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A survey of pocilloporid corals and their endosymbiotic dinoflagellate communities in the Austral and Cook Islands of the South Pacific

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Abstract

It has recently been uncovered that the model reef coral *Pocillopora damicornis* is actually a taxonomic complex comprised of multiple species that are difficult to differentiate *in situ*. However, little is known about the distribution of these species across or within reef ecosystems. To better understand the spatial partitioning between the morphologically-similar sister species *P. damicornis* and *P. acuta*, 88 *Pocillopora* colonies were genotyped across five and three islands/atolls within the Austral and Cook Islands, respectively. A mix of newly described pocilloporid types was found across the two archipelagos, with nearly 33% of the specimens being identified not as the more commonly referenced α genotype of *P. damicornis*, but instead as *P. acuta*. Furthermore, *P. damicornis* was more likely to be found at depths greater than 15 m and in higher coral cover areas relative to *P. acuta*, suggesting that these closely-related species display distinct habitat preferences.

Key words: coral reefs; dinoflagellate; genetics; Pocillopora; South Pacific

Introduction

The model reef-building coral for scientific research, *Pocillopora damicornis* Linnaeus (Fig. 1), assumes a variety of morphologies, from stubby, sub-columnar colonies in certain areas (e.g., high energy environments) to thin, delicately branched growth forms in others (e.g., >20 m). However, recent evidence has found that this species may not actually be as morphologically or geographically diverse as was previously thought; Schmidt-Roach et al. (2014) posed that what is currently classified as "*P. damicornis*" may actually be compromised of 3-4 different species, including *P. acuta* Lamarck, *P. brevicornis* Lamarck, and *P. aliciae* Schmidt-Roach. Given that one of the pivotal reasons for promoting the use of *P. damicornis* as the model reef-building coral is its widespread distribution across the Pacific and Indian Oceans, it will be important to document whether the archetypal *P. damicornis*, from henceforth referred to as genotype α (*sensu* Schmidt-Roach et al., 2014), is indeed found in a diverse array of locations, rather than cryptic sister species that are difficult to distinguish *in situ* (Flot et al., 2010; Marti-Puig et al., 2014).

Since 2011, the Khaled bin Sultan Living Oceans Foundation (LOF) has traversed much of the Caribbean and Pacific Oceans as part of their *Global Reef Expedition* (GRE), the most extensive coral reef survey ever undertaken (www.livingoceansfoundation.org). Given the previously stated need to develop a better picture of the locations within the Pacific Ocean in which the α genotype of *P. damicornis* is found, the LOF GRE research cruises presented an excellent opportunity for addressing this topic. Herein, the findings from the April-May 2013 Austral (French Polynesia) and Cook Islands mission are presented, with a particular emphasis on the genetics of not only the host pocilloporids, but also of the resident dinoflagellate populations of the sampled coral colonies. It was hypothesized that by merging genetic data with ecological data simultaneously obtained during the mission, intra-generic niche partitioning could be elucidated. Specifically, it was hypothesized that the closely related sister species *P. damicornis* and *P. acuta* might demonstrate different habitat preferences over large (inter-island) and small (within reef [i.e., habitat-level]) spatial scales.

Materials and Methods

Sample collection. To determine whether the α genotype of *P. damicornis* is present across a 2,000-km, unexplored region of the South Pacific Ocean (Fig. 2), corals most closely resembling the best described morphologies of *P. damicornis* (Veron, 2000) were collected at each of 30 reef sites across five islands/atolls of French Polynesia's Austral Islands (Table 1 and Fig. 3) and each of 30 reefs from three islands/atolls of the Cook Islands

