Cryptic diversity masks ecologically distinct 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 uncovering a wealth of "cryptic diversity", but the ecological relevance of this diversity remains largely unclear. Here, we present an annotated draft genome for one of the most ubiquitous coral species in the Indo-Pacific (*Pachyseris speciosa*), and establish through extensive resequencing that its broad ecological distribution can be explained by the presence of at least three sympatric cryptic lineages. The lineages lack morphologically diagnostic characters yet exhibit genome-wide divergence with demographic modelling indicating an ancient divergence. Targeted genotyping surveys revealed differences in their ecological distributions, concurrent with differences in physiological and quantitative skeletal traits. Spawning observations highlight the potential role of temporal reproductive isolation in the limited admixture, with consistent observations of fixed differences in genes related to morphogenesis and reproduction. Overall, these findings highlight how our current systematic framework may be greatly underestimating the ecologically relevant diversity on coral reefs, and consequently limiting our ability to protect it 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 zooxanthellate corals –the framework builders of these ecosystems– remains surprisingly moderate with only ~750-850 valid species accepted worldwide (2–4). 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). Molecular studies have also uncovered a wealth of "cryptic diversity" within taxonomic species that cannot be readily linked to morphology (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 cryptic diversity 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 has been exposed through population genetic studies designed to relate genetic patterns to geography (26), and consequently, it usually remains unclear whether and how this cryptic divergence translates to phenotypically and ecologically distinct entities. Besides the detailed studies of the *Orbicella* coral species complex [reviewed in (27)], relatively few studies have since explored ecological or (non-morphological) phenotypic differentiation in sibling species, or assessed potential mechanisms contributing to their divergence (e.g. 28–31). Furthermore, the traditional use of only a few genetic loci has impeded an assessment of the mechanisms underlying cryptic diversification, and the respective roles of neutral processes (e.g. genetic drift) versus selection 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 ecological significance 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 the genetic structure in this species 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 and ecological divergence, and how reproductive isolation may be maintained in these 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. Given that cryptic diversity in scleractinian corals appears to be common and widespread, our findings indicate that conventional skeleton-based taxonomy may be substantially underestimating the ecologically-relevant scleractinian coral diversity on tropical coral reefs.

