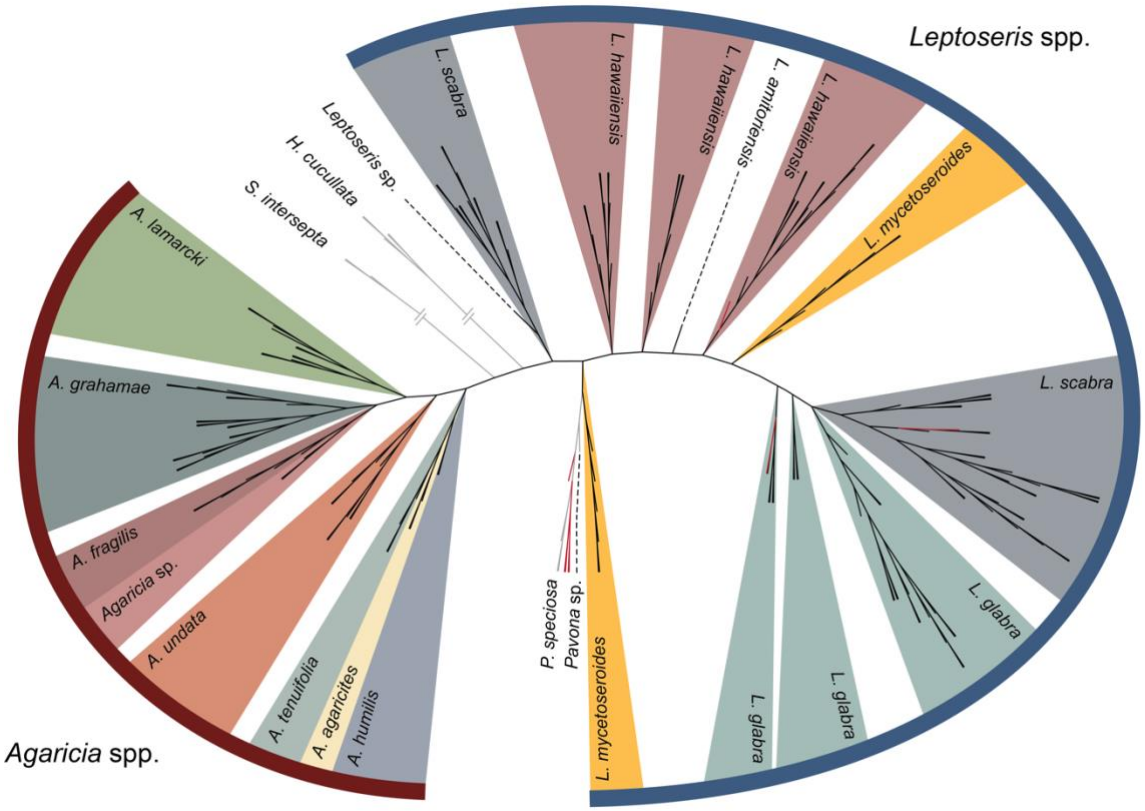

Figure 5. Unrooted tree of the Agariciidae family. R AxML phylogenetic tree, where each color indicates the taxonomic identification of the clade. The red and blue sections in the tree illustrate the *Agaricia* and *Leptoseris* genera respectively, and grey branches illustrate outgroups (*H. cucullata*, *P. speciosa*, *Pavona* sp., *S. intersepta*). Red branches in the tree indicate samples identified as a different taxon from where they grouped.

When combining all samples across the four different genera of the Agariciidae family, we obtained a total of 17,642 RAD loci. The two Agariciid genera (*Helioseris* and *Pavona*) used in this study as outgroups formed separate clades from *Leptoseris* and *Agaricia* (Figure 5). In both phylogenetic trees (RAxML and MrBayes), the *H. cucullata* specimens constituted a cluster outside the focal genera, which supported the separation of the genus *Helioseris* from *Leptoseris*. In addition to the substantial divergence among *Leptoseris* clades, five *Leptoseris* samples from the Red Sea, three of them identified as *L.* cf. *foliosa* and two as *L. mycetoseroides* formed a divergent clade that did not appear to be closely related to any other *Leptoseris* clade. In contrast, this cluster grouped in the same clade of *P. speciosa*, only used in this study as an outgroup, which

indicates that these specimens are most likely part of the *Pachyseris* genus. Moreover, a single unidentified *Leptoseris* sample (DC2000) was lumped with *L. scabra* II in the phylogenetic tree, albeit it represented a single and divergent branch within this clade.

