Comparative genomics explains the evolutionary success of reef-forming corals

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Abstract
Transcriptome and genome data from twenty stony coral species and a selection of reference bilaterians were studied to elucidate coral evolutionary history. We identified genes that encode the proteins responsible for the precipitation and aggregation of the aragonite skeleton on which the organisms live, and revealed a network of environmental sensors that coordinate responses of the host animals to temperature, light, and pH. Furthermore, we describe a variety of stress-related pathways, including apoptotic pathways that allow the host animals to detoxify reactive oxygen and nitrogen species that are generated by their intracellular photosynthetic symbionts, and determine the fate of corals under environmental stress. Some of these genes arose through horizontal gene transfer and comprise at least 0.2% of the animal gene inventory. Our analysis elucidates the evolutionary strategies that have allowed symbiotic corals to adapt and thrive for hundreds of millions of years.

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Introduction
Reef-building stony corals (Scleractinia) and their cnidarian ancestors have created many thousands of square kilometers of biomineralized marine habitat in shallow tropical seas since their extensive radiation in the Middle Triassic period ~240 million years ago (Ma) (Veron, 1995). Coral reefs provide a significant source of ecosystem-based services (Moberg and Folke, 1999) that stabilize coastlines and provide habitat for an astounding variety of flora and fauna (Connell, 1978). To better understand the evolutionary strategies underpinning the evolutionary success of reef-building corals, we analyzed genomic and transcriptomic information from twenty stony corals that contain intracellular photosynthetic dinoflagellate symbionts of the genus Symbiodinium (https://comparative.reefgenomics.org/) (Figure 1, and Figure 1—source data 1). In addition, bilaterian reference gene sets and genomes from other cnidarians, ctenophores, sponges, a choanozoan, and a placozoan were integrated into our analysis. The comprehensive reference database used for our study included 501,991 translated protein sequences from 20 coral species, 98,458 proteins from five other cnidarians such as sea anemone and sea fan, and 91,744 proteins from seven basal marine metazoan lineages such as sponges and ctenophores. These publicly available genomic and transcriptomic data, which showed large disparities in terms of numbers of predicted protein sequences per species were ‘cleaned’ of contaminants and poor quality data with the use of stringent filters and selection criteria (see Materials and methods). This procedure resulted in a reasonably comprehensive coverage of corals (i.e., 20 species in total, 11 robust clade species including 2 genomes, 9 complex clade species including 1 genome) with and average of 21,657 protein sequences per species. Given the challenges associated with inferring conclusions based on the absence of genes (in particular when analyzing transcriptomic data), our approach focused on identifying ortholog groups present in different taxonomic categories to reach conclusions about genes associated with coral specific traits. This analysis yielded a set of 2485 ‘root’ orthologs, 613 ‘Non-Cnidaria’ orthologs, 462 ‘Cnidaria’ orthologs, 1436 ‘Anthozoa’ orthologs, 1810 ‘Hexacorallia’ orthologs, 172 ‘A’ orthologs, 4751 ‘Scleractinia’ orthologs, 1588 ‘complex coral’ orthologs, and 6,970 ‘robust coral’ orthologs (available at http://comparative.reefgenomics.org/). These orthologs were analyzed to address four major issues in coral evolution: 1) the basis of aragonite exoskeletal accretion that results in reef formation; 2) environmental sensing mechanisms of the cnidarian host; 3) evolution of the machinery necessary to accommodate the physiological risks as well as the benefits associated with the photosynthetic algal symbionts that create a hyperoxic environment when exposed to light; and 4) given the rich microbial flora associated with the coral holobiont (Fernando et al., 2015), the contribution of horizontal gene transfer (HGT) to coral evolution. Here we examine novel insights gained in each of these key areas.

Results
Relying on conserved proteins as queries in BLAST searches against our genomic database, we identified major components of the coral biomineralization toolkit and reconstructed their evolutionary origins using standard phylogenetic methods (see Material and methods). These results are presented in the Discussion section below and summarized in Figures 2A and 3. We also identified...
major components of the ion trafficking systems in human genomes, and searched for their orthologs in corals (Figure 2B and Figure 2—source data 1). Finally, using the approach described above, we identified stress response genes in corals and other cnidarians (listed in Supplementary file 1).

To elucidate the impact of foreign gene acquisition in coral evolution, we estimated the extent of HGT in the genomic data using a conservative phylogenomic approach (see Materials and methods). This procedure was followed by localization of key HGT candidates to genomic contigs to validate their provenance (Figure 4). Using the A. digitifera and Seriatopora sp. proteomes independently as queries resulted in 13,256 and 19,700 alignments of which 21 and 41, respectively (i.e., in A. digitifera, Seriatopora sp.), supported HGT (62/32,956 trees = 0.2%). After accounting for gene duplicates and redundancy between the trees, we discovered 41 unique instances of foreign gene acquisition from bacteria and algae (Table 1). Of these candidates, 28 genes were present in the anthozoan common ancestor (i.e., were shared with anemone and/or sea fan) and 13 were specific to corals. In all cases, the HGT-derived genes were shared by multiple anthozoan species and the phylogenies of these genes were largely consistent with the reference tree shown in Figure 1.

Discussion

Coral biomineralization

The most obvious feature of corals over geological time is their fossilized calcium carbonate skeletons, of which the original mineral component is aragonite. It has been hypothesized for many years that the precipitation of aragonite is catalyzed by and organized on an extracellular organic matrix
**Figure 1.** Multigene maximum likelihood (RAxML) tree inferred from an alignment of 391 orthologs (63,901 aligned amino acid positions) distributed among complete genome (boldface taxon names) and genomic data from 20 coral species and 12 outgroups. The PROTGAMMALGF evolutionary...
containing a suite of proteins, lipids, and polysaccharides (Mann, 2001; Watanabe et al., 2003). This process is precisely controlled and occurs in the calcifying fluid lined by the ectodermal calicoblastic cells that initiate and control the precipitation reaction. Four major components are involved in the process and will be described below: a source of inorganic carbon, a source of calcium ions, proteins that catalyze the nucleation reaction, and proteins and other organic molecules that organize the crystals to form macroscopic structures (Figure 2A). In this figure, only the transcellular pathway at the level of the calicoblastic cells is shown. Calcium presumably enters the cells via a calcium channel (Zoccola et al., 1999) and exits through a calcium ATPase which is proposed to remove protons from the site of calcification (Zoccola et al., 2004). Whereas part of the dissolved inorganic carbon (DIC) can enter the cells via a bicarbonate transporter (Furla et al., 2000), the major source of DIC comes from metabolic CO2, which either diffuses out of the cells through the membranes or is intracellularly converted into HCO3 due to a favorable pH (Venn et al., 2009), a reaction which is accelerated by carbonic anhydrases (Bertucci et al., 2013). This bicarbonate can then exit the cells via a bicarbonate transporter (Zoccola et al., 2015). At the site of calcification carbonic anhydrases can also play a role in the kinetics of the interconversion between carbon dioxide and bicarbonate (Bertucci et al., 2013) according to the extracellular pH (Venn et al., 2011). The organic matrix which plays different roles in the biological precipitation of carbonates, comprises a set of proteins including CARPs (Mass et al., 2013; ), collagens (Drake et al., 2013), galaxins (Fukuda et al., 2003), and carbonic anhydrase related proteins (Drake et al., 2013).

More broadly, inorganic carbon in seawater in the upper ocean is approximately 2 mM with 95% in the form of bicarbonate ions and is delivered to the site of calcification from an internal pool within the host animal (Erez, 1978; Furla et al., 2000). This happens either by diffusion of CO2 or by active transport of HCO3 following CO2 hydration (Tambutte et al., 1996). The hydration reaction is catalyzed by an intracellular carbonic anhydrase (CA) (Bertucci et al., 2013). To help facilitate calcification, calicoblastic cells concentrate dissolved inorganic carbon (DIC) in the calcifying fluid (Allison et al., 2014). Analysis of our genome data shows two distinct families of bicarbonate anion transporters (BATs) in the coral Stylophora pistillata (Zoccola et al., 2015). Three isoforms belong to the SLC26 family (Figure 2—figure supplement 1) and 5 isoforms belong to the SLC4 family (Figure 2—figure supplement 2). One isoform, SLC4γ, is restricted to scleractinians and is only expressed in the calicoblastic cells (Zoccola et al., 2015), strongly suggesting that this protein plays a key role in calcification. This bicarbonate transporter could either supply DIC at the site of calcification, or aid in pH regulation in addition to a calcium ATPase (see below). Furthermore, the two BAT gene families are split along phylogenetic lines between the robust and complex clades of scleractinians.

The concentration of calcium ions in seawater is 10 mM, with these ions being actively transported by the calicoblastic cells to the calcifying fluid (Tambutte et al., 1996). Radiocalcium (45Ca) and inhibitor studies demonstrate that calcium entry in calicoblastic cells by facilitated diffusion is dependent on voltage-gated calcium channels (Tambutte et al., 1996). Based on their alpha 1 subunit (Caα1) these channels can be phylogenetically divided into three groups. Specific inhibitors (dihydropyridines) strongly suggest that these channels belong to the voltage-dependent L-type family CaV1 and have been characterized at the molecular level and localized by immunohistochemistry in the calicoblastic cells (Zoccola et al., 1999). We constructed a phylogeny of the alpha 1 subunit of all types of CaV (Figure 2—figure supplement 3) and found orthologs in most of the datasets used here, as previously shown for the actinarian Nematostella vectensis and the scleractinian Acropora millepora (Moran and Zakon, 2014). Calcium efflux from the calicoblastic cells to calcifying fluid likely occurs through a plasma membrane calcium ATPase (Ca-ATPase) (Zoccola et al., 2004). This enzyme is also responsible for removing protons and increasing pH in the calcifying fluid.
in order to increase the aragonite saturation state to promote calcification (Zoccola et al., 2004; Venn et al., 2011; Davy et al., 2012). For this enzyme (Figure 2—figure supplement 4) as well as for calcium channels and bicarbonate transporters, there is a division between the robust and complex clades of scleractinians.

As described in the two previous paragraphs, for the analysis of the source of inorganic carbon and calcium transport for biomineralization, we focused on the molecules which were previously characterized both by pharmacological and physiological studies in order to link molecular characterization to function. Our data clearly show that transporters such as calcium channels and calcium ATPases and some bicarbonate transporter isoforms are ubiquitously present in the calcifying and non-calcifying cnidarians (scleractinian corals and sea anemones). Based on the genomic analysis of...
bicarbonate transporters in two scleractinian corals and one sea anemone, Zoccola et al. (2015) observed that one isoform of the bicarbonate transporter family SLC4, was restricted to scleractinians. The current transcriptomic analysis of calcifying and non-calcifying cnidarian species confirms this result, which underlines the role of this transporter in biomineralization. Additional studies are however needed to localize this transporter in different coral species and to determine whether, as for S. pistillata, it is also specifically expressed in the calicoblastic cells. Another important piece of information is that for all the different enzymes and transporters studied, there is generally a division in the phylogenetic tree between the robust and the complex clades of scleractinian corals. This suggests that the different calcification traits observed for the two clades (for example, complex corals have less heavily calcified skeletons than robust corals), are due to differences in the biochemical characteristics of these enzymes and transporters.

Figure 3. Comparison of robust coral (brown text) and complex coral (green text) and non-coral (blue text) genomes with respect to percent of encoded proteins that contain either >30% or >40% negatively charged amino acid residues (i.e., aspartic acid [D] and glutamic acid [E]). The average composition and standard deviation of D + E is shown for the two cut-offs of these estimates. On average, corals contain >2-fold more acidic residues than non-corals. This acidification of the coral proteome is postulated to result from the origin of biomineralization in this lineage.