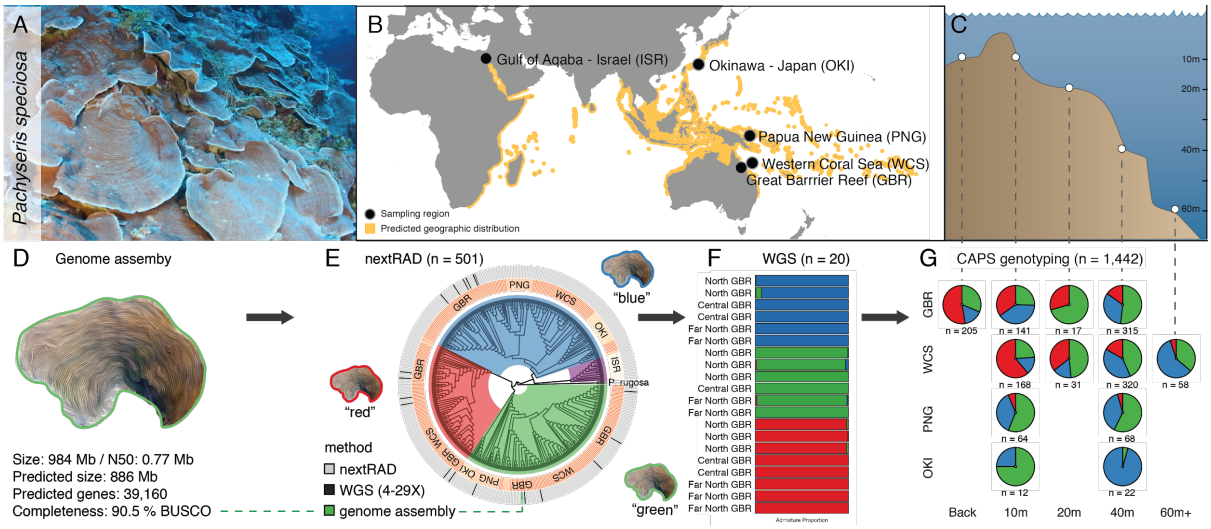

Figure 1. Overview of the study system and sequencing/genotyping approach. (A) *Pachyseris speciosa* at 40 m depth at Holmes Reef (Western Coral Sea). (B) Predicted geographic distribution of *P. speciosa* (*sensu lato*) based on the IUCN Red List (2009), and the location of the five regions included 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 differentiation of sub-tropical Okinawa, the overall structuring demonstrates the presence of genetically distinct lineages within the currently acknowledged species, *P. speciosa*.

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

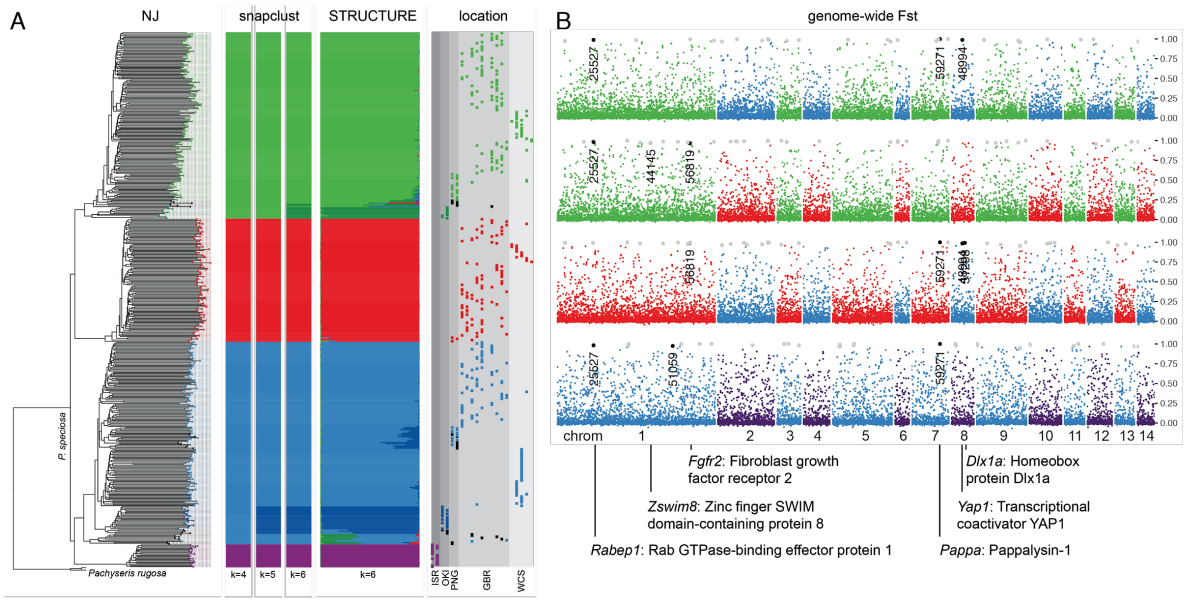

Figure 2. Genetic structuring and genome-wide differentiation of *P. speciosa* lineages. (A) Neighbor-joining tree based on variant nextRAD sites, with matching snapclust (k = 4 to 6) and STRUCTURE (k = 6) assignments for each coral colony. Dots in the "location" column indicate sampling population (geographically 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

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

194 In contrast to the lineages being morphologically indistinguishable, they are divergent
195 genomically with 146 alternatively-fixed SNPs among the three lineages in Australia (0.5 % of
196 29,287 SNPs; Figure 2). This included 8 missense variants (i.e. a nonsynonymous substitution
197 producing a different amino acid) located in genes of which 7 had homology to UniProtDB
198 genes (IDs: *Rabep1*, *Yap1*, *Pappa*, *HecW2*, *Zswim8*, *Fgfr2* and *Dlx1a*). The Fibroblast growth
199 factor receptor 2 gene (*Fgfr2*) is thought to be involved in environmental sensing and larval
200 metamorphosis (43-46). The *Dlx1a* gene is part of the homeobox-containing superfamily
201 generally associated with morphogenesis (47). The *Pappa* gene encodes the pregnancy-
202 associated plasma protein-A, which is upregulated during spawning in *Orbicella franksi* and
203 *Orbicella annularis* (48).