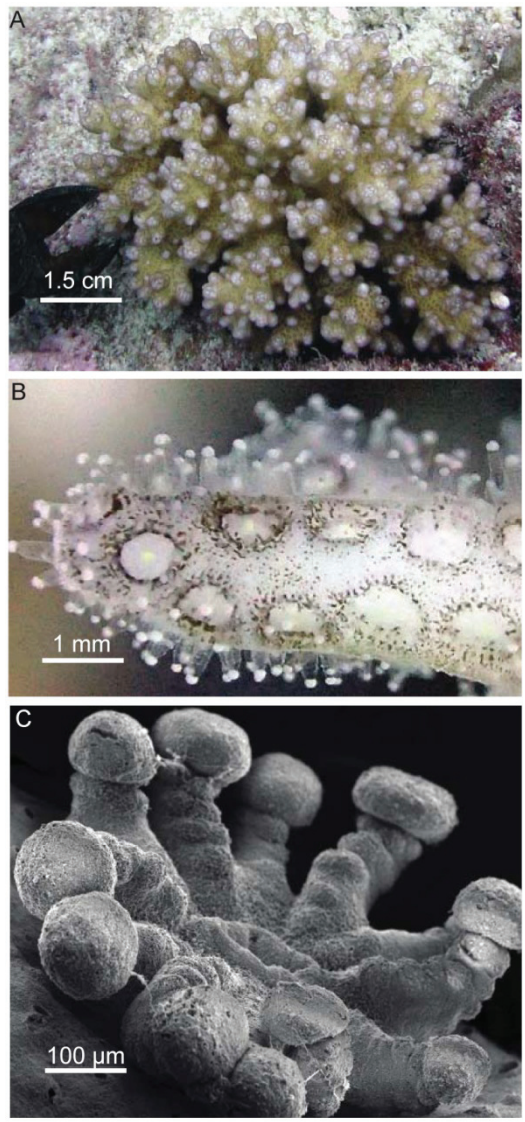

Fig. 1. ***Pocillopora damicornis*** **(genotype α) across two orders of magnitude**. (A) The specimen "*P. damicornis* Rarotonga 81" of Table 4. (B) Using the "microscope" (i.e., macro) mode of an Olympus Tough TG-2 camera in underwater housing (also from Olympus), the ~1-mm diameter polyps are evident. The brown coloration in (A) and (B) represents the endosymbiotic dinoflagellate (genus *Symbiodinium*) populations residing within the coral gastroderm. (C) The mouth and tentacles of an individual *P. damicornis* polyp are more clearly seen with scanning electron microscopy (SEM). The sample was prepared for SEM and imaged as in Mayfield et al. (2013a). Although *P. damicornis* polyps should have 12 tentacles, only 10-11 can be seen due to several having been fused together during the sample preparation process.

