Morphological stasis masks ecologically divergent coral species on tropical reefs

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ABSTRACT

Coral reefs are the epitome of species diversity, yet the number of described scleractinian coral species, the framework-builders of coral reefs, remains moderate by comparison. DNA sequencing studies are rapidly challenging this notion by exposing a wealth of undescribed diversity, but the evolutionary and ecological significance of this diversity remains largely unclear. Here, we present an annotated genome for one of the most ubiquitous corals in the Indo-Pacific (*Pachyseris speciosa*), and uncover through a comprehensive genomic and phenotypic assessment that it comprises morphologically indistinguishable, but ecologically divergent cryptic lineages. Demographic modelling based on whole-genome resequencing disproved that morphological crypsis was due to recent divergence, and instead indicated ancient morphological stasis. Although the lineages occur sympatrically across shallow and mesophotic habitats, extensive genotyping using a rapid diagnostic assay revealed differentiation of their ecological distributions. Leveraging "common garden" conditions facilitated by the overlapping distributions, we assessed physiological and quantitative skeletal traits and demonstrated concurrent phenotypic differentiation. Lastly, spawning observations of genotyped colonies highlighted the potential role of temporal reproductive isolation in the limited admixture, with consistent genomic signatures in genes related to morphogenesis and reproduction. Overall, our findings demonstrate how ecologically and phenotypically divergent coral species can evolve despite morphological stasis, and provide new leads into the potential mechanisms facilitating such divergence in sympatry. More broadly, they indicate that our current taxonomic framework for reef-building corals may be scratching the surface of the ecologically relevant diversity on coral reefs, consequently limiting our ability to protect or restore this diversity effectively.

INTRODUCTION

Tropical coral reefs are known for their high levels of biodiversity, harboring hundreds of thousands of macroscopic and an unknown number of microscopic species (1). Interestingly, the contribution of reef-building corals remains surprisingly moderate with only ~750-850 valid species accepted worldwide (2–5). Under the current taxonomic framework, species are distinguished primarily based on diagnostic skeletal characteristics, an approach that originated from the early days of coral reef science when underwater observations were extremely challenging (6, 7). Skeletal traits at both the corallite and colony level are often highly variable

within species and across environments, posing a major challenge to coral taxonomy (8). Conversely, skeletal traits are known to converge and can even obscure deep phylogenetic divergences at the family level (9, 10), highlighting further challenges to the use of the skeletal morphospace as a taxonomic framework. Molecular approaches have helped resolve some of these difficulties by differentiating morphological plasticity from actual species traits (11, 12), confirming species separation in the context of subtle morphological differences (13–15), and clarifying deeper evolutionary relationships within the order (9, 10). However, molecular studies have also uncovered a wealth of undescribed diversity within taxonomic species that cannot be readily explained (11, 16–24).

The notion that scleractinian coral diversity may be far greater than acknowledged through conventional taxonomy is not novel. A seminal review published over two decades ago highlighted the ubiquitous presence of sibling species in the marine realm, and stressed the importance of exploring its ecological relevance (25). Since then, molecular studies have indeed exposed "taxonomically cryptic diversity" (i.e. genetically distinct taxa that have been erroneously classified under a single species name) within many coral genera (21, 26). However, much of this cryptic diversity is being exposed through population genetic studies designed to relate genetic patterns to geography (26), and consequently, it usually remains unclear to what extent they represent phenotypically and ecologically distinct entities [but see (27–31)], including whether lineages are truly morphologically cryptic. In fact, well-studied examples of coral species complexes are characterized by substantial morphological differentiation (14,16,17,20,27), and we still know little about the potential for ecological or phenotypic differentiation when gross morphology is constrained. The traditional use of only a few genetic loci has also impeded an assessment of the evolutionary context of cryptic diversification, and the respective roles of neutral versus selective processes on ecological or reproductive traits (31, 32). Thus, the ecological relevance of cryptic diversity in corals remains poorly understood and is therefore rarely considered in ecological studies or conservation planning.

To address this knowledge gap, we conducted a comprehensive assessment to evaluate the nature of cryptic diversity in *Pachyseris speciosa* ("Serpent Coral"; Figure 1a), one of the most ubiquitous and abundant species across the Indo-Pacific (3). This zooxanthellate coral has one of the widest bathymetric distributions on tropical coral reefs –from close to the surface to lower mesophotic depths (~5-95 m) (33)– and has therefore been the focus of studies assessing

its ecological opportunism (34–37). Our initial assessment of its genetic structure indicated the presence of undescribed sympatric lineages, and we therefore used this as an opportunity to explore the extent to which cryptic diversity can obscure genomic, ecological and phenotypic divergence, and how reproductive isolation may be maintained in such closely-related lineages. Specifically, our study (summarized in Figure 1) involved the generation of an annotated genome for *P. speciosa* using long-read sequencing, which we used as a reference for reduced-representation sequencing (nextRAD) of populations ranging from across the geographic and bathymetric distribution of this species. Subsequent whole-genome resequencing (WGS) of representative coral colonies was implemented for demographic modelling, and for the development of a cleaved amplified polymorphic sequence (CAPS) assay to assess ecological, phenotypic and reproductive differentiation in the uncovered lineages from Australia. Overall, our findings demonstrate the potential for ecological and phenotypic divergence under morphological stasis, and highlight that conventional skeleton-based taxonomy may be substantially underestimating the ecologically-relevant diversity of scleractinian corals on tropical coral reefs.

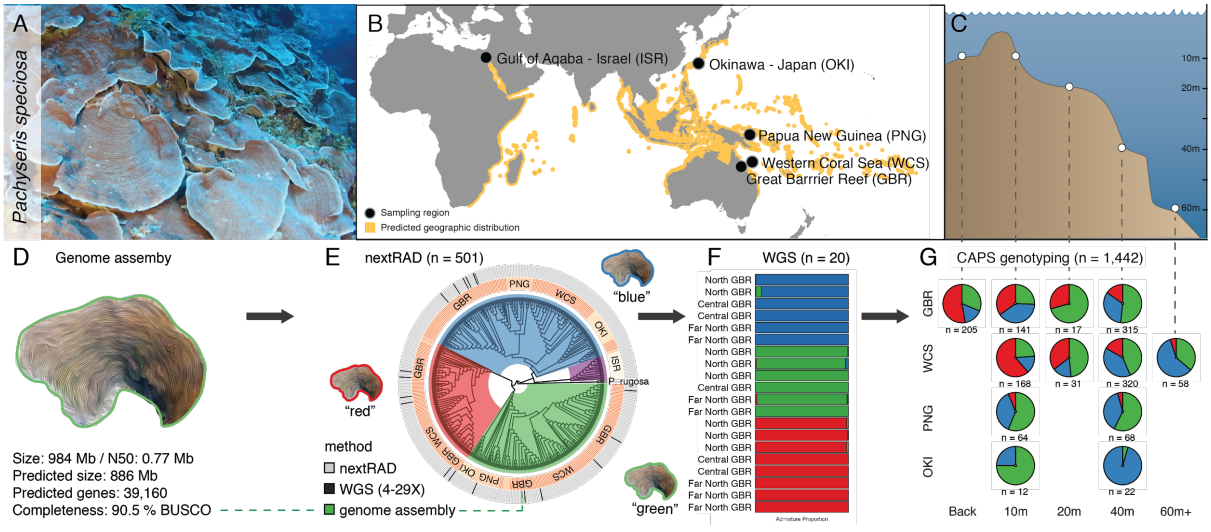

Figure 1. Overview of the study system and sequencing/genotyping approach. (A) *Pachyseris speciosa* at 40 m depth at Holmes Reef (Western Coral Sea). (B) Predicted geographic distribution of *P. speciosa* (*sensu lato*) based on the IUCN Red List (2009), and the location of the five regions included in this study. (C) Sampled habitats and their corresponding water depths (± 3 m). (D) Genome assembly summary statistics. (E) Circular phylogenetic tree summarizing the reduced-representation sequencing (nextRAD) data, and showing the partitioning into four major lineages. Sample region is indicated in the inner surrounding circle, and selection for whole-genome sequencing indicated in the outer surrounding circle. (F) Admixture proportion (PCAngsd) based on whole-genome resequencing (WGS) of representative samples belonging to the three lineages occurring on the

Great Barrier Reef. (G) Geographic and ecological distribution of the lineages across habitats and regions (excluding Israel). Assignments are based on the CAPS genotyping assay merged with the nextRAD data (note that assignments for Okinawa are based exclusively on nextRAD data).