204 Whole-genome resequencing of representative "green", "blue" and "red" colonies from the
205 Great Barrier Reef (n = 20 at 4-29X coverage; Table S10) further confirmed the strong genetic
206 differentiation with minimal admixture (Figure 1f, S8). To assess whether the three lineages
207 have diverged recently and/or experienced differences in demographic history, we used the
208 Pairwise Sequentially Markovian Coalescent [PSMC; (49)] approach for a subset of colonies
209 that were sequenced at greater depth (14-27x coverage; n = 10). Modelled demographic
210 histories showed much greater variation between than within lineages, suggesting gene flow
211 was limited as far back as 10 mya (Figure 3), and that the cryptic nature is due to morphological
212 stasis rather than recent divergence. All lineages peaked in effective population size around 1-
213 3 mya which is consistent with PSMC analyses in recent studies on other robust (50) and
214 complex (51) corals. We also constructed circularized mitochondrial genomes from the whole-
215 genome resequencing data (~19 kbp in length), which in contrast only showed a limited
216 assortment of mitochondrial haplotypes into lineage-specific groups (Figure S9). Given the
217 limited nuclear admixture, this is likely to reflect incomplete lineage sorting and/or ancient
218 introgression, combined with very low mutation rates typical of anthozoans (52, 53). It also
219 reiterates how mitochondrial regions in corals for species-level phylogenetics should be used
220 cautiously (39).

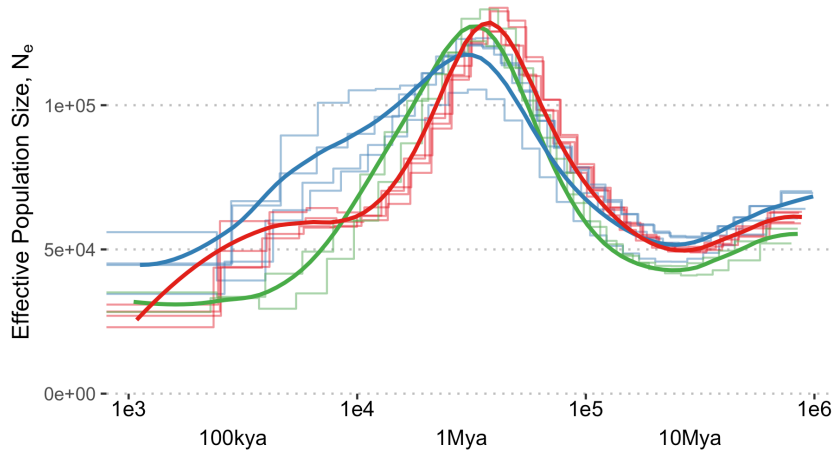

Figure 3. Demographic history as inferred through whole-genome resequencing. Changes in inferred, effective population size as estimated using MSMC for 10 deeply sequenced coral colonies representing the three *Pachyseris* lineages occurring sympatrically in Australia. The timescale is shown in units 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.