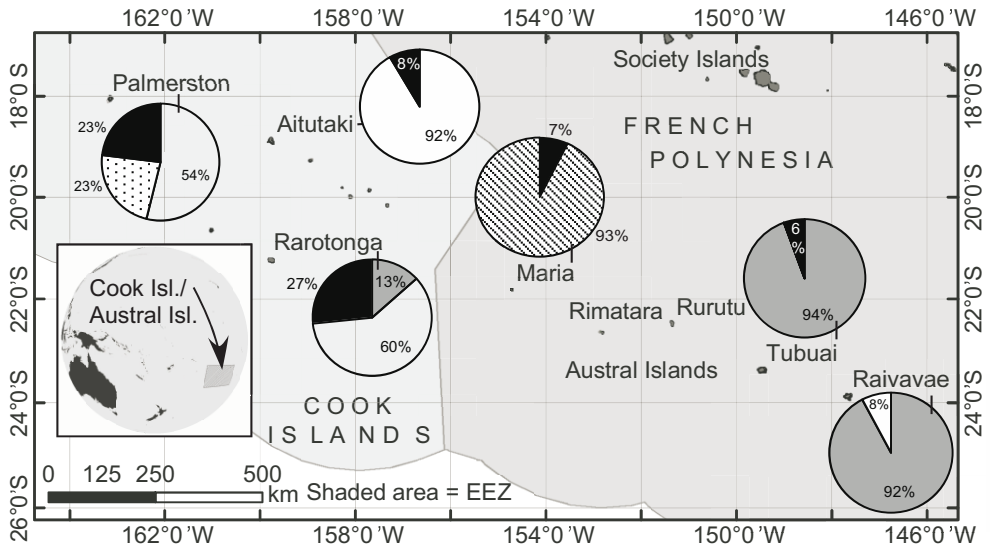

Fig. 2. **Islands visited during the April-May 2013 Austral and Cook Islands cruise**. The cruise began in Raivavae, French Polynesia and crossed eight islands/atolls, ending in Palmerston, Cook Islands. Sixty reefs (Figs. 3-4) spanning ~2,000 km of ocean were visited over the 3.5-week duration of the mission. The pie graphs represent the relative percentages of *P. damicornis* (grey), *P. acuta* (white), *P. meandrina* (black), *P. verrucosa* (undescribed genotype, hatched), and *Pocillopora* sp. haplotype 8A (dotted) at each of the six islands where greater than two samples were genotyped (i.e., excluding Rurutu and Rimatara). "EEZ"= exclusive economic zone.

(Table 2 and Fig. 4) in April and May 2013. In the Austral Islands, Raivavae, Tubuai, Rurutu, Rimatara, and Maria were surveyed over the latter half of April 2013. Herein, only the data from Raivavae, Tubuai, Maria, and, to a lesser extent, Rurutu, are presented; reefs of Rurutu and Rimatara had been devastated by a crown of thorns starfish (COTS) outbreak and so few adult corals were present. Indeed, not a single *P. damicornis*-like coral was found at Rimatara. For a detailed explanation of the site selection process and environmental data acquisition, please see online Appendix I.

Corals were typically collected on SCUBA from depths between 10 and 20 m by random swimming, and depth and time of coral collection were determined by a diving computer (Suunto D4). After taking a picture with an Olympus TG-2 underwater camera, small (50-100 mg) biopsies were removed from coral colonies with bone-cutting pliers and placed into sterile, pre-labeled Whirl-Pak® (Nasco) bags. When present,

Table 1. Austral Islands, French Polynesia sampling site characteristics. The fraction below the island name represents the number of sites (all of which were on the fore reef) at which corals were collected over the total number of sites surveyed, and pocilloporid corals were sampled from 21 out of the 30 sites surveyed. Although five sites at Rimatara were surveyed between April 18 and 19, 2013, no pocilloporids other than *P. eydouxi* were found due to a crown of thorns starfish outbreak. The live coral cover presented for each site represents the average across all survey depths, and the average live coral cover (ALCC) presented for each island (S.E.M.) reflects the average across all sites within an island, not just those from which specimens were taken. A significant island effect on ALCC was detected within the Austral Islands dataset (F=30, p<0.0001), and letters adjacent to ALCC values for each island represent Tukey's honestly significant difference (p<0.05) groups. All statistical analyses in the manuscript were conducted with JMP® (ver. 11), and all error terms presented throughout the manuscript reflect S.E.M. unless otherwise stated.