Discussion

Based on the current taxonomy, most of the reported species at mesophotic depths represent deep-generalists rather than deep-specialists, and the overall richness –particularly at lower mesophotic depths– is low compared to shallow reefs (Muir et al. 2018). Nevertheless, recent population genetic studies focused on MCEs have challenged this notion, indicating the potential for substantial genetic differentiation at mesophotic depths (reviewed in Bongaerts and Smith 2019). Here, we provide a phylogenomic assessment of two Agariciid genera, demonstrating the suitability of reduced representation genome sequencing to resolve taxonomic boundaries in scleractinian corals and exposing substantial undescribed species diversity associated with mesophotic depths.

Genetic structure of the genus *Leptoseris*

In terms of zooxanthellate corals, MCEs across the Indo-Pacific are dominated by the genus *Leptoseris* (Englebert et al. 2017; Kahng et al. 2016; Muir and Pichon 2019). Although some work has been conducted to understand its physiological adaptations to cope with such extreme environments (Fricke et al. 1987; Enriquez et al. 2005; Chan et al. 2009; Padilla-Gamiño et al. 2019; Kahng et al. 2020), it remains unknown how their broad depth gradient and geographic distribution have shaped the evolution of *Leptoseris* species across the Indo-Pacific. Based on the mitochondrial regions of colonies from Hawaii, initial work suggested depth-specialization of *Leptoseris* species with specific endosymbiont-associations (Luck et al. 2013; Pochon et al. 2015). Additionally, studies exposed polyphyletic patterns across the genus, mainly caused by the inclusion of a highly divergent clade of *L. scabra* (Luck et al. 2013; Pochon et al. 2015; Terraneo et al. 2017). Nevertheless, the same studies also indicated that the taxonomic complexity due to homoplasy among distantly related species as well as variability in macromorphological traits between regions might be confounding the delimitation of *Leptoseris* species, highlighting a disagreement between the phylogeny and morphology-based taxonomy, and suggesting that the

current taxonomy does not accurately reflect the evolutionary relationships of this genus (Luck et al. 2013; Terraneo et al. 2017).

Using reduced-representation genome sequencing, we investigated the phylogenetic relationships of the four dominant mesophotic *Leptoseris* species in the Western Coral Sea and Great Barrier Reef and compared them with specimens from the Hawaiian Archipelago, US Minor Outlying Islands, Red Sea, and Australia (GBR and WCS). Initially, the phylogenetic relationships of 101 specimens of these four *Leptoseris* species from Australia based on the *cox*1-1-rRNA marker did not corroborate the taxonomic species. It confirms the generic pattern that while mitochondrial markers sometimes separate species within a certain geographic region, they generally lack resolution for species delimitation or do not accurately reflect overall evolutionary patterns and should be used cautiously (Flot et al. 2011; Bongaerts et al. 2020). Nevertheless, using two phylogenetic approaches (RAxML and MrBayes) on RADseq data, we separate most unambiguously identified samples of our four *Leptoseris* focal species into distinct and well-supported clades. However, for three of the four focal species, *L. scabr*a, *L. mycetoseroides*, and *L. hawaiiensis*, highly divergent clades were retrieved with further genetic differentiation within all clades. Especially *L. scabra* and *L. mycetoseroides* appear to have clades that are distantly related taxa to the rest of the *Leptoseris* spp., which is consistent with the previous observations of these taxa in Hawaii (Luck et al. 2013; Pochon et al. 2015), however, contrary to the patterns observed in these studies, both taxa form well-supported clades and do not group with either *Pavona* or *Agaricia* clades.