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

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

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

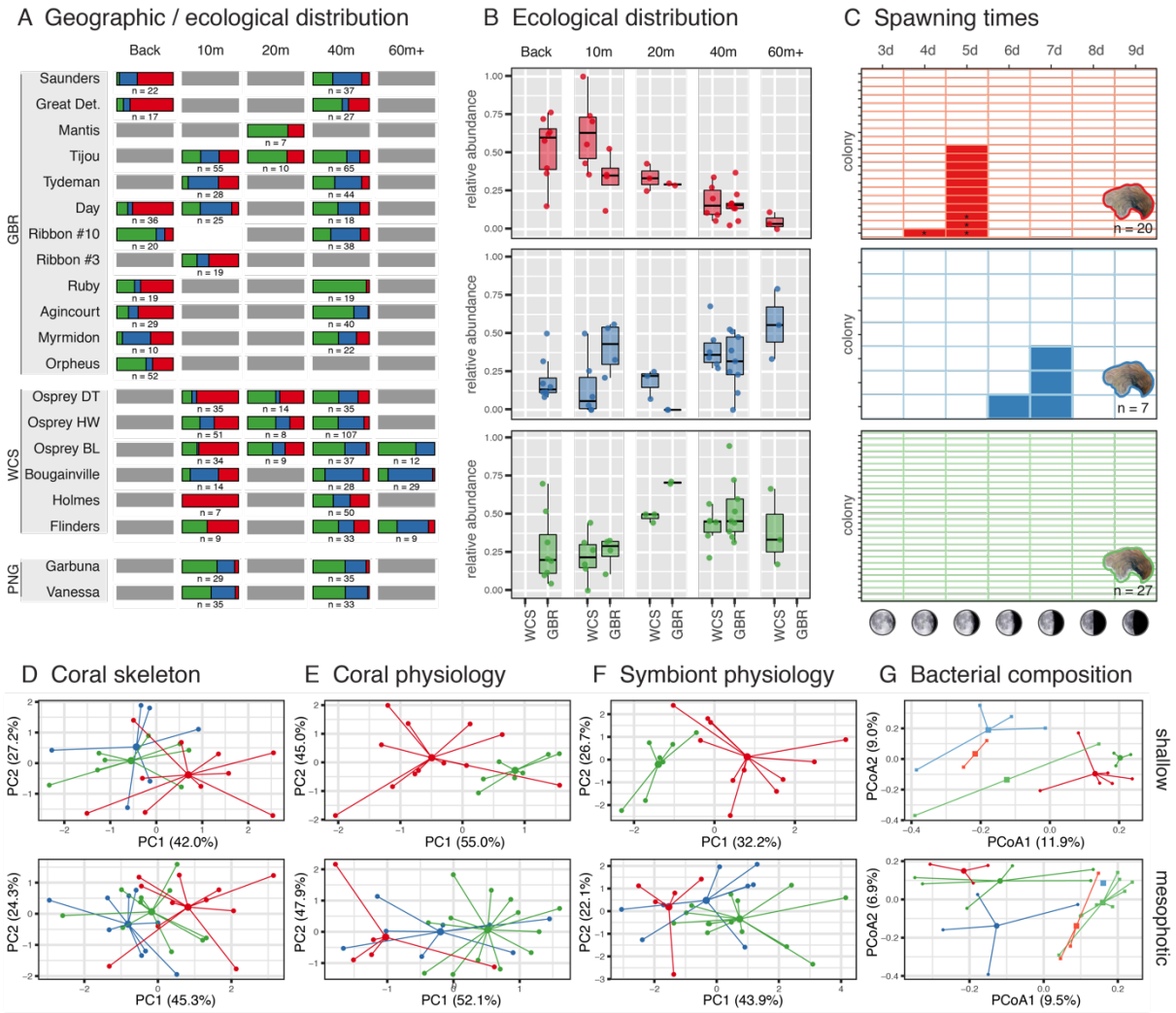

Figure 4. Ecological, phenotypic and reproductive characterization. (A) Proportional composition of *Pachyseris* lineages across geographic locations and depth/habitat. Assignments are based on the CAPS genotyping assay merged with the nextRAD data. Locations within regions are ordered from north to 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.

339 Given the predicted split-spawning (i.e. mass spawning occurring across at least two
340 consecutive months) in 2017, we maintained a subset of the colonies (n = 36) for additional
341 spawning monitoring after the full moon of December in that year. Observations undertaken
342 for those colonies saw gamete release for only one colony of the "blue" lineage and one of the
343 "green" lineages (both on day 4 after the full moon). In total, two-thirds of the colonies from
344 the "red" lineage released gametes, with release observed in the period between day 3 and day
345 8, with most of the release occurring on day 4 (8 colonies versus 1-3 colonies on the other days)
346 (Table S17). While these observations confirmed the occurrence of a split spawning, they may
347 not reflect natural release patterns, given the extended time these colonies were removed from
348 natural cues (e.g. exposure to lunar cycle, lack of exposure to tides). In addition, their prolonged
349 close proximity to one another in the single tank ("raceway") in which they were kept, may
350 have affected the timing of release [e.g. due to chemical signaling; (62)]. The observations
351 confirm the potential for experimental inter-lineage crossings under artificial conditions, to
352 assess whether temporal reproductive isolation is accompanied by gametic incompatibility.