Results and Discussion

Genome assembly and annotation. We generated a highly contiguous reference genome for *Pachyseris speciosa*, assembled through PacBio single-molecule long-read sequencing (Figure 1d; Table S2). The assembled genome size is 984 Mb, representing one of the largest coral genomes reported to date, and comprising 2,368 contigs with a N50 size of 766.6 kb (largest contig 4.6 Mb; Table S3,4). In total, 39,160 protein-coding genes were predicted – a number comparable to that of two other recently sequenced corals from the "robust" clade [i.e. one of the two major clades of extant corals; (38)] – with the gene model being 90.5% complete and 5.1% partial (based on conserved single-copy metazoan orthologous genes) (Table S5,6,7). *De novo* repeat annotation revealed that 52.2% of the assembly is occupied by repetitive elements, which are generally better-resolved in long-read assemblies (39). The dominance of transposable elements compared with other robust corals indicates that the larger genome size may be substantially driven by expansion of those transposons (38) (Table S8).

***Pachyseris speciosa* represents a sympatric species complex.** The annotated genome assembly was used as a reference for reduced-representation sequencing (nextRAD), where we sequenced *P. speciosa* colonies (n = 501) from >30 sites from Australasia [the Great Barrier Reef (GBR), the Western Coral Sea (WCS), and Papua New Guinea (PNG)], Okinawa (OKI), and Israel (ISR) (Figure 1E, S1; Table S1). Genetic structuring based on principal component and neighbor-joining analyses revealed the presence of four highly divergent lineages (Figures 2, S2). One lineage represents the geographically separated Israel population (Gulf of Aqaba) and indicates the existence of a distinct species in the Red Sea region, corroborating a pattern observed in other corals [e.g. *Stylophora pistillata*; (24, 40–41)]. The other three lineages occurred sympatrically on all of the sampled reefs in Australasia, hereafter arbitrarily referred to as "green", "blue" and "red" lineage, with the assembled reference genome representing a genotype belonging to the "green" lineage. Subsequent structure analyses using a maximum likelihood framework (snapclust) indicated an optimum between four and six clusters, and the additional two clusters (for k = 5 and 6) correspond to two populations from Okinawa that grouped with respectively the "blue" and "green" lineages but were substantially differentiated from those in eastern Australia (Figures S2,S3). Beyond this expected geographic

148 differentiation of sub-tropical Okinawa, the overall structuring demonstrates the presence of
149 genetically distinct lineages within the currently acknowledged species, *P. speciosa*.

150 Compared to the divergent, congeneric species *Pachyseris rugosa* [characterized by a more
151 irregular and bifacial morphology (2), outgroup at the bottom of the tree; Figure 2a], the three
152 *P. speciosa* lineages in Australasia represent a closely-related species complex. Differentiation
153 between the "red", "green" and "blue" lineages is observed across the genome and global mean
154 F_{ST} values are much higher (average = 0.1256; range: 0.1032-0.1613) than between geographic
155 regions within these lineages (average = 0.0207; range: 0.0130-0.0339) (Figure 2, S4). The two
156 lineages in Okinawa are more related to the "green" and "blue" lineages, respectively, from
157 Australia than to each other (global mean Fst = 0.1324). Similarly, the "purple" lineage
158 exclusive to Israel (Red Sea) is on average the most divergent of all the lineages, with the
159 closest relatives representing the "blue" lineage from Australia. The apparent restricted
160 distribution of the "red" lineage in Australasia indicates that it may have originated in that
161 region. Indications of admixture (i.e. samples with a maximum assignment < 0.95) between
162 the three lineages were minimal with the exception of one F1 hybrid on the Great Barrier Reef
163 (Figure 2, S5). This coral had a roughly equal assignment to the "green" and "blue" cluster,
164 and a heterozygous genotype for 35 out of 39 genotyped SNPs that were alternatively fixed
165 between corals belonging to these two clusters. Admixture signatures of most other putatively
166 admixed samples were indicative of analytical artefacts (e.g. due to admixture with an
167 unsampled population/lineage; Figure S5). Overall, the limited admixture and presence of only
168 a single F1 hybrid (with its reproductive viability being unknown), indicates that the three
169 lineages have evolved reproductive isolation. This, combined with their widespread sympatric
170 distribution, confirms that these lineages represent distinct species (with their formal
171 description underway; Muir and Bongaerts in preparation), adding to the ever-growing number
172 of cryptic species within the Scleractinia (21, 26).