Specimens of *L. scabra* are scattered across two divergent clades (*L. scabra* I and II), which are further differentiated into seven sympatric subclades (all in Australia). *L. scabra* I, which consists of four sympatric subclades (*L. scabra* Ia - Id), exhibits a wide bathymetric range (10 - 82 m) and forms a sister group to *L. glabra*. Despite its wide bathymetric distribution, specimens from this clade were mostly retrieved from shallower depths, matching the depth ranges of *L. scabra* in the Red Sea (Terraneo et al. 2017), where *L. scabra* is commonly found at shallow depths and appears to be more closely related to other *Leptoseris* taxa (Terraneo et al. 2017). Although, we did not include *L. scabra* specimens from the Red Sea, and our sparse sampling of *L. scabra* I limits interpretation, comparing *L. scabra* I with *L. scabra* in the Red Sea, would shed light on whether these clades represent the same species, which could indicate that *L. scabra* I might be present in

Australasia, the Red Sea, and possibly other Indian Ocean regions (Sheppard 1987). The genetic subdivision of *L. scabra* I into four sympatric subclades in both of the phylogenetic trees was further confirmed by the BFD* analysis; however, the DAPC showed substantial admixture between *L. scabra* Ia and Ib, as well as between *L. scabra* Ic and Id, indicating signs of possibly hybridization within these two genetic groups. Based on these results, we argue that these four subclades represent two distinct evolutionarily units (*L. scabra* Ia + Ib, and *L. scabra* Ic + d) with an overlap in their depth and geographic distributions.

In contrast to *L. scabra* I's bathymetric range, *L. scabra* II was more frequently observed at lower mesophotic depths (≥ 60 m), which matches the depth distribution of *L. scabra* in Hawaii (Dinesen 1980, Luck et al. 2013), suggesting the it may represent a depth-specialist across Australasia and Eastern Pacific. In addition to this, *L. scabra* II appears to be highly distant to other *Leptoseris* taxa, which is also consistent with the patterns observed in Hawaii (Luck et al. 2013), where *L. scabra* was highly divergent from the rest of the *Leptoseris* taxa assessed in that study. Nevertheless, further comparison of this taxon with Hawaiian colonies (not included in this study) is needed to assess whether *L. scabra* II and *L. scabra* in Hawaii are genetically similar and could be considered the same species. The further genetic subdivision within *L. scabra* II was reflected by the three sympatric subclades (*L. scabra* IIa – IIc) found across multiple reefs in the WCS. Species delimitation analyses (BFD* and DAPC) separated these subclades, albeit with minimal admixture of *L. scabra* IIa and *L. scabra* IIb in the DAPC membership graph. These results indicate the presence of two distinct taxa with overlap depth and geographic distribution, and one subclade (*L. scabra* IIb) that perhaps reflects a population-level divergence of *L. scabra* IIa, as this subclade occurs on the GBR as well, as opposed to *L. scabra* IIa and IIc.

Although both *L. scabra* I and *L. scabra* II clades share the same macromorphological traits and were identified as the same taxonomic species, some specimens of *L. scabra* II had an atypical morphology (Dinesen pers. obs.), suggesting there might be diagnostic characters within *L. scabra* II differing from *L. scabra* I, that warrant detailed taxonomic revisions. One of these taxa might be *L. colamna*, described in Palau as a new species, based on its different calices and septo-costae (Yabe and Sugiyama 1941), but later synonymized to *L. scabra* (WoRMS 2020). However, a detailed taxonomic study would be able to assess whether or not they represent, in fact, two taxonomically distinct species.

In the case of *L. glabra*, we observed three sympatric subclades present in Australia (*L. glabra* Ia, c, d), including one that contained two specimens from the Red Sea as well (*L. glabra* Id), indicating extensive distributions of this subclade. A subtle genetic substructure was observed within *L. glabra* Id, which corresponded to the two geographic regions (Australia and the Red Sea); however, differentiation across regions could not be assessed due to our limited sample size after separating the individual subclades. In addition to the three sympatric subclades, the BFD* analysis supported the presence of a fourth one (*L. glabra* Ib) consisting of only two specimens from the Red Sea, although this subclade was grouped with *L. glabra* Ia on the DAPC, which suggests a population-level divergence rather than an additional species.