353 **Geographic and habitat structuring within lineages.** Within each of the three Australasian
354 lineages, principal component analyses identified considerable substructuring, which was
355 driven to a large extent by sampling region (Great Barrier Reef, Western Coral Sea and Papua
356 New Guinea; Figure 5A,C). However, additional substructure was identified (2-3 apparent
357 clusters) in the GBR samples that could not be linked to subregions, locations or habitat (Figure
358 5C). Discriminant analysis of principal components (DAPC) further confirmed the genetic
359 differentiation among the three sampling regions (Figure 5B), indicating restricted gene flow
360 between these regions with a some exceptions indicating recent admixture. Although genetic
361 differentiation between the GBR and WCS has been observed previously in other coral species
362 (64–65), here we identify further differentiation between individual WCS atolls confirming
363 their rather isolated nature as they are surrounded by deep oceanic water. In contrast, only very
364 subtle substructuring was identified between the "Northern" and "Far Northern" regions of the
365 GBR (Figure 5C). The inability to detect distinct location- or region-associated clusters on the
366 GBR could indicate the potential for gene flow over large distances, a pattern observed for
367 other coral species and facilitated by the high density of reefs along the outer shelf (64–68).
368 Nonetheless, given the presence of substructuring within each of the locations [a pattern
369 frequently observed in scleractinian corals; (22)], such "panmixia" should be interpreted with
370 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).