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Figure 4. Analysis of a genomic region in Acropora digitifera that encodes a putative HGT candidate. (A) The genome region showing the position of the HGT candidate (PNK3P) and its flanking genes. (B) Maximum likelihood trees of PNK3P, non-coral metazoans, and the proteins (RNA-binding and GTP-binding proteins) encoded by the flanking genes. Robust and complex corals are shown in Figure supplement 1. DOI: 10.7554/eLife.13288.016

The following figure supplements are available for figure 4:

Figure supplement 1. Maximum likelihood trees of a DEAD-like helicase and the protein encoded by the flanking gene.

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Figure supplement 2. Maximum likelihood tree of an exonuclease-endonuclease-phosphatase (EEP) domain-containing protein (A), an ATP-dependent endonuclease (B), a tyrosyl-DNA phosphodiesterase 2-like protein (C), and DNA mismatch repair (MutS-like) protein (D).

Figure 4 continued on next page
The skeletal structure of corals contains an embedded organic matrix with a set of proteins that have a high proportion of aspartic and glutamic acids (Mitterer, 1978; Weiner, 1979; Mann, 2001; Weiner and Dove, 2003; Gotliv et al., 2005). These coral acid-rich proteins (CARPs) (Mass et al., 2013) show sequence similarity across Scleractinia (Drake et al., 2014) and have functional analogs across the biomineralizing tree of life (Gorski, 1992; Sarashina and Endo, 2001; Kawasaki et al., 2009). CARPs contain >28% aspartic and glutamic acids and have isoelectric points less than pH 5 (Table 1 in Mass et al., 2013). Each of these proteins can individually catalyze the precipitation of calcium carbonate in vitro in natural seawater (Mass et al., 2013), hence, they appear to be responsible for initiating biomineralization. Our results show that the average composition of aspartic and glutamic acids in scleractinian corals is 2-fold higher than in 12 non-calciﬁying invertebrates, with no obvious difference between the robust and complex clades of scleractinians (Figure 3). Moreover, phylogenetic analysis reveals that four CARP genes (CARPs 2–5) are widely distributed among scleractinians, suggesting they are derived from homologs present in non-calciﬁying anthozoans. Extensive duplication of genes encoding CARPs predated the split of robust and complex corals can be seen for CARPs 3–5 (Figure 2—Figure Supplement 5), whereas CARP 2 appears to be unique to robust corals. A previous hypothesis that CARP 1 resulted from a gene (domain) fusion (Mass et al., 2013) is supported by these extensive genome data. CARP 1 is derived from a reticulocalbin-like gene present in all metazoans that underwent the fusion of an acidic N-terminal domain, resulting in a modular gene that is found only in corals (Figure 2—Figure Supplement 5). Our data suggest that the enrichment of highly negatively charged proteins is a major distinguishing feature of stony corals.

At the nanoscale, the biological precipitation of aragonite crystals is insufﬁcient to form the highly organized, stable macrostructures that characterize corals. The crystals are organized by a series of proteins that act as ‘glues’. One of these protein families, found in the skeletons of corals is collagen (Jackson et al., 2010; Drake et al., 2013). In basal invertebrates, there are three families of collagen (fibrillar, multiplexins, and type IV) that are also present in vertebrates. Other than their structural function, collagens play an important role in the regulation of cell-cell adhesion, differentiation, and wound healing (Heino et al., 2009). Collagens in the alpha IV subfamily have been identiﬁed in the organic matrix of coral skeletons (Ramos-Silva et al., 2013; Drake et al., 2013). Alpha IV collagens form networks of ﬁbers that are an important component of the extracellular matrix. Using the complete genome data from S. pistillata and Seriatopora sp., we identiﬁed 230 and 208 predicted open reading frames (ORFs), respectively, that contained a collagen Pfam domain. Of these, 52 S. pistillata proteins contain an extracellular secretion signal, in comparison to 17 from Seriatopora sp. By plotting the isoelectric point (IP) of the secreted collagens from both corals we identiﬁed four acid-rich collagens in Seriatopora sp. and ﬁve in S. pistillata that have an IP < 7 (Figure 2—Figure Supplement 6). This analysis strongly suggests that these collagens play a critical role in tethering aragonite crystals in coral skeletons similar to their role in bone formation (Nudelman et al., 2010).

In addition to collagens, stony corals secrete a variety of other adhesion proteins into the calciﬁying milieu (Ramos-Silva et al., 2013; Drake et al., 2013). These include cadherins, which facilitate cell-cell or cell-substrate adhesion, vitellogenin, and zonadhesin proteins. As part of the biomineralization toolkit, these proteins bind the calicoblastic cells to the newly formed skeleton and may assist in the binding of CARPs to other functional proteins. Interestingly, the ﬁrst protein sequenced from coral skeleton, galaxin, is neither acidic nor calcium binding, and its function remains unknown.
### Table 1. The list of non-redundant anthozoan genes derived via HGT.

| No. | Ancestor | Genes            | Protein products                  | Support | Source(s) |
|-----|----------|------------------|-----------------------------------|---------|-----------|
| 1   | Coral    | A. digitifera_2036 | PNK3P                             | 100     | CA        |
| 2   | Coral    | A. digitifera_8849 | SDR                               | 100     | CA        |
| 3   | Coral    | Seriatopora_31861 | DEAD-like helicase                 | 100     | Bact      |
| 4   | Coral    | Seriatopora_16594 | glyoxalase                        | 100     | CA        |
| 5   | Coral    | Seriatopora_17147 | acyl- dehydrogenase               | 100     | Bact      |
| 6   | Coral    | Seriatopora_17703 | carbonic anhydrase                 | 96      | Dino      |
| 7   | Coral    | Seriatopora_19477 | fatty acid or sphingolipid desaturase | 100 | CA        |
| 8   | Coral    | Seriatopora_3957  | atpase domain-containing protein   | 100     | Bact      |
| 9   | Coral    | Seriatopora_7060  | sam domain-containing protein      | 100     | Bact      |
| 10  | Coral    | Seriatopora_7928  | atp phosphoribosyltransferase      | 100     | CA/Fungi  |
| 11  | Coral    | Seriatopora_8296  | glyoxalase                        | 98      | Bact      |
| 12  | Coral    | Seriatopora_22596 | 2-alkenal reductase                | 92      | Bact      |
| 13  | Coral    | Seriatopora_28321 | histidinol-phosphate aminotransferase | 96    | Unclear   |
| 14  | Anthozoa | A. digitifera_418  | duf718 domain protein             | 100     | CA        |
| 15  | Anthozoa | A. digitifera_15871| peptidase s49                     | 96      | Algae/Bact |
| 16  | Anthozoa | A. digitifera_14520| predicted protein                  | 100     | CA/Bact   |
| 17  | Anthozoa | A. digitifera_7178 | rok family protein/fructokinase    | 93      | Red algae |
| 18  | Anthozoa | A. digitifera_10592| Phospholipid methyltransferase     | 100     | CA/Viri   |
| 19  | Anthozoa | A. digitifera_13390| predicted protein                  | 100     | Bact      |
| 20  | Anthozoa | A. digitifera_313  | malate synthase                   | 98      | CA/Bact   |
| 21  | Anthozoa | A. digitifera_1537 | hypothetical protein               | 100     | Bact      |
| 22  | Anthozoa | A. digitifera_13577| gamma-glutamyltranspeptidase 1-like| 100     | Unclear   |
| 23  | Anthozoa | A. digitifera_5099 | isocitrate lyase (ICL)             | 100     | Bact      |
| 24  | Anthozoa | A. digitifera_13467| uncharacterized iron-regulated protein | 100 | CA        |
| 25  | Anthozoa | A. digitifera_6866 | 3-dehydroquinate synthase          | 98      | CA        |
| 26  | Anthozoa | A. digitifera_11675| intein c-terminal splicing region protein | 100 | Bact      |
| 27  | Anthozoa | Seriatopora_10994 | penicillin amidase                 | 100     | Bact      |
| 28  | Anthozoa | Seriatopora_14009 | nucleoside phosphorylase-like protein | 100 | Bact      |
| 29  | Anthozoa | Seriatopora_14494 | phosphonoacetaldehyde dehydrogenase | 100 | Dino      |
| 30  | Anthozoa | Seriatopora_15303 | exonuclease-endonuclease-phosphatase | 99   | CA/Viri   |
| 31  | Anthozoa | Seriatopora_15772 | fmn-dependent nadh-azoreductase    | 99      | Dino      |
| 32  | Anthozoa | Seriatopora_19888 | had family hydrolase               | 97      | Algae/Bact |
| 33  | Anthozoa | Seriatopora_20039 | chitodextrinase domain protein     | 92      | Dino      |
| 34  | Anthozoa | Seriatopora_20146 | glutamate dehydrogenase            | 100     | CA/Bact   |
| 35  | Anthozoa | Seriatopora_20479 | thf family protein                 | 100     | Bact      |
| 36  | Anthozoa | Seriatopora_21195 | ATP-dependent endonuclease         | 100     | Dino      |
| 37  | Anthozoa | Seriatopora_8585  | chitodextrinase domain protein     | 92      | Bact      |
| 38  | Anthozoa | Seriatopora_24047 | aminotransferase                  | 100     | Bact      |
| 39  | Anthozoa | Seriatopora_25961 | d-alanine ligase                   | 99      | Bact      |
| 40  | Anthozoa | Seriatopora_26478 | quercetin 3-o-methyltransferase    | 100     | Viri      |
| 41  | Anthozoa | Seriatopora_29443 | diaminopimelate decarboxylase      | 100     | CA        |

Bact: Bacteria; CA: chlorophyll c-containing algae; Dino: dinoflagellates; Viri: Viridiplantae.

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(Fukuda et al., 2003). Originally sequenced from Galaxea fascicularis, but more recently identified in the A. millepora skeleton, galaxin is a 30–40 kDa glycosylated protein with a signal peptide, suggesting it is secreted (Fukuda et al., 2003; Ramos-Silva et al., 2013). The primary sequence contains ~20 paired cysteine (CC) residues. Usherin, found in vertebrates has a similar high number of paired cysteine motifs (Baux et al., 2007) and binds type IV collagens (Bhattacharya et al., 2004), suggesting a potential role for this galaxin. Galaxin was originally suggested to be coral-specific (Fukuda et al., 2003), however, galaxin-like proteins are found in non-calcifying taxa outside Cnidaria (e.g., Sanchez et al., 2007; Heath-Heckman et al., 2014). Therefore, it has been proposed that the precursor to modern coral galaxin homologs was recruited as a biomineralization protein when Scleractinia diverged from non-biomineralizing taxa during the Triassic (Foret et al., 2010). Our sequence analysis supports this hypothesis, suggesting that not only is coral galaxin derived from a common ancestor with non-calcifying metazoans, but that it is polyphyletic within corals (Figure 2—figure supplement 7), and independently recruited for a role in biomineralization multiple times in coral evolution. The first evidence for stony corals occurs in the Triassic and fossil evidence shows a rapid proliferation of taxa (reviewed by Stanley, 2003); this was also a time of ‘aragonite seas’ when geochemical conditions were favorable to the formation and evolution of aragonitic coral skeletons (Stanley and Hardie, 1998).