Island	Site	Exposure	Latitude	Longitude	Date	Temp. (°C)	Salinity (psu)	ALCC (%)	# corals genotyped/ # collected
Raivavae	AURV01	leeward	-23.8605	-147.7151	2013-Apr-11	25.7	35.7	23	0/1
(6/8 sites)	AURV03	windward	-23.8318	-147.6574	2013-Apr-11	25.7	35.7	12	1/2
	AURV04	windward	-23.8282	-147.5901	2013-Apr-12	25.5	35.6	55	1/1
	AURV06	leeward	-23.8962	-147.7123	2013-Apr-12	25.6	35.7	46	4/5
	AURV07	leeward	-23.9123	-147.6609	2013-Apr-13	25.5	35.7	34	3/3
	AURV08	leeward	-23.9108	-147.6843	2013-Apr-13	25.5	35.7	41	4/6
			Raivavae ALCC and total genotyped/total collected					**34±4.9(b)**	**13/18**
Tubuai	AUTB09	leeward	-23.4213	-149.4402		25.8	35.6	42	3/4
(7/9 sites)	AUTB10	leeward	-23.3827	-149.5493		25.9	35.6	40	3/3
	AUTB11	leeward	-23.4253	-149.5184		25.9	35.6	33	5/5
	AUTB12	windward	-23.4251	-149.4057		25.8	35.6	32	2/2
	AUTB13	windward	-23.3786	-149.3853		25.8	35.6	43	3/3
	AUTB15	leeward	-23.3485	-149.5313		25.7	35.7	51	1/1
	AUTB16	leeward	-23.3561	-149.5518		25.8	35.6	52	1/1
			Tubuai ALCC and total genotyped/total collected					**41±4.1(ab)**	**18/19**
Rurutu	AURR18	windward	-22.4522	-151.3235		26.5	35.7	4.4	1/3
(3/3 sites)	AURR19	leeward	-22.4323	-151.3760		26.6	35.6	2.6	0/2
	AURR20	windward	-22.5204	-151.3327		26.6	35.7	3.8	1/3
			Rurutu ALCC and total genotyped/total collected					**3.6±0.5(c)**	**2/8**
Maria	AUMA26	windward	-21.8130	-154.6891		26.7		53	3/4
(5/5 sites)	AUMA27	windward	-21.7901	-154.7037		26.8		41	3/3
	AUMA28	leeward	-21.8200	-154.7239		26.8		42	2/3
	AUMA29	windward	-21.7972	-154.6917		26.8		47	3/3
	AUMA30	leeward	-21.8008	-154.7180		26.6		71	3/3
			Maria ALCC and total genotyped/total collected					**51±5.4(a)**	**14/16**
			Austral Islands ALCC and total genotyped/total collected					**32±3.5**	**47/61**
			ALCC minus Rurutu and Rimatara					**40±2.8**	