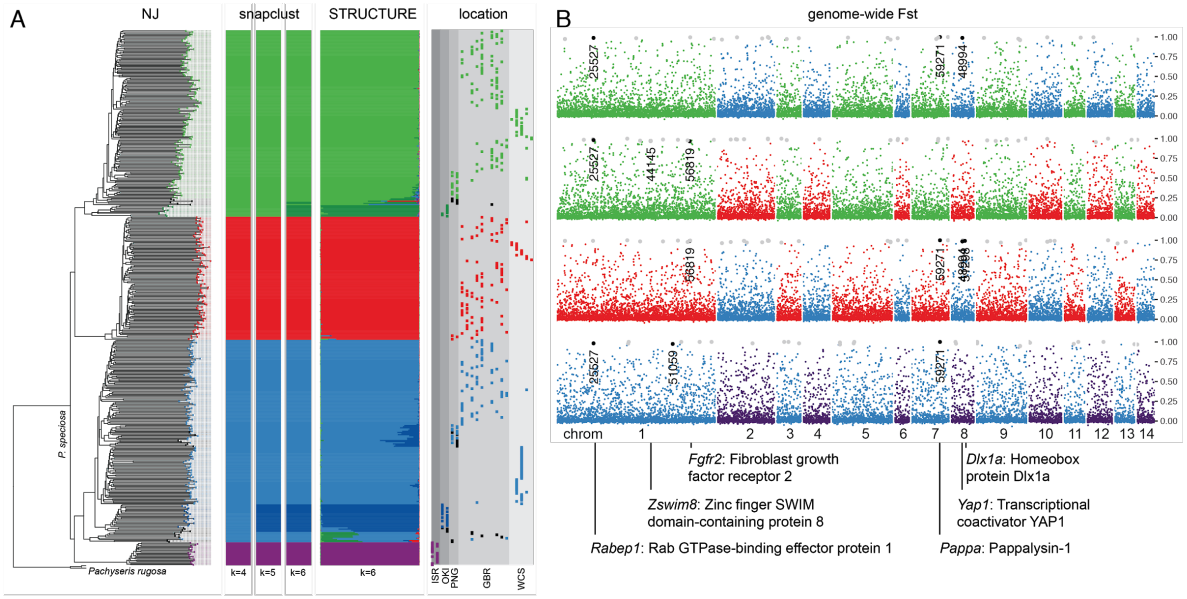

Figure 2. Genetic structuring and genome-wide differentiation of *P. speciosa* lineages. (A) Neighbor-joining tree based on variant nextRAD sites, with matching snapclust (k = 4 to 6) and STRUCTURE (k = 6) assignments for each coral colony. Dots in the "location" column indicate sampling population (geographically arranged within region) with the color indicating STRUCTURE assignment (also indicated in the branch tips of the tree; black indicates potentially admixed samples with a maximum assignment of < 0.95). The bottom clade represents the *Pachyseris rugosa* outgroup (n = 3) and is not included in the adjacent clustering analyses. (B) Manhattan plots showing genome-wide distribution of F_{ST} values for different *P. speciosa* lineage comparisons. Individual single-nucleotide polymorphisms (SNPs) are plotted along pseudochromosomes (mapped to *Acropora millepora* for visualization purposes; showing only those that mapped), with the alternating colors indicating the two lineages compared in each plot ("green", "red", "blue" and "purple"). Gray and black dots indicate SNPs that are alternatively fixed, with black SNPs indicating those that are missense variants (labelled with their gene ID). Missense variants relating to the inter-comparison of the three Australasian lineages are additionally labelled with the corresponding UniProt gene and protein names (note that two variants, identified as located in *Hecw2* and an unidentified gene, are not mentioned here as they did not map to *A. millepora* chromosomes).

Morphologically cryptic lineages despite ancient divergence. An assessment of *in situ* photographs of colonies from the Great Barrier Reef and Western Coral Sea unveiled extensive morphological variability within lineages, but with no apparent distinguishing features between lineages (n = 157; Figure S6). Examination of qualitative traits (including known diagnostic traits for the genus) using skeletal specimens, again found substantial variation but this was not partitioned across the three different lineages (n = 36; Table S9). Although some of the skeletal features overlapped with those described for other *Pachyseris* species (2, 42), the lineages did not align with one of the other five taxonomic species described in this genus [of which only two are reported from Australasian waters; (2)]. An initial assessment of micro-skeletal features

using scanning electron microscopy also did not reveal differentiating characteristics between the three lineages (n = 15; Figure S7).

In contrast to the lineages being morphologically indistinguishable, they are divergent genomically with 146 alternatively-fixed SNPs among the three lineages in Australia (0.5 % of 29,287 SNPs; Figure 2). This included 8 missense variants (i.e. a nonsynonymous substitution producing a different amino acid) located in genes of which 7 had homology to UniProtDB genes (IDs: *Rabep1*, *Yap1*, *Pappa*, *HecW2*, *Zswim8*, *Fgfr2* and *Dlx1a*). The Fibroblast growth factor receptor 2 gene (*Fgfr2*) is thought to be involved in environmental sensing and larval metamorphosis (43-46). The *Dlx1a* gene is part of the homeobox-containing superfamily generally associated with morphogenesis (47). The *Pappa* gene encodes the pregnancy-associated plasma protein-A, which is upregulated during spawning in *Orbicella franksi* and *Orbicella annularis* (48).

Whole-genome resequencing of representative "green", "blue" and "red" colonies from the Great Barrier Reef (n = 20 at 4-29X coverage; Table S10) further confirmed the strong genetic differentiation with minimal admixture (Figure 1f, S8). To assess whether the three lineages have diverged recently and/or experienced differences in demographic history, we used the Pairwise Sequentially Markovian Coalescent [PSMC; (49)] approach for a subset of colonies that were sequenced at greater depth (14-27x coverage; n = 10). Modelled demographic histories showed much greater variation between than within lineages, suggesting an ancient divergence as far back as 10 mya (Figure 3), and that the cryptic nature is due to morphological stasis rather than recent divergence. All lineages peaked in effective population size around 1-3 mya which is consistent with PSMC analyses in recent studies on other robust (50) and complex (51) corals. We also constructed circularized mitochondrial genomes from the whole-genome resequencing data (~19 kbp in length), which in contrast only showed a limited assortment of mitochondrial haplotypes into lineage-specific groups (Figure S9). Given the limited nuclear admixture, this is likely to reflect incomplete lineage sorting and/or ancient introgression, combined with very low mutation rates typical of anthozoans (52, 53). It also reiterates how mitochondrial regions in corals for species-level phylogenetics should be used cautiously (39).

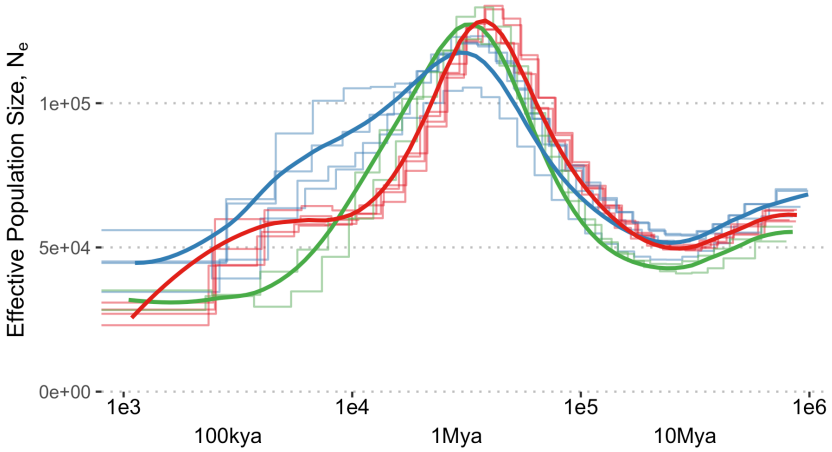

Figure 3. Demographic history as inferred through whole-genome resequencing. Changes in inferred, effective population size as estimated using MSMC for 10 deeply sequenced coral colonies representing the three *Pachyseris* lineages occurring sympatrically in Australia. The timescale is shown in units of numbers of generations in the past. Additional x-axis labels show time in the past assuming a generation time of 35 years. A mutation rate of $4.83e^{-8}$ was assumed for all calculations (50).

Ecological and phenotypic differentiation of the lineages. Given the lack of diagnostic morphological features discriminating the three *Pachyseris* lineages, we designed a cleaved amplified polymorphic sequence (CAPS) assay based on the nextRAD data to allow for high-throughput or field-based genotyping (Figure S10; Table S11). We used the rapid assay to expand the number of identified genotypes in Australia (n = 1,442) to include a broad range of reef habitats and locations, and assessed whether there are other ecological and/or phenotypic traits that distinguish the three lineages. The genotyping confirmed the ubiquitous sympatric distribution, with 39 out of 45 sampled Australasian sites containing representatives of all three lineages (Figure 4A). However, there was a significant overall effect of habitat and region on the relative abundances of the three lineages, with pairwise tests indicating significant differences between shallow (back-reef and 10 m) and mesophotic (40 m and 60 m) habitats (confined to GBR and WCS; Table S12; Figure 1G). When assessing the proportions of individual lineages over depth, the "red" lineage was significantly more abundant on shallow (back-reef and 10 m) as compared to intermediate (20 m) and mesophotic (40 and 60 m) habitats, with the opposite pattern observed for the "green" and "blue" lineages (Figure 4b; Table S13). Although the "red" lineage was not found in Okinawa, the divergent "green" and "blue" lineages (as identified using nextRAD sequencing) appeared to be associated with respectively shallow and mesophotic depths (Figure 1G, 4A), indicating there may also be distinct ecological distributions in Okinawa.