A second type of galaxin, amgalaxin, has an N-terminal acidic domain that precedes the galaxin domain (Reyes-Bermudez et al., 2009). However, unlike galaxin, amgalaxin appears to function only in the early larval stages of biomineralization and has not been observed in the coral skeleton (Reyes-Bermudez et al., 2009; Ramos-Silva et al., 2013). This pattern is similar to the mollusk and coral proteins nacrein and CARP1 (see above), in which an acidic domain is fused to an existing gene (Miyamoto et al., 1996; Mass et al., 2013). Unlike galaxin, the acidic portion of amgalaxin appears to be limited to corals (Figure 2—figure supplement 7). This result suggests that the attachment of an acidic region to galaxin is unique to stony corals and that amgalaxin, like CARP1, emerged from a gene fusion event.

**Environmental and stress response systems**

Corals typically produce planktonic or ‘crawl-away larvae’ that calcify when they settle on an appropriate benthic substrate, and have thereby effectively determined their future physical environment for the life of the organism. Hence, habitat selection is one of the most critical elements in the survival and success of individual corals. To help accommodate variations in habitat on time scales varying from hours to years, corals have evolved a suite of environmental sensing and response systems. One of the most critical environmental cues for coral success is light (Dubinsky and Falkowski, 2011). Stony corals use diel periodicity and light sensing capabilities as cues for spawning, feeding, and orienting the polyps. Perhaps not surprisingly, the host animal has genes encoding a circadian clock. However, the light sensing signal cascades in zooxanthellate corals are particularly complex because of their symbiotic relationship with dinoflagellates, which also have a circadian clock. Coral environmental response genes are coupled to the dinoflagellate circadian clock, anticipating changes in the intracellular milieu such as the coral tissue becoming hypoxic due to zooxanthellate photosynthesis and near-hypoxic at night due to host and symbiont respiration. Numerous chaperones such as heat shock protein (hsp) 40, hsp70, hsp90, grp94, hsp90b1, calreticulin, and protein disulfide isomerase are ‘hard-wired’ to this photosynthesis/respiration clock and the high level of synchrony of circadian transcription of chaperones and antioxidant genes reflects the diurnal preparedness of the coral to the consequences of oxidative protein damage imposed by photosynthesis of the algal symbionts (Levy et al., 2011). Symbiosis also indirectly imposes diurnal gene expression fluctuations, most likely via the hypoxia inducible factor (HIF) system. In a wide array of animals, glycolytic enzymes are regulated by HIF1-alpha transcription factor, a clear ortholog of which is present in the 20 coral genomic datasets. The HIF system is unique to animals, and HIF itself is a target of calpain-mediated degradation in vertebrates. Calpains are Ca^{2+}-dependent regulatory proteases and corals linkage of calpain expression to the HIF system potentially enables them to utilize cellular calcium levels to modulate expression of other HIF targets when hypoxia dominates (Levy et al., 2011).

The casein kinase I (CK1) family consists of serine/threonine protein kinases that are key regulators of circadian timing in bilaterian animals, fungi, and green algae (van Ooijen et al., 2013). CK1-like encoding genes are found in most corals and were suggested to be components of the coral
circadian gene network along with CLOCK, GSK₃B/Sgg, and CSNK1D (Vize, 2009). The proteins ADCl, GNAQ, GNAS, GNB1, CREB1, and NOS1 are related to G-protein coupled receptor signaling and can act on neuropeptide/GPCR-coupled signaling mechanisms. This is consistent with neuropeptides playing a role in synchronized spawning events in tropical abalone (York et al., 2012) and in coral larvae settlement (Grasso et al., 2011). Other proteins such as PPEF1 and GRN1 respond to light stimulus, whereas MTNR1A and MTNR1B are melatonin receptors, whereas PRKAA2 is a protein kinase that responds to peptide hormone stimulus and is responsive to circadian rhythms. The circadian processes are impacted by catabolic process; i.e., S. pistillata glycolysis is controlled by ARNT and HIF1-alpha that provide feedback that affects the circadian loop. Surprisingly, BLASTp analysis of the 20 coral genomic datasets did not turn up the Period gene as reported in other cnidarians. Therefore, the core circadian clock architecture of the negative feedback loop in basal metazoans such as corals may differ significantly from animal lineages that diverge after corals.

Although fluxes of calcium and bicarbonate ions into the calcicoblastic space are part of the biomineralization system, these and other ion pumps also generate electrochemical gradients that allow stony corals to sense the environment and initiate complex and specific signaling cascades (Hille, 1986). This ion trafficking landscape and downstream signaling components are comprised of channels, transporters, exchangers, pumps, second messenger generators, and transcriptional response elements. Many of these ion transporters act as direct physicochemical sensors providing intra-cellular and intra-organismal regulation and the critical linkage between external environmental changes and cytoplasmic and organellar events. Circadian gene network along with CLOCK, GSK₃B/Sgg, and CSNK1D (Vize, 2009). The proteins ADCl, GNAQ, GNAS, GNB1, CREB1, and NOS1 are related to G-protein coupled receptor signaling and can act on neuropeptide/GPCR-coupled signaling mechanisms. This is consistent with neuropeptides playing a role in synchronized spawning events in tropical abalone (York et al., 2012) and in coral larvae settlement (Grasso et al., 2011). Other proteins such as PPEF1 and GRN1 respond to light stimulus, whereas MTNR1A and MTNR1B are melatonin receptors, whereas PRKAA2 is a protein kinase that responds to peptide hormone stimulus and is responsive to circadian rhythms. The circadian processes are impacted by catabolic process; i.e., S. pistillata glycolysis is controlled by ARNT and HIF1-alpha that provide feedback that affects the circadian loop. Surprisingly, BLASTp analysis of the 20 coral genomic datasets did not turn up the Period gene as reported in other cnidarians. Therefore, the core circadian clock architecture of the negative feedback loop in basal metazoans such as corals may differ significantly from animal lineages that diverge after corals.

### Impacts of the environment on the symbiotic life history of corals

Symbiotic corals thrive in oligotrophic tropical and subtropical seas in large part because their intracellular, symbiotic dinoflagellates provide a significant portion of their photosynthesis-derived fixed carbon to the host animal. However, this benefit comes with significant costs. The ecological stability of the symbiotic association is dependent on it being stable in the face of environmental extremes. This symbiosis has been widely described as living close to the upper extremes of thermal tolerance that, when exceeded, leads to a cascade of cellular events resulting in ‘coral bleaching’, whereby corals lose their symbiotic algae and consequently one of their main sources of carbon (Lesser, 2006; 2011). Other environmental extremes can lead to coral bleaching including exposure to ultraviolet radiation (UVR) and ocean acidification (Lesser, 2004; Hoegh-Guldberg et al., 2007). Proximately, in this cascade of events, many physiological studies on bleaching in corals and other symbiotic cnidarians have shown that photosynthetically produced hyperoxic conditions act synergistically with solar radiation, especially UVR, and thermal stress to produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) in both host tissues and Symbiodinium sp. beyond their capacity to quench these toxic products (Lesser, 2006; 2011). Ultimately a series of fairly well described stress response events involving cell cycle arrest and apoptosis, in both the algal symbionts and host, appear to be responsible for the massive expulsion of dinoflagellates from the host, and ultimately, host mortality if the environmental insult is severe enough or of prolonged duration (Lesser, 2006; 2011).

Therefore, the ecological stability of the symbiotic association in zooxanthellate corals requires increased stability in the face of environmental extremes. Previous coral genomic studies have identified genes involved in the stress response of cnidarians (Shinzato et al., 2011), but here we show that corals contain highly conserved genes involved in oxidative stress, DNA repair, the cell cycle and apoptosis (Supplementary file 1). For instance we identify both the extrinsic and intrinsic apoptotic pathways, characteristic of many vertebrates including humans. These genes are not derived...
by HGT in the Cnidaria, because of their presence in poriferans and other sister taxa (see HGT discussion below). Corals exposed to oxidative stress, or UVR, accumulate DNA damage, whereby cell cycle arrest occurs and cell repair is initiated (Lesser and Farrell, 2004). If DNA damage is too severe, then a cellular cascade leading to genetically programmed cell death by apoptosis occurs via an intrinsic, or mitochondrial, pathway. Whereas the intrinsic pathway is considered a response to stress (e.g., thermal stress), the extrinsic, or death-receptor pathway is a cellular process by which cell to cell communication activates apoptosis via ligand binding to cell surface receptors, as in the well described immunological response to cancer cells or pathogens. Genes present in cnidarians and active in the vertebrate intrinsic DNA damage induced apoptotic pathway include: ATM, p53 (and many of its important regulator proteins and transcriptional products), Husp, Bax, Bcl-2, AIF, cytochrome C, APAF1, procaspase 9, procaspase 3, ICAD and CAD (Supplementary file 1). The activity of these genes in cnidarians comprises the cellular machinery necessary to accomplish the following: mitochondrial catastrophe, apoptosome formation, breakdown of the nuclear envelope, intranuclear DNA disassembly and flipping of phosphatidyserine from the inner to the outer leaflet of the plasma membrane that in humans permits macrophage recognition of apoptotic cells. In addition, we identified a complete nitric oxide synthase (NOS; EC 1.14.13.39) in corals. This gene is derived from a metazoan ancestor and is thought to play a key role in the stress response that leads to breakdown of the symbiosis and coral bleaching (Trapidio-Rosenthal et al., 2005; Hawkins et al., 2013). Another significant finding of our analysis of multiple taxa is that Bid (BH3; Bcl-2 domain of homology 3), the only protein that allows the extrinsic and intrinsic pathways in vertebrates to directly communicate with each other, is not present in the coral data. Previous research on apoptosis in invertebrates, particularly on the intrinsic pathway, demonstrated the conserved nature of the molecular machinery in ancestral metazoans (Bender et al., 2012). Cnidarians encode all the genes for both pathways known to be expressed and active in vertebrates, but appear to lack the ability to communicate between them. This function is mediated by p53, the gatekeeper for cell growth and division, through Bid in vertebrates (Sax et al., 2002) that is present in 20 of 25 cnidian datasets examined here. The antiquity of the intrinsic pathway is striking and along with the recent demonstration of a functional extrinsic pathway in cnidarians (Quistad et al., 2014) reveals the importance of these apoptotic pathways in metazoan evolution. Interestingly, tumor necrosis factor (TNF), an essential mediator of the extrinsic death-receptor pathway, was present in only 7 of the 32 datasets examined in this study (Supplementary file 1). Lastly, the presence of the major genes in the human extrinsic and intrinsic pathways suggests that cnidarians may be a potential model system for studying transcriptionally induced apoptosis, when compared to Caenorhabditis elegans and Drosophila melanogaster. In these latter animal models, the available functional data indicate that genes in the cellular senescence, DNA editing, and repair pathways that are governed by the transcriptional activation domain (TAD) of p53 are only 2% (D. melanogaster) and 33% (C. elegans) conserved when compared to human p53 (Walker et al., 2011). This result suggests limited control of somatic cell apoptosis in these organisms perhaps because their adult somatic cells do not divide by mitosis.