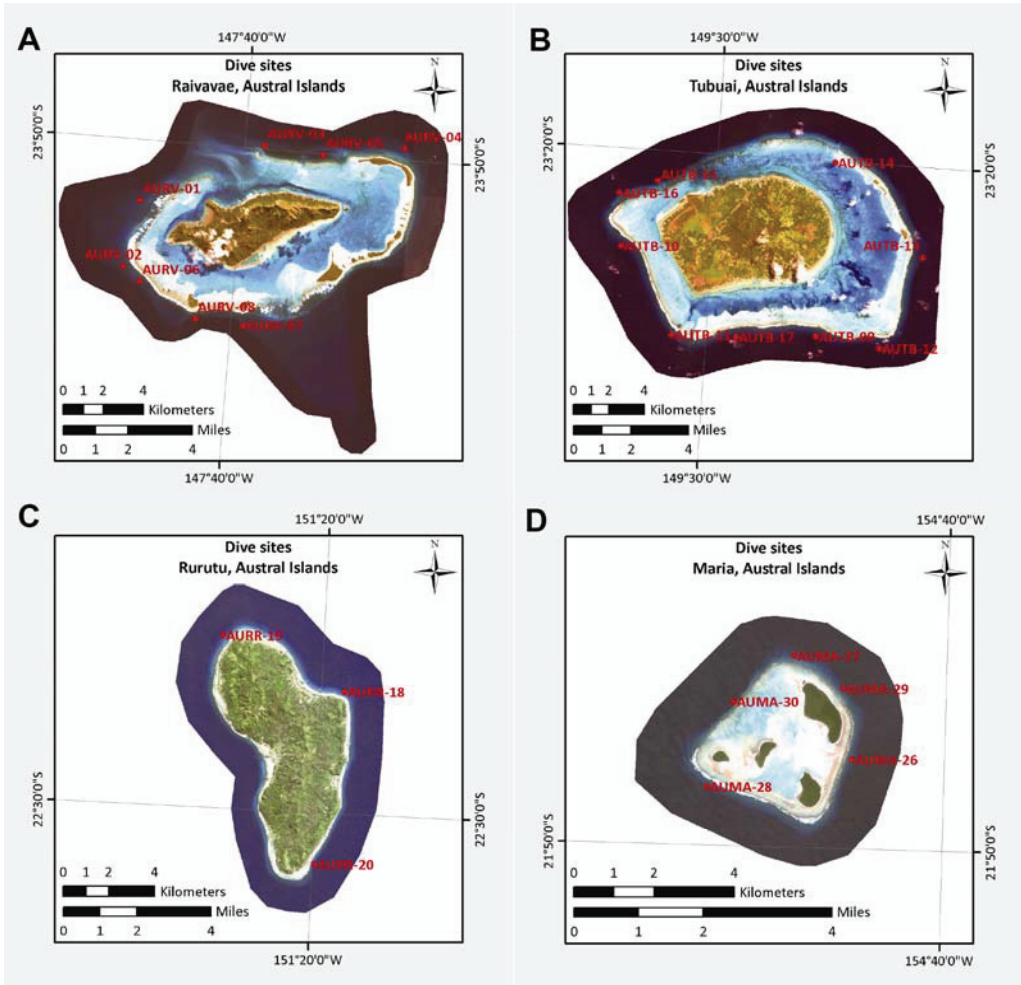

Fig. 3. **Sampling sites of French Polynesia's Austral Islands**. In total 8, 9, 3, 5, and 5 reef sites were surveyed around (A) Raivavae, (B) Tubuai, (C) Rurutu, Rimatara, and (D) Maria, respectively. The map of Rimatara was not presented for reasons described in the text.

typically 1-3 pocilloporid colonies were sampled at each site, and 123 colonies were sampled across 48 of the 60 sites visited over the 3.5 week-long duration of the cruise (Tables 1-2). When corals with a *P. damicornis*-like appearance were not found, *P. verrucosa* Ellis and Solander or *P. meandrina* Dana specimens were instead collected; briefly, although the major aim of the study was to sample only colonies resembling *P. damicornis*, other pocilloporid corals were collected to serve as out-groups in the phylogenetic analyses and to determine whether intra-generic differences in *Symbiodinium* assemblages could be documented.

Table 2. **Cook Islands sampling site characteristics**. The fraction below the island name represents the number of sites at which corals were collected over the total number of sites; all sites except CIAT22 were on the fore reef, and pocilloporid corals were sampled from 27 of the 30 sites visited. The ALCC presented for each site represents the average across all survey depths, and the ALCC presented for each island (S.E.M.) reflects the average across all sites within an island, not just those from which specimens were taken. Letters adjacent to ALCC values represent Tukey's honestly significant difference (p<0.05) groups, as a significant effect of island was detected within the Cook Islands dataset (F=37, p<0.0001). ALCC was similar between the Austral (32%) and Cook (31%) Islands (F=0.23, p=0.64), though differed significantly across all eight islands surveyed during the 3.5-week research cruise (F=46, p<0.0001). "ND"=no data.