To examine potential phenotypic differentiation, we quantitatively assessed skeletal and physiological differences between lineages in Australia. Principal component analyses based on coral host skeletal traits (four traits; n = 54), physiological traits (protein and lipid content; n = 69) and Symbiodiniaceae physiological traits (cell density and five pigment traits; n = 70) revealed separation between lineages despite high levels of variance (Figure 4D-F). The "green" and "red" lineages differed significantly across all three trait groups, whereas the “blue” and “red” lineages differed in skeletal traits and “blue” and “green” in symbiont physiological traits (Table S14). In terms of skeletal traits, the mean density of septa (i.e. vertical blades inside the corallite) was significantly lower in the “red” lineage, but not morphologically diagnostic given the overlapping ranges (Figure S11; Table S15). Observed ranges of physiological traits were similar to those reported in other studies that include *P. speciosa* (34, 35); however separating the three lineages revealed differences in protein density, Symbiodiniaceae density, and photosynthetic pigment concentrations (Figure S12; Table S15). Although *P. speciosa* (*sensu lato*) prefers shaded or deeper environments, it has been considered an efficient autotroph across its entire depth range (36). However, we observed a tendency for different lineages to vary in how they trade symbiont densities for chlorophyll concentration per symbiont cell, where lineages with thicker tissues (greater protein densities) hosted greater symbiont densities (Figure S12; Table S15). Although sample sizes were small relative to the observed variance, the results demonstrate the existence of phenotypic differences between the morphologically cryptic lineages, pointing towards distinct physiological strategies.

To explore whether the phenotypic differences may be attributed to differences in coral-associated bacteria or algal symbionts (Symbiodiniaceae), these microbial communities were compared among the three lineages. Associated bacterial communities were assessed by genotyping the host lineages from samples of a previously published 16S rRNA gene metabarcoding dataset [n = 43; (35)]. No significant differences were found in the bacterial community structure, richness, and diversity of the three lineages (Figure S13), but regional patterns were present (WCS and GBR, Figure 4G, Table S16) confirming earlier findings (37). Three bacterial operational taxonomic units (OTUs) from the genera *Corynebacterium* and *Gluconacetobacter* were consistently found in the three lineages, across regions. Despite the lack of differences in the overall bacterial communities, there were two unique OTUs that were only found in the “blue” and “red” lineage, respectively (Figure S13).

283 To investigate potential differences in lineage-associated Symbiodiniaceae communities, we
284 screened the nextRAD data for contaminating chloroplast or mitochondrial Symbiodiniaceae
285 loci. We found three organellar loci that were genotyped for several hundred samples, but these
286 were largely invariant within Australian samples. This is in line with a previous study (from
287 West Australia) that observed a single Symbiodiniaceae type in *P. speciosa* over depth (34),
288 although some depth partitioning was observed in the Red Sea (35). More detailed
289 mitochondrial characterization was conducted by aligning whole-genome resequencing data
290 from the three lineages to the *Cladocopium goreaui* genome [C1; (52)]; this revealed a highly
291 reticulated haplotype network based on an 8 kb mitochondrial region with 99.7% similarity
292 across samples (n = 16). Out of the eleven haplotypes, two were shared between the "green"
293 and "blue" lineages, whereas the remaining haplotypes were found only once or multiple times
294 but in a single lineage (Figure S14). Overall, microbial associations appeared to be either
295 primarily driven by environment (in the case of the bacterial communities) or were highly
296 consistent (Symbiodiniaceae), indicating that observed phenotypic differences between
297 lineages were unlikely driven by distinct microbial associations.