**Contribution of horizontal gene transfer to coral evolution**
The primary function of the HGT candidates we identified in stony corals is to extend the existing stress related pathways in these animals. These foreign genes encode proteins that provide protection from UVR and stress from reactive species (Banaszak and Lesser, 2009; Nesa et al., 2012). It has already been reported that corals and sea anemones acquired a pathway that produces photosynthetic mycosporine amino acids that absorbs UVR (Shinzato et al., 2011). Our results show additions to the DNA repair pathway, including a polynucleotide kinase 3-phosphatase (PNK3P) of algal origin (Figure 4) and a DEAD-like helicase of bacterial origin (Figure 4—figure supplement 1). These two genes are flanked by eukaryotic or coral-specific genes in their respective contigs in the draft genome of A. digitifera (Figure 4 and Figure 4—figure supplement 1). Two DNA repair genes that were transferred from algal sources were found in the anthozoan ancestor. These encode an exonuclease-endonuclease-phosphatase (EEP) domain-containing protein and an ATP-dependent endonuclease (Figure 4—figure supplement 2). Furthermore, two DNA repair genes are shared between Anthozoa and sponges or choanoflagellates, but are missing from a large diversity of Bilateria; these encode a tyrosyl-DNA phosphodiesterase 2-like protein and a DNA mismatch repair (MutS-like) protein (Figure 4—figure supplement 2). Our results fit in well with the so-called Public
Goods Hypothesis that posits important genetic resources, such as mechanisms of DNA repair, are distributed widely among taxa via both vertical and horizontal evolution (McInerney et al., 2011).

Protection against reactive species in corals, in addition to the multiple homologs we found with antioxidant functions such as superoxide dismutase (Supplementary file 1), is provided by two genes derived via HGT that encode glyoxalase I. One of these has an algal (Figure 4—figure supplement 3) and the other a bacterial provenance (Figure 4—figure supplement 4). Interestingly, the latter gene is physically located between a DNA repair gene (encoding RAD51) and a tRNA modification gene on scaffold 2777 in the A. digitifera draft assembly (Figure 4—figure supplement 4). Glyoxalase I belongs to a system that carries out the detoxification of reactive carbonyls (RC), such as highly cytotoxic methylglyoxal, produced by sugar metabolism and the Calvin cycle (Shimakawa et al., 2014). Methylglyoxal production in plastids increases with light intensity (Takagi et al., 2014). Another gene encoding a putative RC scavenger (Shimakawa et al., 2014) is short-chain dehydrogenase/reductase (SDR) that was derived in corals from an algal source (Figure 4—figure supplement 5). Other alga-derived HGTs were from species containing plastids of red algal secondary endosymbiotic origin (i.e., chlorophyll c-containing lineages such as stromatopiles) (Table 1). Given the coral-Symbiodinium symbiosis, it is also notable that several of the HGT candidates appear to be derived from dinoflagellates (e.g., Figure 4—figure supplement 5). The gene contribution from chlorophyll c-containing lineages suggests a long history of interaction between these algae and the anthozoan lineage.

Conclusions

Cnidarians enter the fossil record about 545 Ma in the latest Ediacaran Period and have been an important component of marine ecosystems throughout the Phanaerozoic, surviving five major mass extinctions and many smaller biotic crises. Although reefs have often disappeared during each of these events, various coral clades have persisted. Our analysis of a subset of coralliform cnidarians, the symbiotic Scleractinia, reveals how their genomic information has provided the basis for adapting to changes in ocean temperature and pH, while maintaining the ability to calcify. This is significant because scleractinians survived throughout the Cenozoic despite atmospheric CO₂ levels reaching 800 ppm 50–34 Ma, and tropical sea temperatures of 30°–34°C from 45 to 55 Ma (Norris et al., 2013). This interval coincides with a reef gap, but reefs were quickly re-established thereafter. The resilience of corals in the face of extraordinary changes in ocean conditions clearly bespeaks a gene inventory that is highly adaptive as exemplified by the diversification of CARPs and genes recruited through HGT. Human activity has the potential to further reduce the abundance of these organisms in coming decades; indeed, there is compelling evidence of human destruction of corals worldwide. However, the diverse genetic repertoire of these organisms will potentially allow them to survive the expected changes in thermal structure and pH in the coming centuries (Stolarski et al., 2011), assuming that their populations and habitats are not physically destroyed by humans.

Materials and methods

Analysis of genome data and construction of coral tree of life

Coral genomic and transcriptome data compiled in this study are summarized in Figure 1—source data 1. All data were filtered to remove assembled contigs <300 bp. ORFs were predicted with TransDecoder (Haas et al., 2013) yielding amino acid sequences. Protein duplicates were subsequently removed with CD-HIT (Fu et al., 2012). With regard to coral sequence datasets, potential contaminant sequences from the algal symbiont, Symbiodinium were removed with script psytrans.py (https://github.com/sylvainforet/psytrans) using training sets retrieved from Symbiodinium microadriaticum (Baumgarten et al., 2013) and Acropora digitifera (Shinzato et al., 2011). Successful separation of coral and algal sequences was validated by GC-content plots that showed a clear bimodal data distribution (results not shown). Filtered sequence data were searched against SwissProt (Boutet et al., 2007), TrEMBL (Bairoch and Apweiler, 2000), NCBI nr databases using BLASTp (Basic Local Alignment Search Tool, e-value cut-off = 1e-03) (Altschul et al., 1990) and retaining annotations from databases in this order. BLAST2GO (Conesa et al., 2005) was queried to provide GO annotations, and KEGG (Kanehisa and Goto, 2000), Pfam (Bateman et al., 2002),
Orthologs were identified using InParanoid (Ostlund et al., 2010) on pairwise BLASTp (e-value cutoff = 1e-05) yielding a list of pairwise orthologs that was subsequently queried with QuickParanoid (http://pl.postech.ac.kr/QuickParanoid/) for automatic ortholog clustering among multiple species. QuickParanoid input files were filtered according to the following rules: A) Only orthologs sets were retained with a confidence score of 1, and B) Pairwise comparisons were retained if only one sequence is present in each of the two involved species. To make more robust inferences based on transcriptomic data, we filtered our ortholog dataset such that any ortholog from a given phylogenetic grouping (i.e., robust corals, complex corals, Scleractinia, Actinaria, Hexacorallia, Anthozoa, Cnidaria, non-cnidarian, root) was considered to be an ortholog in this group if it was present in this group and absent in all other groups. The QuickParanoid-derived ortholog clusters were sorted into the following categories based on the constituent taxa and known species tree (Figure 1): 1.) 2,485 ‘root’ orthologs, 2.) 613 ‘Non-Cnidaria’ orthologs, 3.) 462 ‘Cnidaria’ orthologs, 4.) 1436 ‘Anthozoa’ orthologs, 5.) 1,810 ‘Hexacorallia’ orthologs, 6.) 172 ‘Actinaria’ orthologs, 7.) 4,751 ‘Scleractinia’ orthologs, 8.) 1,588 ‘complex coral’ orthologs, and 9.) 6,970 ‘robust coral’ orthologs (available at http://comparative.reefgenomics.org). For phylogenetic tree building, we selected ‘root’ orthologs that were present in at least 50% of the species of any lineage (i.e. Root, Non-Cnidarian, Cnidarian, Anthozoa, Hexacorallia, Actinaria, Scleractinia, Complex corals, Robust corals) yielding 391 distinct orthologs over 7970 sequences. Orthologs were aligned individually on the protein level via MAFFT (Katoh and Standley, 2013) in ‘LiNSI’ mode. The resulting alignments were concatenated and then trimmed with TrimAl in the automated mode (-automated) (Capella-Gutierrez et al., 2009). The resulting alignment (63,901 amino acids) was used for phylogenetic tree building with RAxML (Stamatakis, 2014) under PROTGAMMALGF model with 100 bootstrap replicates for the estimation of branch supports (-T 32 -f a -x 1234 -p 1234 -N 100 -m PROTGAMMALGF).

Analysis of ion transport

Human ionome protein reference sequences were identified and downloaded from Genbank at NCBI. Using BLASTStation-Local64 (v1.4, TM software, Inc, Arcadia, CA 91007, USA), a coral protein database was generated. This contained all protein sequences available from the reefgenomics website (http://comparative.reefgenomics.org/). The human ionome protein sequences were then used as queries to search (Basic Local Alignment Search Tool, BLAST) against this local database using BLASTp (no filter, Expect: 10; Word Size 3; Matrix: BLOSUM63; Gap Costs: Existence 11 extension 1) using BLASTStation-Local64. The resulting matching coral proteins were saved in multi-FASTA format files, and then re-BLASTed against the NCBI Refseq protein database (Pruitt et al., 2012) limited to human-only proteins (taxid:9606) on the NCBI BLAST webportal (algorithm BLASTp, default parameters; Expect: 10; Word Size 3; Matrix: BLOSUM62; Gap Costs: Existence 11 extension 1) (Camacho et al., 2009). The results were viewed for each coral protein from the input file, and a summary was generated, indicating which human protein was identified as a top hit, and in which coral species it was found. The coral multi-FASTA file was copied and annotated manually with the gene symbols of the human protein identified. If a protein coral sequence was not identified as the original human protein sequence, it was deleted, if other gene family members were identified this information was also annotated, and entered into the summary table. These multi-FASTA files were then stored for future analysis (e.g., generating phylogenetic trees). The results from the coral to human BLASTp alignments were also stored.

Analysis of horizontal gene transfer

Protein sequences in RefSeq (version 58) were downloaded from NCBI FTP site (ftp://ftp.ncbi.nlm.nih.gov/refseq/). When sequences were available from more than one (sub) species in a genus (e.g., Arabidopsis thaliana and A. lyrata in the genus Arabidopsis), the species (e.g., A. thaliana) with largest number of sequence were retained, whereas others (e.g., A. lyrata) were all removed. This dataset was combined with algal sequences collected from Cryptophyta [Guillardia theta (Curtis et al., 2012)], Haptophyceae [Emiliania huxleyi (Read et al., 2013)], Rhizaria [Bigelowiella natans (Curtis et al., 2012) and Reticulomyxa filosa] (Glockner et al., 2014), Stramenopiles [Nannochloropsis gaditana (Radakovits et al., 2012) and Aureococcus anophagefferens (Gobler et al., 2011)] and
dinoflagellates [Alexandrium tamarense (Keeling et al., 2014), Karenia brevis (Keeling et al., 2014), Karlodinium micrum (Keeling et al., 2014), Symbiodinium minutum (Shoguchi et al., 2013)], Glaucophyta [Cyanophora paradoxa (Price et al., 2012)], Viridiplantae [Bathycoccus prasinus (Moreau et al., 2012), Chlorella variabilis (Blanc et al., 2010), Coccomyxa subellipsioidae (Blanc et al., 2012), Micromonas pusilla (Worden et al., 2009), Glycine max (Schmutz et al., 2010)] and all red algal sequences collected in the previous study (Qiu et al., 2015). We further clustered similar sequences (sequence identity ≥85%) among taxa from each order (e.g., Brassocales or Primates), retained the longest sequence and removed all other related sequences in the same cluster using CD-HIT version 4.5.4 (Li and Godzik, 2006). This non-redundant database, combined with protein sequences derived from three coral genomes (Acropora digitifera and Seriatopora sp. and Stylophora pistillata) was designated as ‘Ref58+Coral’ database.

The protein sequences from A. digitifera and Seriatopora sp. genomes were used as query to search against the ‘Ref58+Coral’ database using BLASTp (e-value cut-off = 1e-05). Up to 1000 top hits (query-hit identity ≥27.5%) were recorded. These hits were sorted according to query-hit identity in a descending order among those with query-hit alignment length (≥120 amino acids). Hit sequences were then retrieved from the queried database with no more than three sequences for each order and no more than 12 sequences for each phylum. The resulting sequences were then aligned using MUSCLE version 3.8.31 (Edgar, 2004) under default settings and trimmed using TrimAl version 1.2 (Capella-Gutierrez et al., 2009) in an automated mode (-automated1). Alignment positions with ≥50% gap were discarded. We removed sequence alignments with <80 amino acid sites and those with <10 sequences. The remaining alignments were used for phylogenetic tree building using FastTree version 2.1.7 (Price et al., 2010) under the defaulting settings (except that WAG model was used instead of JTT model). The resulting trees were parsed to search for coral sequences that were nested within metazoan sequences with ≥0.9 local support values estimated using the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) using in-house tools. All such coral sequences were considered to represent metazoan host genes and were discarded from downstream analyses.