For *L. mycetoseroides*, we observed two highly divergent clades (*L. mycetoseroides* I, and *L. mycetoseroides* II) that corresponded to distant geographic locations (the WCS, Hawaiian Archipelago and US Minor Outlying Islands). In both phylogenetic trees, *L. mycetoseroides* I, mainly found at lower mesophotic depths, was placed close to *L. glabra* Id, while *L. mycetoseroides* II, mostly collected from shallow depths, was distantly related from the rest of *Leptoseris* spp., which is consistent with the results of Luck et al. (2013), who attributed the polyphyly of *Leptoseris* spp. to the inclusion of *L. mycetoseroides* into this genus. Besides geographic differences, the BFD* analysis supported the split of *L. mycetoseroides* II into two subclades (*L. mycetoseroides* IIa and IIb), although the DAPC revealed overlap between both subclades, suggesting a population-level differentiation. Moreover, morphological variations have been already documented by Dinesen (1980), who distinguished two extreme morphologies differing mostly by the development of collines, one group having collines with acute tops as opposed to the other that has rounded collines; the holotype from Bikini atolls (Wells 1954) belongs to the first group (Pichon, pers. comm). Based on the widespread sampling locations of *L. mycetoseroides* II across the Central and North Pacific (Gardner Pinnacle, Howland Island, Oahu, and Palmyra Atoll), and taking into account the site of the holotype, we argue that this clade might represent the true *L. mycetoseroides*, while the Australian clade denotes a different undescribed species. Furthermore, the presence of two distinct morphologies correlated with the deep divergence observed in the phylogeny suggests that these clades represent two clearly distinct species with a need for taxonomic revision. It is also important to note that the differences in depths where these specimens were collected do not necessarily represent their depth range, as *L. mycetoseroides* colonies across the Indo-Pacific have been encountered from shallow to the lower-

mesophotic zone (e.g., Englebert et al. 2017; Denis et al. 2019; Pichon 2019; Andradi-Brown et al. 2019).

For *L. hawaiiensis*, a common member of lower mesophotic habitats (e.g., Englebert et al. 2017; Muir and Pichon 2019; Pichon 2019), we observed three sympatric but highly divergent clades across the genus. *L. hawaiiensis* I, from Australia (WCS), appears to be more closely related to other *Leptoseris* species than the other two clades and includes a specimen of *Leptoseris* sp. 1 (*sensu* Luck et al. 2013) from an isolated reef in Hawaii (Nihoa; ~700 km from the Big Island). The eminent resemblance between the skeleton development and macromorphology of *Leptoseris* sp. 1 and *L. hawaiiensis* (Kahng et al. 2020) may have contributed to the lack of reports of the former across the Great Barrier Reef and the Coral Sea; since without examining micromorphological traits such as septal teeth, it is challenging to recognize between these two taxa (Luck et al. 2013). Moreover, the bathymetric distribution of this clade corresponds to the one reported for *Leptoseris* sp. 1 (~10 - 80 m; Luck et al. 2013). Based on these results, we suggest *L. hawaiiensis* I might reflect the occurrence of *Leptoseris* sp. 1 in the reefs of Eastern Australia.

L. hawaiiensis II and III, both sympatrically occurring in Australia and Hawaii, were observed in contrasting depth ranges. While *L. hawaiiensis* II was encountered at shallower depths than previously reported for this species (Rooney et al. 2010; Luck et al. 2013; Pochon et al. 2015), *L. hawaiiensis* III was only found at lower mesophotic depths, which is more consistent to the depth ranges of this species in both Australia and Hawaii (Luck et al. 2013; Englebert et al. 2017). This clade also included a specimen collected at the GBR at 124 m, representing the deepest published report of a zooxanthellate coral collected from the GBR (Englebert et al. 2017). Despite being identified as *L.* cf. *fragilis,* this specimen had morphological characters similar to *L. hawaiiensis* in Luck et al. (2013). This divergence between both clades suggests the presence of a depth-generalist (*L. hawaiiensis* II) and a depth-specialist clade (*L. hawaiiensis* III), although this would have to be explored with larger sample sizes. Furthermore, the BFD* analysis supported the split of *L. hawaiiensis* III into three distinct subclades (*L. hawaiiensis* IIIa – IIIc), two sampled at lower mesophotic depths in Hawaii (*L. hawaiiensis* IIIa and IIIb), and one only sampled in Australia (*L. hawaiiensis* IIIc). However, all three subclades (*L. hawaiiensis* IIIa – IIIc) significantly overlapped in the clustering analysis (DAPC) when comparing all *L. hawaiiensis* clades (I – III), which most likely reflects a population-level divergence of samples from Eastern Australia and Hawaii.