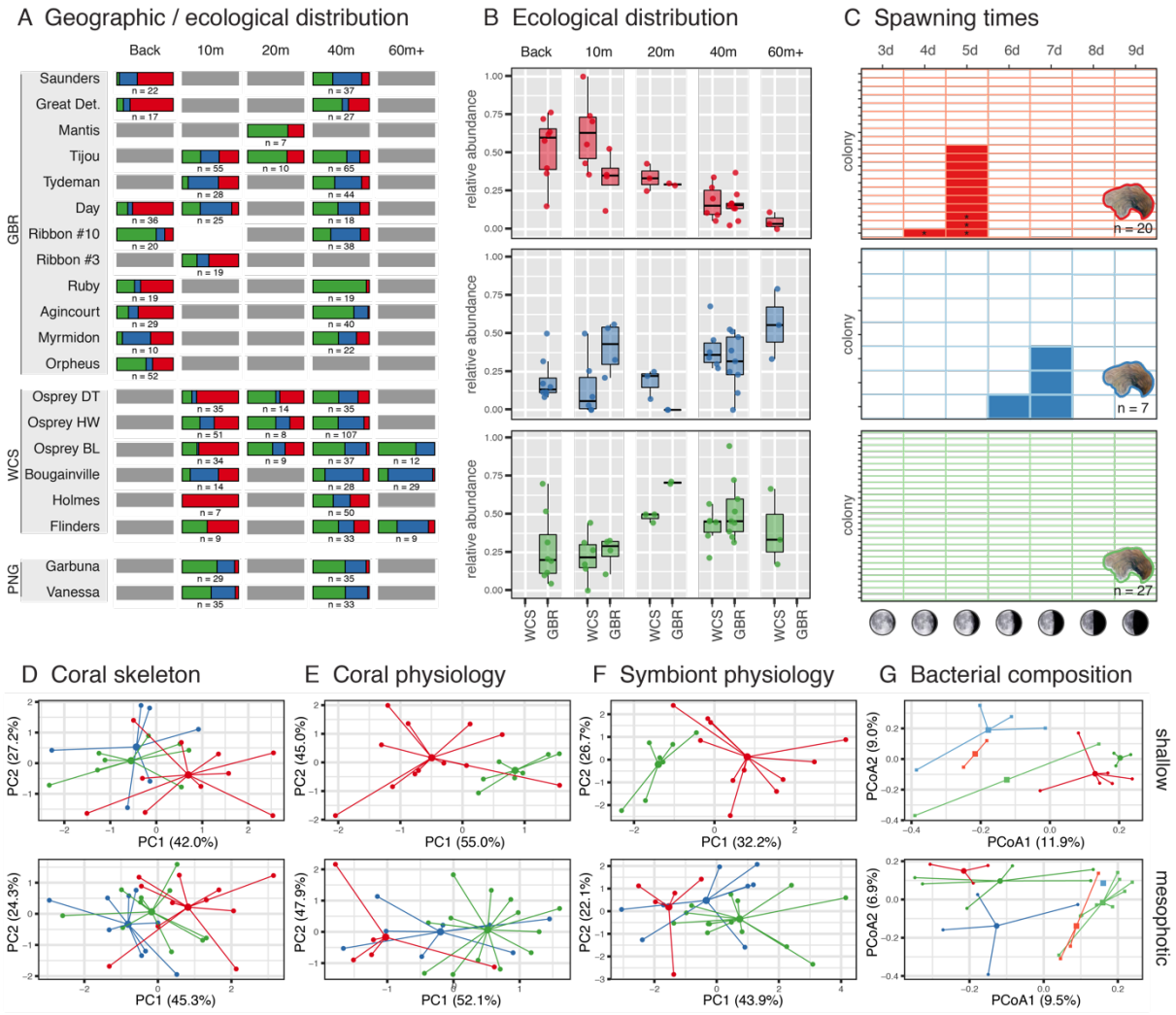

Figure 4. Ecological, phenotypic and reproductive characterization. (A) Proportional composition of *Pachyseris* lineages across geographic locations and depth/habitat. Assignments are based on the CAPS genotyping assay merged with the nextRAD data. Locations within regions are ordered from north to south (top to bottom). (B) Boxplots summarizing relative abundances of *Pachyseris* lineages for each habitat and region (Great Barrier Reef and Western Coral Sea). Dots represent the proportions for each individual population (n = 41; corresponding to those in panel A). (C) Binary heatmap indicating the spawning status of 54 *Pachyseris* colonies (rows) over time (columns) and grouped by lineage. Colonies were collected from shallow depths at Orpheus Island (Great Barrier Reef) and monitored *ex situ* between 3 and 9 days after the full moon in November 2017. Opaque cells indicate the release of eggs (asterisk) or sperm (no symbol). Empty cells indicate that the colony was isolated and monitored, but that no gametes were observed (e.g. no gamete release was observed for the "green" lineage). (D) Principal component analysis for four host skeletal traits. (E) Principal component analysis for two coral host physiological traits: protein and lipid content; PCA). (F) Principal component analysis for six Symbiodiniaceae photophysiological traits. (G) Principal coordinate analysis for bacterial community structure (based on 16S amplicon sequencing). Coral colonies are compared within their own depth groups: shallow (top plots; 10-20 m) and mesophotic (bottom plots; 40-60 m), with most colonies

originating from either 10 or 40 m. Skeletal and physiological traits were measured for samples the WCS, whereas the bacterial included samples from both the GBR (square shapes) and WCS (circle shapes) regions.

Temporal reproductive isolation. Given the limited admixture between the sympatrically-occurring *P. speciosa* lineages, we monitored the reproductive behavior of shallow colonies from Orpheus Island on the Great Barrier Reef. Previous reports from that location indicated spawning of *Pachyseris speciosa* between 5 and 6 days after the full moon (55). Colonies were collected from the field just after the full moon in November 2017, genotyped using the CAPS assay (which identified 20 "red", 7 "blue", and 27 "green" colonies), and monitored *ex situ* for spawning from 3 to 9 days after the full moon. On day 5, half of the colonies of the "red" lineage released gametes (with one colony releasing also on day 4), with no colonies from the other two lineages releasing gametes (Figure 4C). On day 7, nearly half of the colonies from the "blue" lineage released gametes (with one also releasing on day 6; Figure 4c), with again no colonies from the other two lineages releasing gametes. No gamete release was observed for the "green" lineage within the monitoring period, although the "green" colony from which the draft genome was constructed spawned 8 days after the full moon in December 2014 (see Methods). Overall, the average spawning time of male colonies was 19 min after sunset (n = 13), versus an average of 43 min for female colonies (n = 4; Table S18), in line with general observations of males spawning earlier in broadcast spawning coral (56). Attempts to preserve unfertilized eggs in filtered seawater (at ambient temperature) for experimental inter-lineages crosses were not successful (complete degradation within 24 hours).

The temporal segregation in the timing of gamete release observed during the November 2017 spawning provides a potential mechanism to explain the minimal admixture in these sympatrically-occurring lineages. Temporal reproductive isolation is a common strategy for minimizing interspecific gamete encounters in scleractinian corals, with the timing difference ranging from hours (57, 58) to months (59, 60). The two-day difference in gamete release between "red" and "blue" *P. speciosa* points towards differences in processes entrained by the lunar cycles, rather than environmental seasonal or diurnal cycles (61–63). The identification of fixed high-impact gene variants related to environmental sensing, development, and gametogenesis provide initial leads to the genomic basis of this potential prezygotic reproductive barrier.

Given the predicted split-spawning (i.e. mass spawning occurring across at least two consecutive months) in 2017, we maintained a subset of the colonies (n = 36) for additional spawning monitoring after the full moon of December in that year. Observations undertaken for those colonies saw gamete release for only one colony of the "blue" lineage and one of the "green" lineages (both on day 4 after the full moon). In total, two-thirds of the colonies from the "red" lineage released gametes, with release observed in the period between day 3 and day 8, with most of the release occurring on day 4 (8 colonies versus 1-3 colonies on the other days) (Table S17). While these observations confirmed the occurrence of a split spawning, they may not reflect natural release patterns, given the extended time these colonies were removed from natural cues (e.g. exposure to lunar cycle, lack of exposure to tides). In addition, their prolonged close proximity to one another in the single tank ("raceway") in which they were kept, may have affected the timing of release [e.g. due to chemical signaling; (62)]. The observations confirm the potential for experimental inter-lineage crossings under artificial conditions, to assess whether temporal reproductive isolation is accompanied by gametic incompatibility.

Geographic and habitat structuring within lineages. Within each of the three Australasian lineages, principal component analyses identified considerable substructuring, which was driven to a large extent by sampling region (Great Barrier Reef, Western Coral Sea and Papua New Guinea; Figure 5A,C). However, additional substructure was identified (2-3 apparent clusters) in the GBR samples that could not be linked to subregions, locations or habitat (Figure 5C). Discriminant analysis of principal components (DAPC) further confirmed the genetic differentiation among the three sampling regions (Figure 5B), indicating restricted gene flow between these regions with a some exceptions indicating recent admixture. Although genetic differentiation between the GBR and WCS has been observed previously in other coral species (64–65), here we identify further differentiation between individual WCS atolls confirming their rather isolated nature as they are surrounded by deep oceanic water. In contrast, only very subtle substructuring was identified between the "Northern" and "Far Northern" regions of the GBR (Figure 5C). The inability to detect distinct location- or region-associated clusters on the GBR could indicate the potential for gene flow over large distances, a pattern observed for other coral species and facilitated by the high density of reefs along the outer shelf (64–68). Nonetheless, given the presence of substructuring within each of the locations [a pattern frequently observed in scleractinian corals; (22)], such "panmixia" should be interpreted with caution.

Where other studies found a clear partitioning of cryptic lineages across habitats (28, 29), we observed substantial overlap between the depth distributions of the three *Pachyseris* lineages (e.g. the "red" lineage is present at shallow and mesophotic depths, but at lower relative abundances in the latter). Surveys during the 2016 mass bleaching found that most *Pachyseris* colonies down to 25 m were bleached, while fewer than half of the colonies at 40 m depth were bleached (69). Opportunistic genotyping of 14 healthy and 3 bleached colonies collected from 40 m during this event found healthy representatives of all three lineages (8 "green", 5 "red", and 1 "blue" healthy colony; versus 2 "green" and 1 "blue" bleached colony). The broad depth range of these lineages combined with the strong depth-attenuation of bleaching in *Pachyseris* (69), raises the question whether deep populations may benefit those at shallow depth by acting as a refuge and source of reproduction (70). Unfortunately, genetic differentiation between habitats (within each lineage) could not be adequately assessed at individual locations given the small sample sizes after splitting into cryptic lineages. When merging individual locations on the GBR, DAPC showed some discrimination between habitat clusters (Figure 5D, S15). This separation was driven by many low-contributing and several high-contributing alleles indicating there may be potential limitations to such vertical connectivity (Figure S15).