Island	Site	Exposure	Latitude	Longitude	Date	Temp. (°C)	Salinity (psu)	ALCC (%)	# corals genotyped/ # collected
Rarotonga	CIRR01[a]	leeward	-21.1941	-159.8091	2013-Apr-22	27.4	35.7	38	4/5
(12/12 sites)	CIRR02[a]	windward	-21.2513	-159.8290	2013-Apr-23	ND	ND	28	2/2
	CIRR03[a]	windward	-21.2136	-159.8331	2013-Apr-23	ND	ND	30	0/4
	CIRR04[a]	leeward	-21.2417	-159.7225	2013-Apr-24	27.4	35.5	19	1/3
	CIRR05[a]	windward	-21.2136	-159.7330	2013-Apr-24	27.4	35.7	29	2/3
	CIRR06[a]	leeward	-21.1993	-159.7569	2013-Apr-24	ND	ND	45	1/3
	CIRR07[b]	windward	-21.2642	-159.8165	2013-Apr-26	27.3	35.7	16	3/3
	CIRR08[b]	windward	-21.2745	-159.7725	2013-Apr-26	27.3	35.7	21	2/4
	CIRR09[a]	windward	-21.2719	-159.7299	2013-Apr-26	27.4	35.7	21	0/1
	CIRR10[a]	windward	-21.2007	-159.7714	2013-Apr-27	27.4	35.7	39	0/2
	CIRR11[b]	windward	-21.2300	-159.8335	2013-Apr-27	27.3	35.7	29	0/3
	CIRR12[a]	leeward	-21.1935	-159.7965	2013-Apr-27	27.4	35.7	33	0/1
			Rarotonga ALCC and total genotyped/total collected					**29±2.6(b)**	**15/34**
Aitutaki	CIAT13[a]	windward	-18.9043	-159.7236	2013-Apr-28	28.6	35.5	26	3/3
(8/10 sites)	CIAT15[a]	leeward	-18.8897	-159.8272	2013-Apr-29	28.4	35.5	7.7	1/1
	CIAT16[a]	leeward	-18.8672	-159.8188	2013-Apr-29	28.4	35.5	13	2/2
	CIAT17[a]	leeward	-18.8331	-159.7941	2013-Apr-30	28.4	35.3	8.5	2/2
	CIAT18[a]	windward	-18.9173	-159.8452	2013-Apr-30	28.3	35.4	17	1/1
	CIAT19[a]	leeward	-18.8517	-159.8054	2013-Apr-30	27.7	35.4	24	1/1
	CIAT20[a]	windward	-18.9283	-159.7943	2013-May-1	28.2	35.5	12	2/2
	CIAT22[b]	windward	-18.9271	-159.7250	2013-May-1	28.3	35.1	7.1	0/1
			Aitutaki ALCC and total genotyped/total collected					**15±2.8(c)**	**12/13**
Palmerston	CIPA23[a]	windward	-17.9926	-163.1535	2013-May-3	27.9	35.5	56	2/2
(7/8 sites)	CIPA24[a]	windward	-18.0291	-163.1178	2013-May-3	28.0	35.5	31	1/1
	CIPA25[a]	windward	-18.0489	-163.1128	2013-May-3	28.2	35.5	47	2/2
	CIPA26[a]	windward	-18.0885	-163.1521	2013-May-4	28.0	35.5	60	2/3
	CIPA27[a]	windward	-18.0697	-163.1293	2013-May-4	28.0	35.5	57	2/2
	CIPA28[a]	leeward	-18.0412	-163.1876	2013-May-5	27.9	35.5	41	3/3
	CIPA29[a]	leeward	-18.0057	-163.1757	2013-May-5	28.0	35.5	64	2/2
			Palmerston ALCC and total genotyped/total collected					**51±3.1(a)**	**14/15**
			Cook Islands ALCC and total genotyped/total collected					**30±3.2**	**41/62**
			ALCC and total genotyped/total collected from both archipelagos					**31±2.3**	**88/123**

[a]fringing reef. [b]reef flat.