Although based on our limited sampling size, this interpretation should be taken with caution. It is also important to note that most of the specimens of *L. hawaiiensis* IIIc were identified as *L.* cf. *hawaiiensis* due to their atypical morphology, which could be attributed to the different habitats these subclades occupy. Nevertheless, it warrants a detailed taxonomic description to assess differences in diagnostic characters.

Overall our results corroborate the complexity of *Leptoseris* across the Indo-Pacific. Based on genetic data, we confirm the species boundaries within this genus are not congruent with the current taxonomic classification and that *Leptoseris* is in a clear need of a taxonomic revision. Based on the that we suggest the presence of several undescribed species with similar macromorphological traits that might be masking the broad diversity within this genus. One issue why this diversity may have been overlooked is that the holotypes' descriptions were primarily based on shallow colonies (Wells 1954; Dinesen 1980), which do not share the same environmental variables with deeper reefs and may therefore show substantial morphological divergence. From a biogeographic perspective, landmasses, broad ocean basins, and temperature gradients separate the Indo-Pacific into three regions, Western, Central, and Eastern Indo-Pacific (Spalding et al. 2007). As previously observed for other genera, such as *Acropora* or *Pocillopora* (Ladner and Palumbi 2012; Schmidt-Roach et al. 2013), it is not surprising that we observe such variability in *Leptoseris* across regions. Many island archipelagos across the Indo-Pacific realm are relatively isolated, which often translates to high levels of endemism and evolutionary isolation of species (Spalding et al. 2007; Veron et al. 2013). Nevertheless, geographic isolation cannot account for all of the diversity of *Leptoseris* that we exposed, as many sympatrically occurring clades were observed. This further subdivision of clades of all four focal species into sympatric subclades could reflect isolation of taxa occupying distinct ecological niches, even in the absence of geographic barriers (Beltman and Metz 2005; Slagsvold and Wiebe 2006; Ladner and Palumbi 2012), which should be further explored in more targeted follow-up studies.

Genetic structure of the genus *Agaricia*

Despite being an ecologically important reef-builder across Western Atlantic and Caribbean reefs, *Agaricia*'s phylogeny remains rather unresolved, in part due to their complex taxonomic history (Wells 1956, 1973; Stemann 1991; Budd et al. 1994) and the molecular markers until recently

available (Kitahara et al. 2012; Bongaerts et al. 2013, 2015). Using traditional sequence markers, such as *atp*6, *nad*5, and *cox*1-1-rRNA regions, previous studies could distinguish between two major clades that differed in bathymetric distribution (Bongaerts et al. 2013, 2015). However, these markers failed to discriminate between different species, such *A. grahamae* and *A. lamarcki* (Bongaerts et al. 2015). Using reduced-representation genome sequencing, we were able to resolve the phylogenetic relationships of all our four focal species and detect additional genetic subdivision within most taxa, highlighting the resolving power of RAD-sequencing to explore coral diversity and giving us insight into the undescribed diversity within this genus across Caribbean reefs. Similar to the patterns observed by Bongaerts et al. (2013, 2015), we could identify two major clades within the *Agaricia* genus using two phylogenetic approaches (RAxML and MrBayes). The divergence between these two clades corresponds to their bathymetric distributions (Bongaerts et al. 2013) and the two morphologies previously observed across *Agaricia* spp. (Stemann 1991; Budd et al. 1994): "deep" and unifacial plates (*A. grahamae*, *A. lamarcki*, *A. fragilis*, and *A. undata*), and "shallow" and bifacial colonies (*A. agaricites*, *A. humilis*, and *A. tenuifolia*). In addition to the separation of the genus into two groups, a third deep divergence was observed within the "deep" clade, separating *A. undata* as a distinct clade.