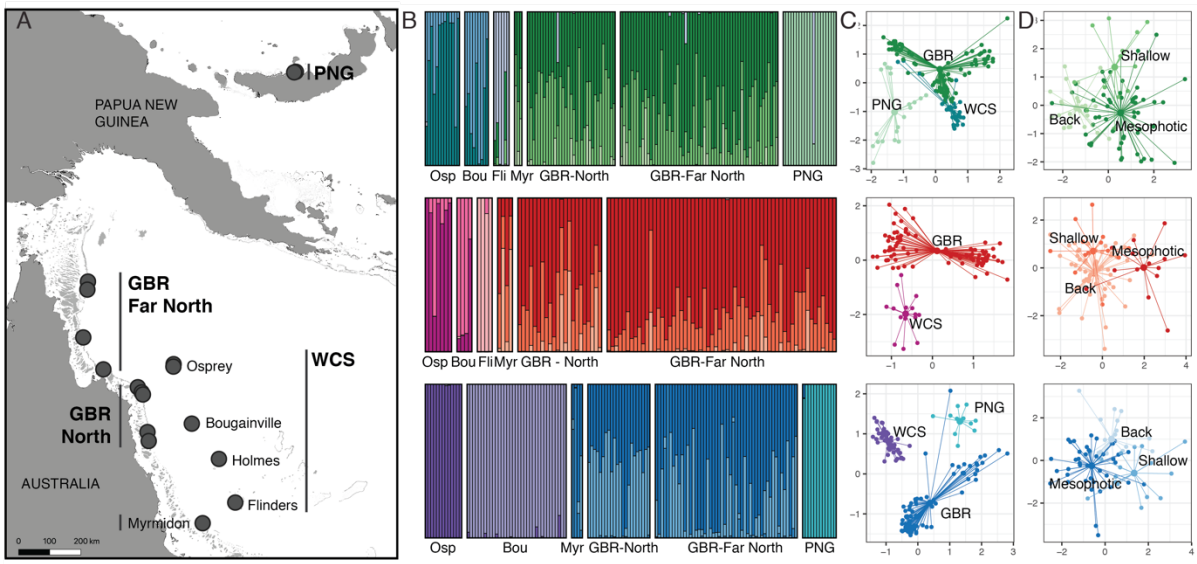

Figure 5. Genetic structuring within lineages by region and habitat. (A) Map showing the Australasian sampling locations/regions included in the DAPC plots. (B) DAPC assignment plots for each of the three lineages (from top to bottom: "green", "red" and "blue") using location as prior (but ignoring habitat and grouping "North" and "Far North" locations on the Great Barrier Reef). (C) Principal component analyses for each of the three lineages (from top to bottom: "green", "red" and "blue"), showing structuring by region as well as unexplained substructuring within the Great Barrier Reef populations. (D) DAPC scatter plot for the Great Barrier Reef using

habitat as prior. PCA and DAPC scatter plots show individual colonies connected to respectively region and habitat centroids, and analyses are based on nextRAD data after the removal of outliers.

Conclusions. Tens of thousands of multicellular species have been described in association with tropical coral reefs, yet this is estimated to represent <10% of their actual species richness (1). Reef-building corals (Scleractinia) have been considered to contribute relatively little to this estimated discrepancy, with the taxonomy considered to be relatively complete compared to other less-studied coral reef taxa (1). Molecular approaches have indeed corroborated many of the current taxonomic species (71), but have also unveiled extensive undescribed diversity within the traditional morphological boundaries (21, 26). This pattern is likely to be accelerated given the transition to genome-wide sequencing approaches, solving the resolution issues associated with traditional sequence markers (30, 72, 73). The inability to discriminate this diversity as readily identifiable taxonomic units in the field (or even through close skeletal examination) has raised concerns about the practicality and necessity of incorporating it into our systematic framework, particularly as the lack of morphological differentiation can lead to the assumption of negligible physiological differentiation. While we found no diagnostic morphological characters distinguishing the three *Pachyseris* lineages in this study, the genome-wide differentiation was accompanied by differences in ecological, physiological, and reproductive traits, demonstrating how morphological stasis can mask substantial ecological divergence. Failure to recognize such cryptic diversity will result in erroneous interpretations of species distributions, extinction risk, and spatial genetic differentiation (21, 26), all with critical ramifications for conservation management. In light of the rapid degradation of the world's coral reefs, it is critical to acknowledge and start capturing this hidden diversity to improve our understanding and ability to protect these fragile ecosystems.

Methods overview

We assembled and annotated a *de novo* reference genome using PacBio sequencing (~100x coverage) of a sperm sample from a *Pachyseris speciosa* colony from Orpheus Island on the Great Barrier Reef. This was used as a reference for reduced-representation sequencing (nextRAD) of *P. speciosa* colonies (n = 501) from shallow and mesophotic habitats in five different regions (Great Barrier Reef, Western Coral Sea, Papua New Guinea, Okinawa and Israel), sequenced across 4 Illumina NextSeq 500 lanes. Whole-genome re-sequencing of representative *P. speciosa* samples (n = 20) was then undertaken for historic demographic

modelling, using the Illumina Nextera protocol at ~5X or ~20X coverage across 8 Illumina HiSeq 2500 lanes. Given the lack of diagnostic morphological characteristics distinguishing the lineages, we designed a cleaved amplified polymorphic sequence (CAPS) assay to increase the number of genotyped samples (n = 1,442), and assess ecological, phenotypic and reproductive differences. Morphological differences between lineages were assessed through *in situ* photographs (n = 157), examination of qualitative traits in bleach-dried skeletons (n = 36), and micro-skeletal features using scanning electron microscopy (n = 15). Quantitative measurements were undertaken for five skeletal characters (n = 54). Physiological characterization was undertaken through protein and lipid quantification, *Symbiodiniaceae* cell counts, and photopigment quantification using high-performance liquid chromatography (HPLC) (n = 73). Spawning behavior was assessed through reproductive monitoring of colony fragments (n = 54) during November/December 2017 at the Orpheus Island Research Station. Microbiome characterization was undertaken by genotyping host lineages (n = 43) of a previous study based on 16S rRNA amplicon sequencing (37). See *SI Appendix, Supplementary Methods* for a detailed explanation of all the methods.

Data Availability. Raw sequence data for the genome and transcriptome assembly are available through ENA accession number PRJEB23386, the reduced-representation data through XXXX, and the whole-genome resequencing through XXXX. Genome assembly, variant call datasets, electronic notebooks, and scripts are accessible through https://github.com/pimbongaerts/pachyseris.

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References

1. R. Fisher, *et al.*, Species richness on coral reefs and the pursuit of convergent global estimates. *Current Biology* **25**, 500–505 (2015).
2. J.E.N. Veron, Stafford-Smith M.G., Turak E. and DeVantier L.M. Corals of the World. Accessed 16 Aug 2020. http://www.coralsoftheworld.org
3. L. DeVantier, E. Turak, L. DeVantier, E. Turak, Species richness and Relative abundance of reef-building corals in the Indo-West Pacific. *Diversity* **9**, 25 (2017).
4. T. Horton, *et al.*, World Register of Marine Species. Accessed 4 Sep 2020. https://www.marinespecies.org
5. J. S. Madin, *et al.*, The Coral Trait Database, a curated database of trait information for coral species from the global oceans. *Scientific Data* **3**, 1–22 (2016).
6. M. B. Best, G. J. Boekschoten, A. Oosterbaan, Species concept and ecomorph variation in living and fossil Scleractinia. *Palaeontographica Americana* **54**, 70–79 (1984).
7. N. Knowlton, J. B. Jackson, New taxonomy and niche partitioning on coral reefs: jack of all trades or master of some? *Trends in Ecology & Evolution* **9**, 7–9 (1994).
8. P. A. Todd, Morphological plasticity in scleractinian corals. *Biological Reviews* **83**, 315–337 (2008).