We conducted a second run of phylogenomic analysis using an expanded database comprising ‘Ref58+Coral’ database and all metazoan sequences collected in this study (http://comparative.reefgenomics.org/datasets.html). The analyses were performed following the aforementioned procedure except that phylogenetic trees were constructed using RAxML (Stamatakis, 2014) under PROTGAMMALGF model with branch supports estimated using 100 bootstrap replicates. With these RAxML trees, we searched for coral sequences that were nested within non-metazoan sequences (with ≥60% bootstrap support). The resulting phylogenetic trees were manually inspected to identify HGT candidates. HGT cases that were unique to the query species (not shared with any other coral taxa) were discarded. The tree topologies for the resulting candidates were confirmed by re-building the trees using IQtree version 0.96 (Nguyen et al., 2015) under the best amino acid substitution model selected by the build-in model-selection function. Branch supports were estimated using ultrafast bootstrap (UFboot) approximation approach (Minh et al., 2013) using 1500 bootstrap replicates (-bb 1500). Coral sequences were considered to have a HGT origin if they were nested within non-metazoan sequences with ≥90% UFboot support. When phylogenetic trees derived from the A. digitifera data and those derived from Seriatopora sp. showed the same HGT event (i.e., an ancient transfer that occurred before the split of these two species), they were manually grouped into a shared non-redundant group. The same was the cases for phylogenetic trees that resulted from recent gene duplications. This process gave rise to 21 A. digitifera sequences and 41 Seriatopora sp. sequences that represent 41 independent HGTs from non-metazoan sources (Table 1).

The key HGT genes involved in stress response were mapped to A. digitifera genome browser using the BLAST function therein (http://marinegenomics.oist.jp/acropora_digitifera). The corresponding phylogenetic trees were rebuilt with inclusion of representative sequences (if available) from more algal taxa (Pyrodinium bahamense pbaa01, Gambierdiscus australis CAWD149, Goniononas Pacifica CCMP1869, Togula jolla CCCM725, Pleurochrysis carterae CCMP645, Ceratium fusus PA161109) that were generated from the Marine Microbial Eukaryote Transcriptome Sequencing Project (Keeling et al., 2014). The alignments were carried out using MUSCLE version 3.8.31 (Edgar, 2004) followed by manual trimming and curation (e.g., with the removal of highly divergent sequences and redundant sequences from highly sampled groups). The corresponding ML trees
were built using IQtree (Nguyen et al., 2015) as aforementioned. The phylogenetic trees for the flanking genes (if any) were generated likewise.

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Additional information

Competing interests
PGF: Reviewing editor, eLife. The other authors declare that no competing interests exist.

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Author contributions
DB, Conceived and coordinated the project and helped write the paper; SA, MA, SB, MB, SF, YJL, SM, CRV, Overall coral genome database and website, Comparative genomic analyses and the coral tree of life; JLD, BK, TM, DZ, HMP, ST, Biomineralization pathway; DE, MMe, CS, ES, APMW, VW, Design of the study; RDG, AJS, Ion transporters; DFG, MPL, OL, MMacMa, EM, DT, CWW, Environmental stress response systems; DCP, Bioinformatic analyses; HQ, Horizontal gene transfer analysis; NW, Seriatopora sp. coral genome data production; EZ, Negatively charged amino acids and other bioinformatic analyses; PGF, Conceived and coordinated the project and helped write the paper, Analysis and interpretation of data, Drafting or revising the article.

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Additional files

Supplementary files
- Supplementary file 1. Taxonomic compilation and presence/absence in each taxon for genes involved in oxidative stress, DNA repair, cell cycle and apoptosis. The values in parentheses show the number of taxa in which the gene sequence was recovered in the genomic database.

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## Major datasets

The following previously published datasets were used:

| Author(s)                                                                 | Year | Dataset title                                                                                                                                  | Dataset URL                                                                 | Database, license, and accessibility information |
|--------------------------------------------------------------------------|------|-----------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|-----------------------------------------------|
| Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M, Fujiwara M, Koyanagi R, Ikuta T, Fujiyama A, Miller DJ, Satoh N | 2011 | Data from: Acropora digitifera genome to understand coral responses to environmental change                                                   | http://www.ncbi.nlm.nih.gov/bioproject/PRJDA67425                           | Publicly available at NCBI Bioproject (accession no. PRJDA67425) |
| Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traynor-Knowles N, Palumbi SR | 2013 | Data from: Genomic basis for coral resilience to climate change                                                                               | http://www.ncbi.nlm.nih.gov/bioproject/PRJNA177515                           | Publicly available at NCBI GenBank (accession no. PRJNA177515) |
| Moya A, Huismann L, Ball EE, Hayward DC, Grasso LC, Chua CM, Woo HN, Gattuso JP, Foret S, Miller DJ | 2012 | Data from: Whole transcriptome analysis of the coral Acropora millepora reveals complex responses to CO2-driven acidification during the initiation of calcification | http://www.ncbi.nlm.nih.gov/bioproject/PRJNA149513                           | Publicly available at NCBI Bioproject (accession no. PRJNA149513) |
| Srivastava M, Simakov O, Chapman J, Fahey B, Gauthier ME, Mitros T, Richards GS, Conaco C, Dacre M, Hellsten U, Larroux C, Putnam NH, Stanke M, Adamska M, Darling A, Degnan SM, Oakley TH, Plachetzkii DC, Zhai Y, Adamski M, Calcino A, Cummins SF, Goodstein DM, Harris C, Jackson DJ, Leys SP, Shu S, Woodcroft BJ, Vervoort M, Kosik KS, Manning G, Degnan BM, Roikhsar DS | 2010 | Data from: The Amphimedon queenslandica genome and the evolution of animal complexity                                                              | http://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA66531                         | Publicly available at NCBI BioProject (accession no. PRJNA66531) |
| Sabourault C, Gannot P, Deleury E, Allemand D, Furla P                   | 2009 | Data from: Comprehensive EST analysis of the symbiotic sea anemone, Anemone viridis                                                             | http://www.ncbi.nlm.nih.gov/nucest/FK719875                                  | Publicly available at NCBI EST (accession no. FK719875-FK759813)         |
| Kitchen SA, Crowder CM, Poole AZ, Weis VM, Meyer E                       | 2015 | Data from: De Novo Assembly and Characterization of Four Anthozoan (Phylum Cnidaria) Transcriptomes                                              | http://www.ncbi.nlm.nih.gov/bioproject/PRJNA295078                           | Publicly available at NCBI BioProject (accession no. PRJNA295078)       |
| Stefanik DJ, Lubinski TJ, Granger BR, Byrd AL, Reitzel AM, DeFilippo L, Lorenz A, Finnerty JR | 2014 | Data from: Production of a reference transcriptome and transcriptomic database (EdwardsiellaBase) for the lined sea anemone                       | http://cnidarians.bu.edu/EdwardsiellaBase/cgi-bin/knock.cgi                  | Publicly available at EdwardsiellaBase (Edwardsiella lineata genomics database) |
| Pooyaei Mehr SF, Desalle R, Kao HT, Narechania A, Han Z, Tchernov D, Pieribone V, Gruber DF | 2013 | Data from: Transcriptome deep-sequencing and clustering of expressed isoforms from Favia corals                                                | http://www.ncbi.nlm.nih.gov/bioproject/PRJNA176860                           | Publicly available at NCBI BioProject (accession no. PRJNA176860)       |
| Author(s) | Year | Data from: | BIoproject | Accession No. | Availability |
|-----------|------|------------|-------------|--------------|--------------|
| Burge CA, Mouchka ME, Harvell CD, Roberts S | 2013 | Immune response of the Caribbean sea fan, Gorgonia ventalina, exposed to an Aplanochytrium parasite as revealed by transcriptome sequencing | [http://www.ncbi.nlm.nih.gov/bioproject/PRJNA172986](http://www.ncbi.nlm.nih.gov/bioproject/PRJNA172986) | PRJNA172986 | Publicly available at NCBI Bioproject (accession no. PRJNA172986) |
| Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T, Ratteei, Balasubramanian PG, Borman J, Busam D, Diabennett K, Pfannkoch C, Suman N, Sutton GG, Viswanathan LD, Walenz B, Goodstein DM, Hellsten U, Kawasaki T, Putnam NH, Shu S, Blumberg B, Dana CE, Gee L, Kibler DF, Law L, Lindgens D, Martinez DE, Peng J, Wigge PA, Bertulat B, Guder C, Nakamura Y, Ozbek S, Watanabe H, Khalturin K, Hemmrich G, Franke A, Augustin R, Fraune S, Hayakawa E, Hayakawa S, Hirose M, Hwang JS, Ikeo K, Nishimiya-Fujisawa C, Ogura A, Takahashi T, Steinmetz PR, Zhang X, Aufschneider R, Eder MK, Gorny AK, Salvenmoser W, Heimberg AM, Wheeler BM, Peterson KJ, Böttger A, Tischler P, Wolf A, Gojobori T, Remington KA, Strausberg RL, Venter JC, Technau U, Hobmayer B, Bosch TC, Holstein TW, Fujisawa T, Bode HR, David CN, Rokhsar DS, Steele RE | 2010 | The dynamic genome of Hydra | [http://www.ncbi.nlm.nih.gov/bioproject/PRJNA12876](http://www.ncbi.nlm.nih.gov/bioproject/PRJNA12876) | PRJNA12876 | Publicly available at NCBI Bioproject (accession no. PRJNA12876) |
| Ryan JF, Pang K, Schnitzler CE, Nguyen AD, Moreland RT, Simmons DK, Koch BJ, Francis WR, Havlak P, NISC Comparative Sequencing Program, Smith SA, Putnam NH, Haddock SH, Dunn CW, Wolfsberg TG, Mullikin JC, Martindale MQ, Baxevanis AD | 2013 | The genome of the ctenophore Mnemiopsis leidyi and its implications for cell type evolution | [http://www.ncbi.nlm.nih.gov/bioproject/PRJNA64405](http://www.ncbi.nlm.nih.gov/bioproject/PRJNA64405) | PRJNA64405 | Publicly available at NCBI Bioproject (accession no. PRJNA64405) |
| Year | Authors                                                                 | Data from:                                                                 | Source Link                                                                 | Accession No.       |
|------|-------------------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|---------------------|
| 2008 | King N, Westbrook MJ, Young SL, Kuo A, Abedin M, Chapman J, Fairclough S, Hellsten U, Isogai Y, Letunic I, Marr M, Pincus D, Putnam N, Rokas A, Wright KJ, Zuzow R, Dirks W, Good M, Goodstein D, Lemons D, Li W, Lyons JB, Morris A, Nicholas S, Richter DJ, Salamov A, Sequecing JG, Bork P, Lim WA, Manning G, Miller WT, McGinnis W, Shapiro H, Tjian R, Grigoriev IV, Rokhsar D | The genome of the choanoflagellate Monosiga brevicollis and the origin of metazoans | http://www.ncbi.nlm.nih.gov/bioproject/PRJNA19045 | Publicly available at NCBI Bioproject (accession no. PRJNA19045) |
| 2007 | Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, Salamov A, Terry A, Shapiro H, Lindquist E, Kapitonov VV, Jurka J, Genikovich G, Grigoriev IV, Lucas SM, Steele RE, Finnerty JR, Technau U, Martin-dale MQ, Rohksar DS | Data from: Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization | http://www.ncbi.nlm.nih.gov/bioproject/PRJNA19965 | Publicly available at NCBI Bioproject (accession no. PRJNA19965) |
| 2012 | Nichols SA, Roberts BW, Richter DJ, Fairclough SR, King N               | Data from: Origin of metazoan cadherin diversity and the antiquity of the classical cadherin/beta-catenin complex | http://www.ncbi.nlm.nih.gov/bioproject/PRJNA230415 | Publicly available at NCBI Bioproject (accession no. PRJNA230415) |
| 2013 | Sun J, Chen Q, Lun JC, Xu J, Giu JW                                     | Data from: PcarnBase: development of a transcriptomic database for the brain coral Platygyra carnosus | http://www.ncbi.nlm.nih.gov/bioproject/PRJNA81573 | Publicly available at NCBI Bioproject (accession no. PRJNA81573) |
| 2014 | Moroz LL, Kocot KM, Citarella MR, Dosung S, Norekian TP, Povolotskaya IS, Grigorenko AP, Dailey C, Berezikov E, Buckley KM, Pitsyn A, Reshetov D, Mukherjee K, Moroz TP, Bobkova Y, Yu F, Kapitonov VV, Jurka J, Bobkov W, Swore JJ, Girardo DO, Fodor A, Gusev F, Sanford R, Bruders R, Kistler E, Mills CE, Rast JP, Derelle R, Solovyev VV, Kondrashov FA, Swalla BJ, Sweedler JV, Rogaei El, Halanych KM, Kohn AB | Data from: The ctenophore genome and the evolutionary origins of neural systems | http://www.ncbi.nlm.nih.gov/bioproject/PRJNA213480 | Publicly available at NCBI Bioproject (accession no. PRJNA213480) |
| 2011 | Traylor-Knowles N, Granger BR, Lubinski TJ, Panikh JR, Garamszegi S, Xia Y, Marto JA, Kaufman L, Finnerty JR | Data from: Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral, Pocillopora damicornis | http://cnidarians.bu.edu/PocilloporaBase/cgi-bin/pdamdata.cgi | Publicly available at PocilloporaBase (Pocillopora Transcriptomics Database) |
| Name 1 | Name 2 | Year | Data from: | URL | Availability |
|-------|--------|------|------------|-----|-------------|
| Shinzato C | Inoue M, Kusakabe M | 2014 | A snapshot of a coral | [NCBI Bioproject](http://www.ncbi.nlm.nih.gov/bioproject/PRJDB731) | Publicly available at NCBI Bioproject (accession no. PRJDB731) |
| Srivastava M, Begovic E, Chapman J, Putnam NH, Hellsten U, Kawashima T, Kuo A, Mitros T, Salamov A, Carpenter ML, Signorovitch AY, Moreno MA, Kamm K, Grimwood J, Schmutz J, Shapiro H, Grigoriev IV, Buss LW, Schierwater B, Dellaporta SL, Rokhsar DS | 2008 | The Trichoplax genome and the nature of placozoans | [NCBI Bioproject](http://www.ncbi.nlm.nih.gov/bioproject/PRJNA30931) | Publicly available at NCBI Bioproject (accession no. PRJNA30931) |
| Schwarz JA, Brokstein PB, Voolstra C, Terry AY, Manohar CF, Miller DJ, Szmant AM, Cof froth MA, Medina M | 2008 | Coral life history and symbiosis: functional genomic resources for two reef building Caribbean corals | [NCBI EST](http://www.ncbi.nlm.nih.gov/nucest/DR982333) | Publicly available at NCBI EST (accession no. DR982333-DR988505) |
| Schwarz JA, Brokstein PB, Voolstra C, Terry AY, Manohar CF, Miller DJ, Szmant AM, Cof froth MA, Medina M | 2008 | Coral life history and symbiosis: functional genomic resources for two reef building Caribbean corals | [NCBI EST](http://www.ncbi.nlm.nih.gov/nucest/EY021828) | Publicly available at NCBI EST (accession no. EY021828-EY031784) |