In both phylogenetic trees, each taxonomic species formed a distinct and well-supported clade, yet, additional genetic differentiation was found across our four focal species (*A. grahamae*, *A. lamarcki*, *A. fragilis*, and *A. undata*). The extent of this genetic substructure and the divergence between subclades varies across these taxa. Individuals of *A. grahamae* are differentiated into three large clades that occur sympatrically in Southern Caribbean reefs. Two of the *A. grahamae* clades (*A. grahamae* II and III) were found at the same depth (~ 50 m) and same island (Bonaire and Curaçao) with no apparent genetic structure between both islands, despite being separated by a deep-water trench (4000 - 5000 m; Frade et al. 2019). Furthermore, the BFD* analysis supported an additional split within *A. grahamae* I, which corresponds to two deeper clades (*A. grahamae* Ia and Ib) that occur sympatrically in the South-western Caribbean. *A. grahamae* Ia included mostly specimens from San Andrés (South-western Caribbean), although one sample from Bonaire grouped with this subclade as well, which was unexpected, as these two islands are located in two different ecoregions (Williams et al. 2015). *A. grahamae* Ib consists exclusively of samples from San Andrés collected at lower mesophotic depths (> 60 m), and its divergence with *A. grahamae* I and II are most likely the result of allopatric separation, due to the distance of ~1,300 km between

this oceanic island and the island of Curaçao (Southern Caribbean). When analyzing all four *A. grahamae* subclades (*A. grahamae* Ia, Ib, II, III), assignments of DAPC and the gene trees (SNAPP) corroborate this divergence between the clades *A. grahamae* Ia-b and *A. grahamae* II-III, which strongly support the species-level differentiation of these taxa.

In the case of the specimens of *A. lamarcki*, there is a split that mostly corresponds to the separation between upper-mesophotic depths (30-60 m) and shallow habitats of the reef (< 30 m). This genetic subdivision of *A. lamarcki* in Curaçao contrasts the patterns observed in Puerto Rico, where strong population connectivity was found across mesophotic and shallow populations of this taxon (Hammerman et al. 2018). Nevertheless, the small sampling size and depth range included in that study could have possibly masked the genetic structure in these reefs, since significant depth zonation in the endosymbiont associations of *A. lamarcki* has been documented in the same region and Curaçao (Bongaerts et al. 2013; Lucas et al. 2016). From a phylogenetic perspective, our results indicate the presence of two sympatrically occurring clades in the reefs of Curaçao that might be associated with distinct microhabitats, as previously observed within this taxon (Laverick et al. 2019). The genetic substructure observed in both taxa, *A. grahamae* and *A. lamarcki*, stresses the need to assess environmental factors that might be driving the formation of these sympatric clades, such as the presence of microhabitats or differences in spawning times. Nevertheless, in both cases, the clades of *A. grahamae* and *A. lamarcki* represent genetically distinct individuals and should be considered separate evolutionarily significant units.

For *A. undata*, we observed genome-wide divergence between the Southern Caribbean (Curaçao) and the South-western Caribbean (Colombia and San Andrés) regions, but not depth, which is consistent with the results of Gonzalez-Zapata et al. (2018a). Although their results also showed a divergence between locations within the South-western Caribbean region, that divergence might be masked by our limited sampling size of those locations. A possible explanation for the regional difference between the Southern and South-western Caribbean might be attributed to environmental and community structure differences between both regions (Chollett et al. 2012; Williams et al. 2015). Regional differences have also been documented in the type of endolithic algae *Ostreobium* associated with *A. undata*, which has been hypothesized to be essential for this taxon to cope with lower-mesophotic habitats (Land et al. 1975; Schlichter et al. 1995, Gonzalez-Zapata et al. 2018b).