9. H. Fukami, *et al.*, Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature* **427**, 832–835 (2004).
10. D. Huang, W. Y. Licuanan, A. H. Baird, H. Fukami, Cleaning up the 'Bigmessidae': Molecular phylogeny of scleractinian corals from Faviidae, Merulinidae, Pectiniidae and Trachyphylliidae. *BMC Evolutionary Biology* **11**, 37 (2011).
11. S. Schmidt-Roach, K. J. Miller, P. Lundgren, N. Andreakis, With eyes wide open: a revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zoological Journal of the Linnean Society* **170**, 1–33 (2014).
12. Z. H. Forsman, *et al.*, Coral hybridization or phenotypic variation? Genomic data reveal gene flow between *Porites lobata* and *P. compressa*. *Molecular Phylogenetics and Evolution* **111**, 132–148 (2017).
13. N. Knowlton, E. Weil, L. A. Weigt, H. M. Guzman, Sibling species in *Montastraea annularis*, coral bleaching, and the coral climate record. *Science* **255**, 330–333 (1992).
14. E. Weil, N. Knowton, A multi-character analysis of the Caribbean coral *Montastraea annularis* (Ellis and Solander, 1786) and its two sibling species, *M. faveolata* (Ellis and Solander, 1786) and *M. franksi* (Gregory, 1895). *Bulletin of Marine Science* **55**, 151–175 (1994).
15. Z. H. Forsman, D. J. Barshis, C. L. Hunter, R. J. Toonen, Shape-shifting corals: molecular markers show morphology is evolutionarily plastic in *Porites*. *BMC Evolutionary Biology* **9**, 45 (2009).
16. P. Bongaerts, *et al.*, Genetic divergence across habitats in the widespread coral *Seriatopora hystrix* and its associated *Symbiodinium*. *PLoS one* **5**, e10871 (2010).
17. P. R. Frade, *et al.*, Semi-permeable species boundaries in the coral genus *Madracis*: introgression in a brooding coral system. *Molecular Phylogenetics and Evolution* **57**, 1072–1090 (2010).
18. F. Stefani, *et al.*, Comparison of morphological and genetic analyses reveals cryptic divergence and morphological plasticity in *Stylophora* (Cnidaria, Scleractinia). *Coral Reefs* **30**, 1033 (2011).
19. J. T. Ladner, S. R. Palumbi, Extensive sympatry, cryptic diversity and introgression throughout the geographic distribution of two coral species complexes. *Molecular Ecology* **21**, 2224–2238 (2012).
20. P. A. Warner, M. J. van Oppen, B. L. Willis, Unexpected cryptic species diversity in the widespread coral *Seriatopora hystrix* masks spatial-genetic patterns of connectivity. *Molecular Ecology* **24**, 2993–3008 (2015).

21. Z. T. Richards, O. Berry, M. J. H. van Oppen, Cryptic genetic divergence within threatened species of *Acropora* coral from the Indian and Pacific Oceans. *Conservation Genetics* **17**, 577–591 (2016).
22. P. Gélin, C. Fauvelot, L. Bigot, J. Baly, H. Magalon, From population connectivity to the art of striping Russian dolls: the lessons from *Pocillopora* corals. *Ecology and Evolution* **8**, 1411–1426 (2018).
23. F. Sinniger, R. Prasetia, M. Yorifuji, P. Bongaerts, S. Harii, *Seriatopora* diversity preserved in upper mesophotic coral ecosystems in Southern Japan. *Frontiers in Marine Science* **4**, 155 (2017).
24. R. Arrigoni, M. L. Berumen, J. Stolarski, T. I. Terraneo, F. Benzoni, Uncovering hidden coral diversity: a new cryptic lobophylliid scleractinian from the Indian Ocean. *Cladistics* **35**, 301–328 (2019).
25. N. Knowlton, Sibling species in the sea. *Annual Review of Ecology and Systematics* **24**, 189–216 (1993).
26. E. A. Sheets, P. A. Warner, S. R. Palumbi, Accurate population genetic measurements require cryptic species identification in corals. *Coral Reefs* **37**, 549–563 (2018).
27. A. M. González, C. A. Prada, V. Ávila, M. Medina, "Ecological speciation in corals" in *Population Genomics: Marine Organisms*, (Springer, 2018), pp. 303–324.
28. P. Bongaerts, *et al.*, Adaptive divergence in a scleractinian coral: physiological adaptation of *Seriatopora hystrix* to shallow and deep reef habitats. *BMC Evolutionary Biology* **11**, 303 (2011).
29. C. Prada, M. E. Hellberg, Long prereproductive selection and divergence by depth in a Caribbean candelabrum coral. *Proceedings of the National Academy of Sciences* **110**, 3961–3966 (2013).
30. N. L. Rosser, *et al.*, Phylogenomics provides new insight into evolutionary relationships and genealogical discordance in the reef-building coral genus *Acropora*. *Proceedings of the Royal Society: Biological Sciences* **284**, 20162182 (2017).
31. N. H. Rose, R. A. Bay, M. K. Morikawa, S. R. Palumbi, Polygenic evolution drives species divergence and climate adaptation in corals. *Evolution* **72**, 82–94 (2018).
32. P. Bongaerts, *et al.*, Deep reefs are not universal refuges: Reseeding potential varies among coral species. *Science Advances* **3**, e1602373 (2017).
33. P. Bongaerts, *et al.*, *Symbiodinium* diversity in mesophotic coral communities on the Great Barrier Reef: a first assessment. *Marine Ecology Progress Series* **439**, 117–126 (2011).

34. T. F. Cooper, *et al.*, Niche specialization of reef-building corals in the mesophotic zone: metabolic trade-offs between divergent *Symbiodinium* types. *Proceedings of the Royal Society B: Biological Sciences* **278**, 1840–1850 (2011).
35. M. Ziegler, C. M. Roder, C. Büchel, C. R. Voolstra, Mesophotic coral depth acclimatization is a function of host-specific symbiont physiology. *Frontiers in Marine Science* **2**, 4 (2015).
36. V. Z. Radice, O. Hoegh-Guldberg, B. Fry, M. D. Fox, S. G. Dove, Upwelling as the major source of nitrogen for shallow and deep reef-building corals across an oceanic atoll system. *Functional Ecology* **33**, 1120–1134 (2019).
37. A. Hernandez-Agreda, W. Leggat, P. Bongaerts, T. D. Ainsworth, The microbial signature provides insight into the mechanistic basis of coral success across reef habitats. *mBio* **7**, e00560-16 (2016).
38. H. Ying, *et al.*, Comparative genomics reveals the distinct evolutionary trajectories of the robust and complex coral lineages. *Genome Biology* **19**, 175 (2018).
39. S. Koren, *et al.*, Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Research* **27**, 722–736 (2017).
40. J.-F. Flot, *et al.*, Incongruence between morphotypes and genetically delimited species in the coral genus *Stylophora*: phenotypic plasticity, morphological convergence, morphological stasis or interspecific hybridization? *BMC Ecology* **11**, 22 (2011).
41. R. Arrigoni, *et al.*, Towards a rigorous species delimitation framework for scleractinian corals based on RAD sequencing: the case study of *Leptastrea* from the Indo-Pacific. *Coral Reefs* doi:10.1007/s00338-020-01924-8 (2020).
42. J. E. N. Veron, M. Pichon, Scleractinia of Eastern Australia. III. Families Agariciidae, Siderastreidae, Fungiidae, Oculiniidae, Merulinidae, Pectiniidae, Caryophylliidae, Dendrophylliidae. *Australian Institute of Marine Science Monograph Series* **4**, 471 (1980).
43. F. Rentzsch, J. H. Fritzenwanker, C. B. Scholz, U. Technau, FGF signalling controls formation of the apical sensory organ in the cnidarian *Nematostella vectensis*. *Development* **135**, 1761–1769 (2008).
44. A. Reyes-Bermudez, A. Villar-Briones, C. Ramirez-Portilla, M. Hidaka, A. S. Mikheyev, Developmental progression in the coral *Acropora digitifera* is controlled by differential expression of distinct regulatory gene networks. *Genome Biology and Evolution* **8**, 851–870 (2016).