**References**

Allison N, Cohen I, Finch AA, Erez J, Tudhope AW, Edinburgh Ion Microprobe Facility. 2014. Corals concentrate dissolved inorganic carbon to facilitate calcification. *Nature Communications* 5:5741. doi: 10.1038/ncomms6741

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215:403–410. doi: 10.1016/S0022-2836(05)80360-2

Bairoch A, Apweiler R. 2000. The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000. *Nucleic Acids Research* 28:45–48. doi: 10.1093/nar/28.1.45

Banaszak AT, Lesser MP. 2009. Effects of solar ultraviolet radiation on coral reef organisms. *Photochemical & Photobiological Sciences* 8:1276–1294. doi: 10.1039/b902763g

Bateman A, Birney E, Cerruti L, Durbin R, Etwiller L, Eddy SR, Griffiths-Jones S, Howe KL, Marshall M, Sonnhammer EL. 2002. The pfam protein families database. *Nucleic Acids Research* 30:276–280. doi: 10.1093/nar/30.1.276

Baumgarten S, Bayer T, Aranda M, Liew YJ, Carr A, Micklem G, Voolstra CR. 2013. Integrating microRNA and mRNA expression profiling in *symbiodinium microadriaticum*, a dinoflagellate symbiont of reef-building corals. *BMC Genomics* 14:704. doi: 10.1186/1471-2164-14-704

Baud P, Larrieu L, Blanchet C, Hamel C, Ben Salah S, Viele A, Gilbert-Dussardier B, Holder M, Calvas P, Philip N, Edery P, Bonneau D, Claustres M, Malcolm S, Roux AF. 2007. Molecular and in silico analyses of the full-length isoform of usherin identify new pathogenic alleles in Usher type II patients. *Human Mutation* 28:781–789. doi: 10.1002/humu.20513

Bender CE, Fitzgerald P, Tait SW, Llambi F, McStay GP, Tupper DO, Pellettiere J, Sánchez Alvarado A, Salvesen GS, Green DR. 2012. Mitochondrial pathway of apoptosis is ancestral in metazoans. *Proceedings of the National Academy of Sciences of the United States of America* 109:4904–4909. doi: 10.1073/pnas.1120680109

Bertucci A, Moya A, Tambutté S, Allemand D, Supuran CT, Zoccola D. 2013. Carbonic anhydrases in anthozoan corals—a review. *Bioorganic & Medicinal Chemistry* 21:1437–1450. doi: 10.1016/j.bmc.2012.10.024

Bhattacharyya G, Kalluri R, Orten DJ, Kimberling WJ, Cosgrove D. 2004. A domain-specific usherin/collagen IV interaction may be required for stable integration into the basement membrane superstructure. *Journal of Cell Science* 117:233–242. doi: 10.1242/jcs.00850

Blanc G, Duncan G, Agarkova I, Borodovsky M, Gurnon J, Kuo A, Lindquist E, Lucas S, Pangilinan J, Polle J, Salamov A, Terry A, Yamada T, Dunigan DD, Grigoriev IV, Claverie JM, Van Etten JL. 2010. The chlorella variabilis NC64A genome reveals adaptation to photosymbiosis, coevolution with viruses, and cryptic sex. *The Plant Cell* 22:2943–2955. doi: 10.1105/tpc.110.076406

Blanc G, Agarkova I, Grimwood J, Kuo A, Brueggeaman A, Dunigan DD, Gurnon J, Ladunga I, Lindquist E, Lucas S, Pangilinan J, Pröschold T, Salamov A, Schmutz J, Weeks D, Yamada T, Lomsadze A, Borodovsky M, Claverie...
et al. 2012. The genome of the polar eukaryotic microalga coccomyxa subellipsoidea reveals traits of cold adaptation. Genome Biology 13:R39. doi: 10.1186/gb-2012-13-5-r39

Boutet E, Lieberher D, Tognolli M, Schneider M, Bairoch A. 2007. Uniprokt/kb/Swiss-prot. Methods in Molecular Biology 406:89–112. doi: 10.1007/978-1-59745-355-0_4

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: Architecture and applications. BMC Bioinformatics 10:421. doi: 10.1186/1471-2105-10-421

Cassell-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25:1972–1973. doi: 10.1093/bioinformatics/btp348

Conesa A, Gótz S, García-Gómez JM, Terol J, Talón M, Robles M. 2005. Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21:3674–3676. doi: 10.1093/bioinformatics/bt610

Connell JH. 1978. Diversity in tropical rain forests and coral reefs. Science 199:1302–1310. doi: 10.1126/science.199.4335.1302

Curtis BA, Tanifuji G, Burki F, Gruber A, Irimia M, Maruyama S, Arias MC, Ball SG, Gile GH, Hirakawa Y, Hopkins JF, Kuo A, Rensing SA, Schmutz J, Symeonidi A, Elias M, Eveleigh RJ, Herman EK, Klute MJ, Nakayama T, et al. 2012. Algal genomes reveal evolutionary mosaicism and the fate of nucleomorphs. Nature 492:59–65. doi: 10.1038/nature11681

Davy SK, Allemend D, Weis VM. 2012. Cell biology of cnidarian-dinoflagellate symbiosis. Microbiology and Molecular Biology Reviews 76:229–261. doi: 10.1128/MMBR.05014-11

Debrenne F, Retnner J Zhuravlev AY. 2001. Sponges, cnidarians and eponorphs 99999. In: Riding R. The Ecology of the Cambrian Radiation. New York: Columbia University Press; 301–325.

Drake JL, Mass T, Haramaty L, Zelzien E, Bhattacharya D, Falkowski PG. 2013. Proteomic analysis of skeletal organic matrix from the stony coral Stylopora pistillata. Proceedings of the National Academy of Sciences of the United States of America 110:3788–3793. doi: 10.1073/pnas.1301419110

Drake JL, Mass T, Falkowski PG. 2014. The evolution and future of carbonate precipitation in marine invertebrates: Witnessing extinction or documenting resilience in the Anthropocene? Elementa: Science of the Anthropocene 2:000026. doi: 10.12952/journal.elementa.000026

Dubinsky Z, Falkowski P. 2011. Light as a Source of Information and Energy in Zooxanthellate Corals. In: Dubinsky Z, Stambler N. Coral Reefs: An Ecosystem in Transition. Dordrecht: Springer Netherlands; 107–118. doi: 10.1007/978-94-007-0114-4_8

Edgar RC. 2004. MUSCLE: A multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:113. doi: 10.1186/1471-2105-5-113

Erez J. 1978. Vital effect on stable-isotope composition seen in foraminifera and coral skeletons. Nature 273:199–202. doi: 10.1038/273199a0

Fernando SC, Wang J, Sparling K, Garcia GD, Francini-Filho RB, de Moura RL, Paranhos R, Thompson FL, Thompson JR. 2015. Microbiota of the major South Atlantic reef building coral Mussismilia. Microbial Ecology 69:267–280. doi: 10.1007/s00248-014-0474-6

Forrét S, Knock B, Houlston E, Momose T, Manuel M, Quéméneur E, Hayward DC, Ball EE, Miller DJ. 2010. New tricks with old genes: The genetic bases of novel cnidarian traits. Trends in Genetics 26:154–158. doi: 10.1016/j.tig.2010.01.003

Fu L, Niu B, Zhu Z, Wu S, Li W. 2012. CD-HIT: Accelerated for clustering the next-generation sequencing data. Bioinformatics 28:3150–3152. doi: 10.1093/bioinformatics/bts565

Fukuda I, Okii S, Fujita T, Murayama E, Nagasawa H, Isa Y, Watanabe T. 2003. Molecular cloning of a cDNA encoding a soluble protein in the coral exoskeleton. Biochemical and Biophysical Research Communications 304:11–17. doi: 10.1016/S0006-291X(03)00527-8

Furla P, Galgani I, Durand I, Allemend D. 2000. Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis. The Journal of Experimental Biology 203:3445–3457.