Driven by the results of Bongaerts et al. (2017), representatives were included in this study to compare the genetic structure observed in the *A. fragilis* population from Bermuda with its congeners. The shallow and deep populations of this taxon did separate in both phylogenetic trees (RAxML and MrBayes); however, this divergence was minimal in both phylogenetic trees and the gene trees (SNAPP), compared to other focal species. The membership graph of the DAPC showed both clades are substantially admixed, suggesting a population-level divergence rather than species differentiation, as previously observed by Bongaerts et al. (2017). In addition to the subtle genetic structure observed within our *A. fragilis* clade, three of the unidentified samples from Colombia, referred here as *Agaricia* sp., form a separate clade sister to *A. fragilis* in the phylogenetic trees, although phenotypically they looked similar to *A. agaricites* (González-Zapata and Sánchez, pers. obs.). This divergence between the *A. fragilis* clades and the *Agaricia* sp. clade was supported by the SNAPP tree, where the split of these clades is deeper than the split between both *A. fragilis* clades (*A. fragilis* I and II). Similarly, the genetic divergence between *Agaricia* sp. and *A. fragilis* was supported by the DAPC membership graph, where *Agaricia* sp. and *A. fragilis* specimens are assigned to separate clades. This group (*Agaricia* sp.) could reflect an allopatric clade of *A. fragilis*, suggesting a strong geographical divergence between the *A. fragilis* in Bermuda and the South-western Caribbean, or possibly an undescribed taxon related to *A. fragilis*. The extent of the divergence is comparable to the genetic structure observed in *A. grahamae* and *A. lamarcki*, which stresses the importance of including multiple regions and taxa to assess undescribed diversity within corals.

The similarity of *A. tenuifolia* and *A. agaricites*, based on their location in the tree, the short branches in the SNAPP tree, suggests that these specimens might represent two clades of the same taxon. Given that location and species are confounded in this study, we cannot distinguish whether the observed genetic differentiation corresponds to inter-specific or geographic variation. The resemblances of these taxa and the great morphological variation within both of them have been observed across several Caribbean reefs (Zlatarski and Estalella 1982; Fenner 1999) and could also mean that the *A. tenuifolia* specimens were misidentified. However, given the morphological similarity of these species, it warrants further investigation.

Our results confirm that the genus *Agaricia* represents a taxonomically and genetically diverse group and corroborate the partitioning into two major clades. These results support the

morphological work done by Stemann (1991), where, based on intraspecific characters of modern and fossil representatives, they classified the seven *Agaricia* spp. into two different groups, *Agaricia* (*A. grahamae*, *A. fragilis*, *A. lamarcki*, *A. undata*) and *Undaria* (*Undaria tenuifolia*, *U. agaricites*, *U. humilis*) and demonstrated no overlap between the two groups. However, these groups are not adopted or formally acknowledged. In terms of undescribed diversity beyond described species, the extent of divergence seems to vary greatly. We observed species-level (*A. grahamae* and *A. lamarcki*), regional (*A. undata*), and population-level divergence (*A. fragilis*), which highlights the different evolutionary processes that would have shaped this genus through geographical and ecological factors. While the fragmentation of populations by geographic barriers might have led to an allopatric divergence of some *Agaricia* species, we also found the occurrence of sympatric clades, suggesting disruptive selection, ecological partitioning, or temporal reproductive isolation might be driving this divergence (Carlon and Budd 2002; Frade et al. 2010). Distinguishing between species- and population-level divergence remains challenging as speciation is a continuum process and species delimitation analyses often tend to oversplit genetic groups (Derkarabetian et al. 2019). Here, we demonstrate that substantial undescribed diversity is present across this genus and that strong regional divergence might have shaped the phylogeny of this genus. Furthermore, our results highlight the urgency to assess the ecological factors involved in the differentiation of *Agaricia* species, including their reproductive strategies, which remain unknown in the case of our focal species.

Conclusions

Our findings shed light on the diversity of two understudied mesophotic keystone genera, *Leptoseris* and *Agaricia*, and confirm that species richness in MCEs may be higher than previously acknowledged, exposing at least seven undescribed *Leptoseris* species and three undescribed *Agaricia* species. Detailed taxonomic studies are now needed to address whether there are diagnostic macro- or micromorphological characters that delineate the uncovered genetic clades. In addition, the presence of genetic groups associated with different depth ranges highlights the urgency to further explore biogeographic patterns and ecological niches of mesophotic taxa to understand the actual species richness of these ecosystems. Our work contributes to the growing number of studies in which undescribed diversity in scleractinian corals has been found using

molecular approaches (e.g., Richards et al. 2016; Sheets et al. 2018; Bongaerts et al. 2020; Oury et al. 2020; Prata et al. in preparation) and demonstrates the utility of reduced-representation genome sequencing approaches in coral systematics. Assessments of species boundaries and understanding the genetic divergence patterns involved are critical for protecting these ecosystems, particularly considering the fast decline corals reefs are facing as a result of climate change.

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