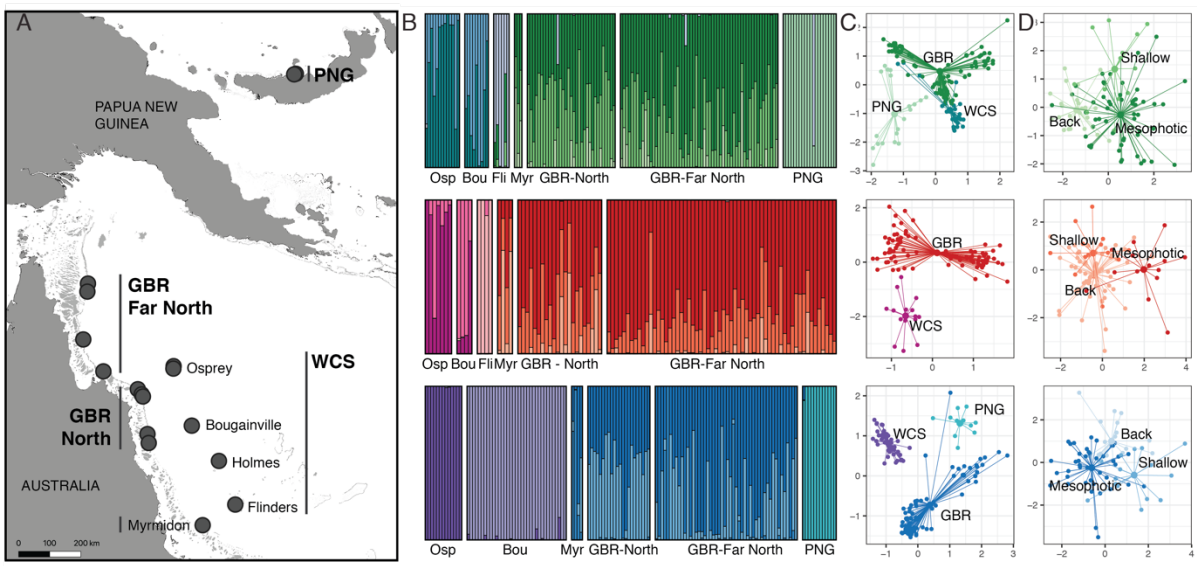

Figure 5. Genetic structuring within lineages by region and habitat. (A) Map showing the Australasian sampling locations/regions included in the DAPC plots. (B) DAPC assignment plots for each of the three lineages (from top to bottom: "green", "red" and "blue") using location as prior (but ignoring habitat 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). However, the growing number of molecular assessments, such as the one presented here, are revealing widespread cryptic diversity within the traditional morphological boundaries (21, 26). Further support for this pattern is likely to be unveiled given the transition to genome-wide sequencing approaches, solving the resolution issues associated with traditional sequence markers (30, 72, 73). The inability to discriminate cryptic species as readily identifiable taxonomic units in the field (or even through close skeletal examination) has raised concerns about the practicality and necessity of recognizing this newly exposed diversity, 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 ecological, physiological, and reproductive differences, demonstrating how cryptic diversity can mask 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

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

441 **Data Availability.** Raw sequence data for the genome and transcriptome assembly are
442 available through ENA accession number PRJEB23386, the reduced-representation data
443 through XXXX, and the whole-genome resequencing through XXXX. Genome assembly,
444 variant call datasets, electronic notebooks, and scripts are accessible through
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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).

488 8. P. A. Todd, Morphological plasticity in scleractinian corals. *Biological Reviews* **83**,
489 315–337 (2008).
490 9. H. Fukami, *et al.*, Conventional taxonomy obscures deep divergence between Pacific
491 and Atlantic corals. *Nature* **427**, 832–835 (2004).
492 10. D. Huang, W. Y. Licuanan, A. H. Baird, H. Fukami, Cleaning up the 'Bigmessidae':
493 Molecular phylogeny of scleractinian corals from Faviidae, Merulinidae, Pectiniidae
494 and Trachyphylliidae. *BMC Evolutionary Biology* **11**, 37 (2011).
495 11. S. Schmidt-Roach, K. J. Miller, P. Lundgren, N. Andreakis, With eyes wide open: a
496 revision of species within and closely related to the *Pocillopora damicornis* species
497 complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zoological*
498 *Journal of the Linnean Society* **170**, 1–33 (2014).
499 12. Z. H. Forsman, *et al.*, Coral hybridization or phenotypic variation? Genomic data
500 reveal gene flow between *Porites lobata* and *P. compressa*. *Molecular Phylogenetics*
501 *and Evolution* **111**, 132–148 (2017).
502 13. N. Knowlton, E. Weil, L. A. Weigt, H. M. Guzman, Sibling species in *Montastraea*
503 *annularis*, coral bleaching, and the coral climate record. *Science* **255**, 330–333
504 (1992).
505 14. E. Weil, N. Knowton, A multi-character analysis of the Caribbean coral *Montastraea*
506 *annularis* (Ellis and Solander, 1786) and its two sibling species, *M. faveolata* (Ellis
507 and Solander, 1786) and *M. franksi* (Gregory, 1895). *Bulletin of Marine Science* **55**,
508 151–175 (1994).
509 15. Z. H. Forsman, D. J. Barshis, C. L. Hunter, R. J. Toonen, Shape-shifting corals:
510 molecular markers show morphology is evolutionarily plastic in *Porites*. *BMC*
511 *Evolutionary Biology* **9**, 45 (2009).
512 16. P. Bongaerts, *et al.*, Genetic divergence across habitats in the widespread coral
513 *Seriatopora hystrix* and its associated *Symbiodinium*. *PLoS one* **5**, e10871 (2010).
514 17. P. R. Frade, *et al.*, Semi-permeable species boundaries in the coral genus *Madracis*:
515 introgression in a brooding coral system. *Molecular Phylogenetics and Evolution* **57**,
516 1072–1090 (2010).
517 18. F. Stefani, *et al.*, Comparison of morphological and genetic analyses reveals cryptic
518 divergence and morphological plasticity in *Stylophora* (Cnidaria, Scleractinia). *Coral*
519 *Reefs* **30**, 1033 (2011).
520 19. J. T. Ladner, S. R. Palumbi, Extensive sympatry, cryptic diversity and introgression
521 throughout the geographic distribution of two coral species complexes. *Molecular*
522 *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).