Glöckner GH, Hülsmann N, Schleicher M, Noegel AA, Eichinger L, Gallinger C, Pawlowski J, Sierral R, Euteneuer U, Pellet L, Moustafa A, Platzer M, Groth O, Schliwa M. 2014. The genome of the foraminiferan reticulomyxa filosa. Current Biology 24:11–18. doi: 10.1016/j.cub.2013.11.027

Gobler CJ, Berry DL, Dyhrman ST, Wilhelm SW, Salamov A, Lobanov AV, Zhang Y, Collier JL, Wurch LL, Kurtza AB, Dill BD, Shah M, Ver Berkmoes NC, Kuo A, Terry A, Pangilinan J, Lindquist EA, Lucas S, Paulsen IT, Hattenrath-Lehmann TK, et al. 2011. Niche of harmful alga aureococcus anophagefferens revealed through ecogenomics. Proceedings of the National Academy of Sciences of the United States of America 108:4352–4357. doi: 10.1073/pnas.1016106108

Gorski JP. 1992. Acidic phosphoproteins from bone matrix: A structural rationalization of their role in mineralization. Calcified Tissue International 50:391–396. doi: 10.1007/BF00296767

Gotliv BA, Kessler N, Sumerel JL, Morse DE, Tuross N, Addadi L, Weiner S. 2005. Asprich: A novel aspartic acid-rich protein family from the prismatic shell matrix of the bivalve ascotrigonia rigida. Chembiochem: A European Journal of Chemical Biology 6:304–314. doi: 10.1002/cbic.200400221

Grasso LL, Negri AP, Pöfert S, Saint R, Hayward DC, Miller DJ, Ball EE. 2011. The biology of coral metamorphosis: Molecular responses of larvae to inducers of settlement and metamorphosis. Developmental Biology 353:411–419. doi: 10.1016/j.ydbio.2011.02.010

Haas BJ, Papanicolaou A, Yasour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Lieber M, Macmanes MD, Ott M, Orvis J, Pochet N, Stambler N, Sullivan MB, Westerman R, Williams T, Dewey CN, Henschel R, et al. 2013. De novo transcript sequence reconstruction from rna-seq using the trinity platform for reference generation and analysis. Nature Protocols 8:1494–1512. doi: 10.1038/nprot.2013.084
Hawkins TD, Bradley BJ, Davy SK. 2013. Nitric oxide mediates coral bleaching through an apoptotic-like cell death pathway: Evidence from a model sea anemone-dinoflagellate symbiosis. *FASEB Journal* 27:4790–4798. doi: 10.1096/fj.13-235051

Heath-Heckman EA, Gillette AA, Augustin R, Gillette MX, Goldman WE, McFall-Ngai MJ. 2014. Shaping the microenvironment: Evidence for the influence of a host galaxan on symbiont acquisition and maintenance in the squid-Vibrio symbiosis. *Environmental Microbiology* 16:3669–3682. doi: 10.1111/1462-2920.12496

Heine J, Huhtala M, Käpylä J, Johnson MS. 2009. Evolution of collagen-based adhesion systems. *The International Journal of Biochemistry & Cell Biology* 41:341–348. doi: 10.1016/j.biocel.2008.08.021

Hicks M. 2006. A new genus of early Cambrian coral in esmeralda county, southwestern nevada. *Journal of Paleontology* 80:609–615. doi: 10.1666/0022-3360(2006)80<609:ANGOEC>2.0.CO;2

Hille B. 1986. Ionic channels: Molecular pores of excitable membranes. *Harvey Lectures* 82:47–69.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield S, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatzllos ME. 2007. Coral reefs under rapid climate change and ocean acidification. *Science* 318:1737–1742. doi: 10.1126/science.1152509

Horton JS, Wakano CT, Speck M, Stokes AJ. 2015. Two-pore channel 1 interacts with citron kinase, regulating completion of cytokinesis. *Channels* 9:21–29. doi: 10.4161/ch.3936950.2014.978676

Jackson DJ, McDougall C, Woodcroft B, Moase P, Rose RA, Kube M, Reinhardt R, Rokhsar DS, Montagnani C, Joubert C, Piquemal D, Degnan BM. 2010. Parallel evolution of nacre building gene sets in molluscs. *Molecular Biology and Evolution* 27:591–608. doi: 10.1093/molbev/msp278

Katoh K, Kihara S, Kuma KI, Hasegawa M, Hasegawa M, Goto S. 2000. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* 28:27–30. doi: 10.1093/nar/28.1.27

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30:772–780. doi: 10.1093/molbev/msmt010

Kawahara K, Buchanan AV, Weiss KM. 2009. Biomimeralization in humans: Making the right choices in life. Annual *Review of Genetics* 43:119–142. doi: 10.1146/annurev-genet-102108-134242

Keeling PJ, Burki F, Wilcox HM, Allam B, Allen EE, Amaral-Zettler LA, Armburst EV, Archibald JM, Bharti AK, Bell CJ, Beszteri B, Biddle KD, Cameron CT, Campbell L, Caron DA, Catholico RA, Collier JL, Coyne K, Davy SK, Deschamps P, et al. 2014. The marine microbial eukaryote transcriptome sequencing project (MMETSP): Illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. *PLoS Biology* 12:e1001889. doi: 10.1371/journal.pbio.1001889

Krishtal O. 2015. Receptor for protons: First observations on acid sensing ion channels. *Neuropsychopharmacology* 94:4–8. doi: 10.1016/j.neuroph.2014.12.014

Lesser MP. 2004. Experimental biology of coral reef ecosystems. *Journal of Experimental Marine Biology and Ecology* 300:217–252. doi: 10.1016/j.jembe.2003.12.027

Lesser MP, Farrell JH. 2004. Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. *Coral Reefs* 23:367–377. doi: 10.1007/s00338-004-0392-z

Lesser MP. 2006. Oxidative stress in marine environments: Biochemistry and physiological ecology. *Annual Review of Physiology* 68:253–278. doi: 10.1146/annurev.physiol.68.040104.110001

Lesser MP. 2011. Coral bleaching: causes and mechanisms. In: Dubinsky Z, Stambler N. *Coral Reefs: An Ecosystem in Transition*. Netherlands, Springer: 405–419. doi: 10.1007/978-94-007-0114-4_23

Levy O, Kaniewska P, Alon S, Eisenberg E, Karako-Lampert S, Bay LK, Reef R, Rodriguez-Lanetty M, Miller DJ, Hoegh-Guldberg O. 2011. Complex diel cycles of gene expression in coral-algal symbiosis. *Science* 331:175. doi: 10.1126/science.1196419

Li W, Godzik A. 2006. Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22:1658–1659. doi: 10.1093/bioinformatics/btl158

Mann S. 2001. Biomimeralization: Principles and concepts in bioinorganic materials chemistry. New York: Oxford Univ. press.

Mass T, Drake JL, Haramaty L, Kim JD, Zelzion E, Bhattacharya D, Falkowski PG. 2013. Cloning and characterization of four novel coral acid-rich proteins that precipitate carbonates in vitro. *Current Biology* 23:1126–1131. doi: 10.1016/j.cub.2013.05.007

Mass T, Drake JL, Peters EC, Jiang W, Falkowski PG. 2014. Immunolocalization of skeletal matrix proteins in tissue and mineral of the coral stylophora pistillata. *Proceedings of the National Academy of Sciences of the United States of America* 111:12728–12733. doi: 10.1073/pnas.1408621111

McInerney JO, Pisanį D, Baptiste E, O’Connell MJ. 2011. The public goods hypothesis for the evolution of life on earth. *Biology Direct* 6:41. doi: 10.1186/1745-6150-6-41

Minh BQ, Nguyen MA, von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30:1188–1195. doi: 10.1093/molbev/mst024

Mitterer RM. 1978. Amino acid composition and metal binding capability of the skeletal protein of corals. *Bulletin of Marine Science* 28:173–180.

Miyamoto H, Miyashita T, Okushima M, Nakano S, Morita T, Matsumoto A. 1996. A carbonic anhydrase from the nacreous layer in oyster pearls. *Proceedings of the National Academy of Sciences of the United States of America* 93:9657–9660. doi: 10.1073/pnas.93.18.9657

Moberg F, Folke C. 1999. Ecological goods and services of coral reef ecosystems. *Ecological Economics* 29:215–233. doi: 10.1016/S0921-8009(99)00009-9
Moran Y, Zakon HH. 2014. The evolution of the four subunits of voltage-gated calcium channels: Ancient roots, increasing complexity, and multiple losses. *Genome Biology and Evolution* 6:2210–2217. doi: 10.1093/gbe/evu177

Moreau H, Verheist B, Couloux A, Derelle E, Rombauds S, Grimsley N, Van Bel M, Poulain J, Katinka M, Hohmann-Marriott MF, Piganeau G, Rouzé P, Da Silva C, Wincker P, Van de Peer Y, Vandepoele K. 2012. Gene functionalities and genome structure in bathycoccus prasinos reflect cellular specializations at the base of the green lineage. *Genome Biology* 13. doi: 10.1186/gb-2012-13-8-74

Nesa B, Baird AH, Harii S, Yakovleva I, Hidaka M. 2012. Algal symbionts increase DNA damage in coral planulae exposed to sunlight. *Zoological Studies* 51:12–17.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32:268–274. doi: 10.1093/molbev/msv300

Nillius B, Szallasi A. 2014. Transient receptor potential channels as drug targets: From the science of basic research to the art of medicine. *Pharmacological Reviews* 66:674–814. doi: 10.1124/jpet.113.008268

Norris RD, Turner SK, Hull PM, Ridgwell A. 2013. Marine ecosystem responses to cenozoic global change. *Science* 341:492–498. doi: 10.1126/science.1240543

Nudelman F, Pieterse K, George A, Bomans PH, Friedrich H, Brylka LJ, Hilbers PA, de With G, Sommerdijk NA. 2010. The role of collagen in bone apatite formation in the presence of hydroxyapatite nucleation inhibitors. *Nature Materials* 9:1004–1009. doi: 10.1038/nmat2875

Östlund G, Schmitt T, Forsslund K, Köstler T, Messina DN, Roopra S, Frings O, Sonnhammer EL. 2010. Inparanoid 7: New algorithms and tools for eukaryotic orthology analysis. *Nucleic Acids Res* 38:D196–203. doi: 10.1093/nar/gkp931

Pandolfi JM. 2011. The paleoecology of coral reefs. In: Dubinsky Z, Stambler N. *Coral Reefs: An Ecosystem in Transition*. Netherlands: Springer; 13–24. doi: 10.1007/978-94-007-0114-4_2

Petrucco S, Volpi G, Bolchi A, Rive C, Ottonello S. 2002. A nick-sensing DNA 3’-repair enzyme from arabidopsis. *The Journal of Biological Chemistry* 277:23675–23683. doi: 10.1074/jbc.M201411200