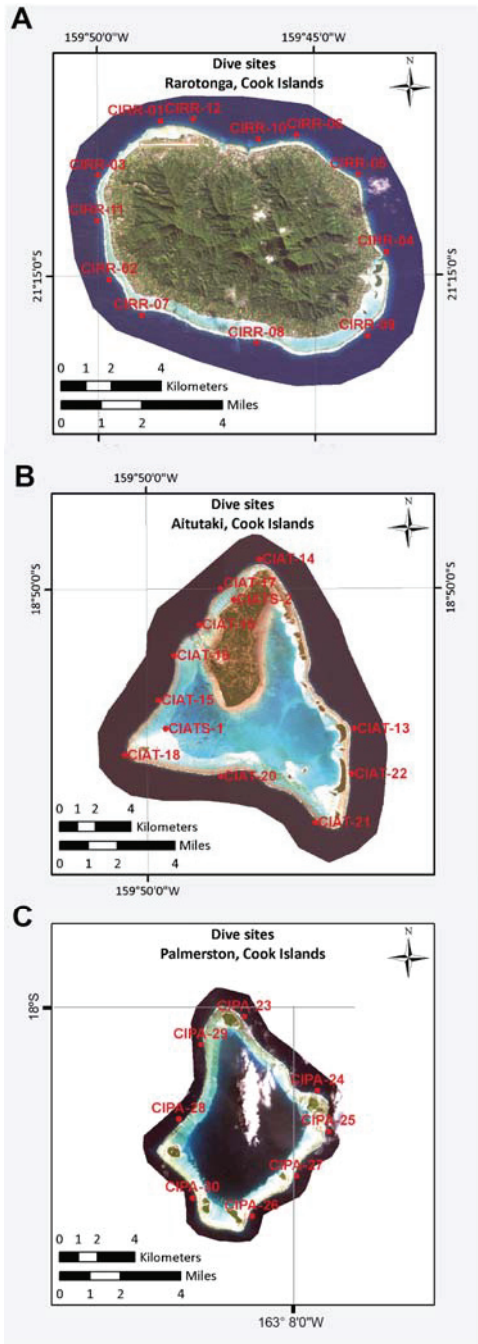

Fig. 4. **Sampling sites of the Cook Islands**. In total 12, 10, and 8 reef sites were surveyed on SCUBA around (A) Rarotonga, (B) Aitutaki (atoll), and (C) Palmerston (atoll), respectively. Two snorkel survey sites (CIATS-1 and CIATS-2) have also been marked on the map of Aitutaki.

Immediately upon returning to the small craft vessel after a dive, coral biopsies were minced with bone-cutting pliers or tweezers, immersed in RNALater® (Life Technologies), and stored at -20 or 4°C for several hours. Upon return to the *M.Y. Golden Shadow*, samples were homogenized in RNALater with mortars and pestles as in Mayfield et al. (2013b). Homogenized tissues + skeleton in RNALater were frozen in an -80°C freezer onboard the ship for several weeks. Then, they were transferred to a -150°C liquid nitrogen dry shipper (Doble 20 model, Chart Biomedical), exported under CITES permit FR1398700102-E (to ABM), and transferred on a commercial airline to a -80°C freezer at Taiwan's National Museum of Marine Biology and Aquarium (NMMBA), where extractions were later performed.

Nucleic acid extractions, host coral genotyping, phylogenetic analyses, and* Symbiodinium *genotyping. For a detailed description of the nucleic acid extraction protocol, as well as the host coral and *Symbiodinium* genotyping assays and corresponding phylogenetic analyses, readers are referred to Appendix II.

Statistical analyses. Given the collection of abiotic (e.g., temperature), ecological (e.g., coral cover), and genetic (i.e., host coral and *Symbiodinium* genotypes) data, an attempt was made to elucidate whether certain members of the *P. damicornis* species complex tended to reside in specific habitats or display particular phenotypes. A likelihood ratio-based contingency analysis was conducted to determine whether the five host genotypes/species documented across the ~2,000-km oceanic transect showed distinct preferences for temperature, salinity, latitude, longitude, country (Austral Islands, French Polynesia vs. Cook Islands), island (six of the seven islands from which samples were collected [excluding Rurutu]), reef exposure (windward vs. leeward), reef type (fore reef vs. reef flat), depth (5-10 m, 10-15 m, and 15-20 m), or coral cover (0-15%, 15-30%, 30-45%, 45-60%, and >60%). A similar analysis was conducted to determine whether each host coral genotype was equally likely to demonstrate the same color (healthy, pale, very pale, or bleached) and possess the same *Symbiodinium* type (clade C only, clades A and C, or clades C and D). Fisher's exact tests were used to compare the proportions of each pocilloporid genotype at each of these environmental and physiological comparisons, and statistical significance was set at an α level of 0.05. Only environmental and physiological parameters that varied significantly across species/genotypes are discussed below.