599 45. M. E. Strader, G. V. Aglyamova, M. V. Matz, Molecular characterization of larval
600 development from fertilization to metamorphosis in a reef-building coral. *BMC*
601 *Genomics* **19**, 1–17 (2018).
602 46. P. A. Cleves, M. E. Strader, L. K. Bay, J. R. Pringle, M. V. Matz, CRISPR/Cas9-
603 mediated genome editing in a reef-building coral. *Proceedings of the National*
604 *Academy of Sciences* **115**, 5235–5240 (2018).
605 47. M. Takechi, N. Adachi, T. Hirai, S. Kuratani, S. Kuraku, The Dlx genes as clues to
606 vertebrate genomics and craniofacial evolution in *Seminars in Cell & Developmental*
607 *Biology*, (Elsevier, 2013), pp. 110–118.
608 48. A. M. Gonzalez Angel, Genomic and Transcriptomic Insights on the *Orbicella*
609 Species Complex (2019). PhD Thesis. Penn State University.
610 49. S. Schiffels, R. Durbin, Inferring human population size and separation history from
611 multiple genome sequences. *Nature Genetics* **46**, 919–925 (2014).
612 50. C. Prada, *et al.*, Empty niches after extinctions increase population sizes of modern
613 corals. *Current Biology* **26**, 3190–3194 (2016).
614 51. Y. Mao, E. P. Economo, N. Satoh, The roles of introgression and climate change in
615 the rise to dominance of *Acropora* corals. *Current Biology* **28**, 3373-3382. e5 (2018).
616 52. M. J. van Oppen, B. L. Willis, D. J. Miller, Atypically low rate of cytochrome b
617 evolution in the scleractinian coral genus Acropora. *Proceedings of the Royal Society*
618 *Series B: Biological Sciences* **266**, 179–183 (1999).
619 53. T. L. Shearer, M. J. H. Van Oppen, S. L. Romano, G. Wörheide, Slow mitochondrial
620 DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology* **11**, 2475–
621 2487 (2002).
622 54. H. Liu, *et al.*, *Symbiodinium* genomes reveal adaptive evolution of functions related
623 to coral-dinoflagellate symbiosis. *Communications Biology* **1**, 1–11 (2018).
624 55. R. C. Babcock, *et al.*, Synchronous spawnings of 105 scleractinian coral species on
625 the Great Barrier Reef. *Marine Biology.* **90**, 379–394 (1986).
626 56. D. R. Levitan, Sex-specific spawning behavior and its consequences in an external
627 fertilizer. *The American Naturalist* **165**, 682–694 (2005).
628 57. H. Fukami, M. Omori, K. Shimoike, T. Hayashibara, M. Hatta, Ecological and
629 genetic aspects of reproductive isolation by different spawning times in *Acropora*
630 corals. *Marine Biology* **142**, 679–684 (2003).
631 58. D. R. Levitan, *et al.*, Mechanisms of reproductive isolation among sympatric
632 broadcast-spawning corals of the *Montastraea annularis* species complex. *Evolution*
633 **58**, 308–323 (2004).

59. N. L. Rosser, Asynchronous spawning in sympatric populations of a hard coral reveals cryptic species and ancient genetic lineages. *Molecular Ecology* **24**, 5006–5019 (2015).
60. J. P. Gilmour, J. N. Underwood, E. J. Howells, E. Gates, A. J. Heyward, Biannual spawning and temporal reproductive isolation in *Acropora* corals. *PloS One* **11**, e0150916 (2016).
61. A. H. Baird, J. R. Guest, B. L. Willis, Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. *Annual Review of Ecology, Evolution, and Systematics* **40**, 551–571 (2009).
62. D. R. Levitan, N. D. Fogarty, J. Jara, K. E. Lotterhos, N. Knowlton, Genetic, spatial, and temporal components of precise spawning synchrony in reef building corals of the *Montastraea annularis* species complex. *Evolution: International Journal of Organic Evolution* **65**, 1254–1270 (2011).
63. P. Kaniewska, S. Alon, S. Karako-Lampert, O. Hoegh-Guldberg, O. Levy, Signaling cascades and the importance of moonlight in coral broadcast mass spawning. *Elife* **4**, e09991 (2015).
64. M. J. van Oppen, A. Lutz, G. De'ath, L. Peplow, S. Kininmonth, Genetic traces of recent long-distance dispersal in a predominantly self-recruiting coral. *PLoS One* **3**, e3401 (2008).
65. V. Lukoschek, C. Riginos, M. J. van Oppen, Congruent patterns of connectivity can inform management for broadcast spawning corals on the Great Barrier Reef. *Molecular Ecology* **25**, 3065–3080 (2016).
66. M. J. van Oppen, L. M. Peplow, S. Kininmonth, R. Berkelmans, Historical and contemporary factors shape the population genetic structure of the broadcast spawning coral, *Acropora millepora*, on the Great Barrier Reef. *Molecular Ecology* **20**, 4899–4914 (2011).
67. G. Torda, P. Lundgren, B. L. Willis, M. J. van Oppen, Revisiting the connectivity puzzle of the common coral *Pocillopora damicornis*. *Molecular Ecology* **22**, 5805–5820 (2013).
68. Z. L. Fuller, *et al.*, Population genetics of the coral *Acropora millepora*: Toward genomic prediction of bleaching. *Science* **369** (2020).
69. P. R. Frade, *et al.*, Deep reefs of the Great Barrier Reef offer limited thermal refuge during mass coral bleaching. *Nature Communications* **9**, 1–8 (2018).
70. P. Bongaerts, T. B. Smith, "Beyond the 'Deep Reef Refuge' Hypothesis: A Conceptual Framework to Characterize Persistence at Depth" in *Mesophotic Coral*

669 *Ecosystems*, Coral Reefs of the World., Y. Loya, K. A. Puglise, T. C. L. Bridge, Eds.
670 (Springer International Publishing, 2019), pp. 881–895.
671 71. J. Veron, Overview of the taxonomy of zooxanthellate Scleractinia. *Zoological*
672 *Journal of the Linnean Society* **169**, 485–508 (2013).
673 72. A. M. Quattrini, *et al.*, Universal target-enrichment baits for anthozoan (Cnidaria)
674 phylogenomics: New approaches to long-standing problems. *Molecular Ecology*
675 *Resources* **18**, 281–295 (2018).
676 73. R. Z. Quek, S. S. Jain, M. L. Neo, G. W. Rouse, D. Huang, Transcriptome-based
677 target-enrichment baits for stony corals (Cnidaria: Anthozoa: Scleractinia).
678 *Molecular Ecology Resources* **20** (2020).