Price MN, Dehal PS, Arkin AP. 2010. Fasttree 2—approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490. doi: 10.1371/journal.pone.0009490

Price DC, Chan CX, Yoon HS, Yang EC, Qiu H, Weber AP, Schwake R, Gross J, Blouin NA, Lane C, Reyes-Prieto A, Dunnford DG, Neilson JA, Burger G, Steiner JM, Löffelhardt W, Meuser JE, Posewitz MC, Ball S, et al. 2012. *Cyanophora paradoxa* genome elucidates origin of photosynthesis in algae and plants. *Science* 335:843–847. doi: 10.1126/science.1213517

Prütt KD, Tatusova T, Brown GR, Maglott DR. 2012. NCBI reference sequences (refseq): Current status, new features and genome annotation policy. *Nucleic Acids Research* 40:D130–135. doi: 10.1093/nar/gkr1079

Qiu H, Price DC, Yang EC, Yoon HS, Bhattacharya D. 2015. Evidence of ancient genome reduction in red algae (rhodophyta). *Journal of Phycology* 51:624–636. doi: 10.1111/jpy.12294

Quistad SD, Stotland A, Baretot KL, Smurthwaite CA, Hilton BJ, Grasía JS, Wolkowicz R, Rohwer FL. 2014. Evolution of trn-f-induced apopptosis reveals 550 my of functional conservation. *Proceedings of the National Academy of Sciences of the United States of America* 111:9567–9572. doi: 10.1073/pnas.1405912110

Radakovits R, Jinkerson RE, Fuerstenberg SI, Tae H, Settlage RE, Moore JL, Posewitz MC. 2012. Draft genome sequence and genetic transformation of the oenocystis alga nanochloropsis gaditana. *Nature Communications* 3:686. doi: 10.1038/ncomms1688

Ramos-Silva P, Kaandorp JH, Huismann Lee M, Barie B, Zanella-Cléon I, Guichard N, Miller DJ, Marin F. 2013. The skeletal proteome of the coral acropora millepora: The evolution of calcification by co-option and domain shuffling. *Molecular Biology and Evolution* 30:2099–2112. doi: 10.1093/molbev/mst109

Ramsey IS, Delling M, Clapham DE. 2006. An introduction to TRP channels. *Annual Review of Physiology* 68:619–647. doi: 10.1146/annurev.physiol.68.040204.100431

Read BA, Kegel J, Klute MJ, Kuo A, Lefebvre SC, Maumus F, Mayer C, Miller J, Monier A, Salamon A, Young J, Aguilar M, Cleaver JM, Frickenhaus T, Gonzalez K, Herman EK, Lin YC, Napier J, Ogata H, Sarno AF, et al. 2013. Pan genome of the phytoplankton emiliania underpins its global distribution. *Nature* 499:209–213. doi: 10.1038/nature12221

Reyes-Bermudez A, Lin Z, Hayward DC, Miller DJ, Ball EE. 2009. Differential expression of three galaxin-related genes during settlement and metamorphosis in the scleractinian coral acropora millepora. *BMC Evolutionary Biology* 9:178. doi: 10.1186/1471-2148-9-178

Sanchez S, Hourdez S, Lallier FH. 2007. Identification of proteins involved in the functioning of riftia pachyptila symbiosis by subtractive suppression hybridization. *BMC Genomics* 8:337. doi: 10.1186/1471-2164-8-337

Sarashina I, Endo K. 2001. The complete primary structure of molluscan shell protein 1 (MSP-1), an acidic calcium-binding protein from murexid mussels. *Zoological Studies* 40:178–183. doi: 10.1007/s10126-001-0013-6

Sax JK, Fei P, Murphy ME, Bernhard E, Korsmeyer SJ, El-Deiry WS. 2002. BID regulation by p53 contributes to chemosensitivity. *Nature Cell Biology* 4:842–849. doi: 10.1038/nclb866

Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J, Xu D, Hellsten U, May GD, Yu Y, Sakurai T, Umezawa T, Bhattacharrya MK, Sandhu D, Vallyordan B, Lindquist E, et al. 2010. Genome sequence of the palaeopolyploid soybean. *Nature* 463:179–183. doi: 10.1038/nature08670

Shimakawa G, Suzuki M, Yamamoto E, Saito R, Iwamoto T, Nishi A, Miyake C. 2014. Why don’t plants have diabetes? Systems for scavenging reactive carbonyls in photosynthetic organisms. *Biochemical Society Transactions* 42:543–547. doi: 10.1042/BST20130273
Shimodaira H, Hasegawa M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* 16:1114–1116. doi: 10.1093/oxfordjournals.molbev.a026201

Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M, Fujwara M, Koyanagi R, Ikuta T, Fujiyama A, Miller DJ, Satoh N. 2011. Using the acropora digitifera genome to understand coral responses to environmental change. *Nature* 476:320–323. doi: 10.1038/nature10249

Shoguchi E, Shinzato C, Kawashima T, Gyoja F, Mungpakdee S, Koyanagi R, Takeuchi T, Hisata K, Tanaka M, Fujwara M, Hamada M, Seidi A, Fujie M, Usami T, Goto H, Yamasaki S, Arakaki N, Suzuki Y, Sugano S, Toyoda A, et al. 2013. Draft assembly of the symbiondium minutum nuclear genome reveals dinoflagellate gene structure. *Current Biology* 23:1399–1408. doi: 10.1016/j.cub.2013.05.062

Stamatakis A. 2014. Raxml version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. doi: 10.1093/bioinformatics/btu033

Stanley SM, Hardie LA. 1998. Secular oscillations in the carbonate mineralogy of reef-building and sediment-producing organisms driven by tectonically forced shifts in seawater chemistry. *Palaeogeography, Palaeoclimatology, Palaeoecology* 144:3–19. doi: 10.1016/S0031-0182(98)00109-6

Stanley GD. 2003. The evolution of modern corals and their early history. *Earth-Science Reviews* 60:195–225. doi: 10.1016/S0012-8252(02)00104-6

Stolarski J, Kitahara MV, Miller DJ, Cairns SD, Mazur M, Melbom A. 2011. The ancient evolutionary origins of scleractinia revealed by azooxanthellate corals. *BMC Evolutionary Biology* 11:316. doi: 10.1186/1471-2148-11-316

Takagi D, Inoue H, Odawara M, Shimakawa G, Miyake C. 2014. The Calvin cycle inevitably produces sugar-derived reactive carbonyl methylglyoxal during photosynthesis: A potential cause of plant diabetes. *Plant & Cell Physiology* 55:333–340. doi: 10.1093/pcp/pcu007

Tambutté É, Allemand D, Mueller M, Jaubert J. 1996. A compartmental approach to the mechanism of calcification in hermatypic corals. *The Journal of Experimental Biology* 199:1029–1041.

Trapidillo-Rosenthal H, Zielke S, Owen R, Buxton L, Boeing B, Bhagoori R, Archer J. 2005. Increased zooxanthellae nitric oxide synthase activity is associated with coral bleaching. *The Biological Bulletin* 208:3–6. doi: 10.2307/3593094

van Ooijen G, Hindle M, Martin SF, Barrios-Llerena M, Sanchez F, Bouget FY, O’Neill JS, Le Bihan T, Millar AJ. 2013. Functional analysis of casein kinase 1 in a minimal ecosystem system. *PloS One* 8:e70021. doi: 10.1371/journal.pone.0070021

Venn AA, Tambutté E, Lotto S, Zoccola D, Allemand D, Tambutté S. 2009. Imaging intracellular pH in a reef coral and symbiotic anemone. *Proceedings of the National Academy of Sciences of the United States of America* 106:16574–16579. doi: 10.1073/pnas.0902894106

Venn A, Tambutté E, Holcomb M, Allemand D, Tambutté S. 2011. Live tissue imaging shows reef corals elevate ph under their calcifying tissue relative to seawater. *PloS One* 6:e20013. doi: 10.1371/journal.pone.0020013

Veron JEN. 1995. Corals in Space and Time: The Biogeography and Evolution of the Scleractinia. Sydney: UNSW Press.

Vize PD. 2009. Transcriptome analysis of the circadian regulatory network in the coral acropora millepora. *The Biological Bulletin* 216:131–137.

Walker CW, Van Beneden RJ, Muttray AF, Böttger SA, Kelley ML, Tucker AE, Thomas WK. 2011. p53 superfamily proteins in marine bivalve cancer and stress biology. *Advances in Marine Biology* 59:1–36. doi: 10.1016/B978-0-12-385536-7.00001-7

Wang X, Zhang X, Dong XP, Samie M, Li X, Cheng X, Goschka A, Shen D, Zhou Y, Harlow J, Zhu MX, Clapham DE, Ren D, Xu H. 2012. TPC proteins are phosphoinositide-activated sodium-selective ion channels in endosomes and lysosomes. *Cell* 151:372–383. doi: 10.1016/j.cell.2012.08.036

Watanabe T, Fukuda I, China K, Isa Y. 2003. Molecular analyses of protein components of the organic matrix in the exoskeleton of two scleractian coral species. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology* 136:767–774. doi: 10.1016/S1095-9993(03)00177-5

Weiner S. 1979. Aspartic acid-rich proteins: Major components of the soluble organic matrix of mollusk shells. *Calciﬁed Tissue International* 29:163–167. doi: 10.1007/BF02408072

Weiner S, Dove PM. 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry* 54:1–29. doi: 10.2113/GS540001

Worden AZ, Lee JH, Mock T, Rouzé P, Simmons MP, Aerts AL, Allen AE, Cuvelier ML, Derelle E, Everett MV, Foulon E, Grimwood J, Gundlach H, Henrisat B, Napoli C, McDonald SM, Parker MS, Rombauts S, Salamov A, Von Dassow P, et al. 2009. Green evolution and dynamic adaptations revealed by genomes of the marine picocyanobacteria. *The Journal of Experimental Biology* 212:157–167. doi: 10.1242/jeb.044540

York PS, Cummins SF, Degnan SM, Woodcroft BJ, Degnan BM. 2012. Marked changes in neuroepitope expression accompany broadcast spawning in the gastropod halisiasis asinina. *Frontiers in Zoology* 9:9. doi: 10.1186/1742-9994-9-9

Zdobnov EM, Apweiler R. 2001. Interproscan—an integration platform for the signature-recognition methods in Interpro. *Bioinformatics* 17:847–848. doi: 10.1093/bioinformatics/17.9.847

Zoccola D, Tambutté E, Sénégal-Balas F, Michiels JF, Failla JP, Jaubert J, Allemand D. 1999. Cloning of a calcium channel alpha1 subunit from the reef-building coral, stophora pistillata. *Gene* 227:157–167. doi: 10.1016/S0378-1119(98)00602-7

Zoccola D, Tambutté E, Kulhanek E, Puverel S, Scimeca JC, Allemand D, Tambutté S. 2004. Molecular cloning and localization of a PMCA p-type calcium ATPase from the coral *stophora pistillata*. *Biochimica Et Biophysica Acta* 1663:117–126. doi: 10.1016/j.bbamem.2004.02.010
Zoccola D, Ganot P, Bertucci A, Caminiti-Segonds N, Techer N, Voolstra CR, Aranda M, Tambutte E, Allemand D, Casey JR, Tambutte S. 2015. Bicarbonate transporters in corals point towards a key step in the evolution of cnidarian calcification. *Scientific Reports* 5:9983. doi: 10.1038/srep09983