558 33. P. Bongaerts, *et al.*, *Symbiodinium* diversity in mesophotic coral communities on the
559 Great Barrier Reef: a first assessment. *Marine Ecology Progress Series* **439**, 117–126
560 (2011).
561 34. T. F. Cooper, *et al.*, Niche specialization of reef-building corals in the mesophotic
562 zone: metabolic trade-offs between divergent *Symbiodinium* types. *Proceedings of*
563 *the Royal Society B: Biological Sciences* **278**, 1840–1850 (2011).
564 35. M. Ziegler, C. M. Roder, C. Büchel, C. R. Voolstra, Mesophotic coral depth
565 acclimatization is a function of host-specific symbiont physiology. *Frontiers in*
566 *Marine Science* **2**, 4 (2015).
567 36. V. Z. Radice, O. Hoegh-Guldberg, B. Fry, M. D. Fox, S. G. Dove, Upwelling as the
568 major source of nitrogen for shallow and deep reef-building corals across an oceanic
569 atoll system. *Functional Ecology* **33**, 1120–1134 (2019).
570 37. A. Hernandez-Agreda, W. Leggat, P. Bongaerts, T. D. Ainsworth, The microbial
571 signature provides insight into the mechanistic basis of coral success across reef
572 habitats. *mBio* **7**, e00560-16 (2016).
573 38. H. Ying, *et al.*, Comparative genomics reveals the distinct evolutionary trajectories
574 of the robust and complex coral lineages. *Genome Biology* **19**, 175 (2018).
575 39. S. Koren, *et al.*, Canu: scalable and accurate long-read assembly via adaptive k-mer
576 weighting and repeat separation. *Genome Research* **27**, 722–736 (2017).
577 40. J.-F. Flot, *et al.*, Incongruence between morphotypes and genetically delimited
578 species in the coral genus *Stylophora*: phenotypic plasticity, morphological
579 convergence, morphological stasis or interspecific hybridization? *BMC Ecology* **11**,
580 22 (2011).
581 41. R. Arrigoni, *et al.*, Towards a rigorous species delimitation framework for
582 scleractinian corals based on RAD sequencing: the case study of *Leptastrea* from the
583 Indo-Pacific. *Coral Reefs* doi:10.1007/s00338-020-01924-8 (2020).
584 42. J. E. N. Veron, M. Pichon, Scleractinia of Eastern Australia. III. Families
585 Agariciidae, Siderastreidae, Fungiidae, Oculiniidae, Merulinidae, Pectiniidae,
586 Caryophylliidae, Dendrophylliidae. *Australian Institute of Marine Science*
587 *Monograph Series* **4**, 471 (1980).
588 43. F. Rentzsch, J. H. Fritzenwanker, C. B. Scholz, U. Technau, FGF signalling controls
589 formation of the apical sensory organ in the cnidarian *Nematostella vectensis*.
590 *Development* **135**, 1761–1769 (2008).
591 44. A. Reyes-Bermudez, A. Villar-Briones, C. Ramirez-Portilla, M. Hidaka, A. S.
592 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).

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

58. D. R. Levitan, *et al.*, Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the *Montastraea annularis* species complex. *Evolution* **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 Ecosystems*, Coral Reefs of the World., Y. Loya, K. A. Puglise, T. C. L. Bridge, Eds. (Springer International Publishing, 2019), pp. 881–895.
71. J. Veron, Overview of the taxonomy of zooxanthellate Scleractinia. *Zoological Journal of the Linnean Society* **169**, 485–508 (2013).
72. A. M. Quattrini, *et al.*, Universal target-enrichment baits for anthozoan (Cnidaria) phylogenomics: New approaches to long-standing problems. *Molecular Ecology Resources* **18**, 281–295 (2018).
73. R. Z. Quek, S. S. Jain, M. L. Neo, G. W. Rouse, D. Huang, Transcriptome-based target-enrichment baits for stony corals (Cnidaria: Anthozoa: Scleractinia). *Molecular Ecology Resources* **20** (2020).