Results

Molecular characteristics and phylogenetic analysis. Of the 91 host coral partial

mitochondrial ATP synthase subunit 6 (*atp6*) and putative control region (*pmapcr*) PCR amplicons sequenced, 88 had high quality trace files that could be used for phylogenetic analyses. The final, trimmed DNA alignment was 784 bp in length and contained a partial portion of *atp6* (102 bp) at the 5'-end, followed by a partial portion of the *pcr* at the 3'-end (682 bp). In total, there were 33 variable sites, and 27 of these were informative for parsimony-based analyses. This high percentage of parsimoniously informative sites indicates adequate nucleotide evolutionary rates of the partial mitochondrial *atp6* and the *pcr* genes for resolving recent divergence among sister species and in delineating species boundaries of pocilloporid corals (Chen et al., 2008a, b).

Of these 88 successfully genotyped samples (Tables 3-4), 32, 28, and 11 were found to be *P. damicornis* (genotype α), *P. acuta* (genotype β), and *P. meandrina* (genotype m), respectively. The additional 4 and 13 samples were found to be *Pocillopora* sp. haplotype 8a (Pinzon et al., 2013) and an undescribed genotype of *P. verrucosa*, respectively, based on BLAST analyses. Un-rooted phylogenetic trees for the 88 sequences showed five distinct genetic lineages, all of which were supported by bootstrap values exceeding 70 (Fig. 5). Excepting for changes in node position within each lineage, identical topological patterns were generated by the neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML), and BAYsian analyses (data not shown). Estimated evolutionary divergence rate calculations revealed genetic distances among lineages ranging from 0.0078 to 0.0248 (Table 5), and these were higher than those of the intra-lineage comparisons (<0.0003, data not shown).

Pocilloporid distribution and environmental characteristics. For a detailed description of the spatial distribution of the 88 pocilloporid haplotypes (summarized in Fig. 2), as well as the environmental characteristics of each site, please see Appendix III.

Pocilloporid niche partitioning. There was a statistically significant effect of country (X^2=85, p<0.0001) on pocilloporid distribution; 94% of the *P. damicornis* specimens were from the Austral Islands, while 97% of the *P. acuta* samples were from the Cook Islands. There were also statistically significant effects of island (X^2=151, p<0.0001) and exposure (X^2=15, p<0.01) on species distribution. Regarding the latter, *P. damicornis* was two times more likely to be found on leeward reefs than windward reefs (Fisher's exact test, p<0.05), while *P. acuta* was found in similar proportions at windward vs. leeward reefs (Fisher's exact test, p>0.05). On the other hand, *P. verrucosa* was two times more likely to be found on windward reefs, and *Pocillopora*

Table 3. **Austral Islands, French Polynesia pocilloporid samples (*n*=47).** Additional site characteristics can be found in Table 1. "ND" = no data.

Sample (locus) name	NCBI accession	Site	Depth (m)	*Symbiodinium* clade(s)
Pocillopora damicornis (genotype α[a])				
P. damicornis Raivavae 3	KJ720218	AURV03	11.5	C only
P. damicornis Raivavae 4	KM975289	AURV04	16.0	C only
P. damicornis Raivavae 5	KM975290	AURV06	14.0	C only
P. damicornis Raivavae 6	KM975291	AURV06	13.0	C only
P. damicornis Raivavae 7	KJ790160	AURV06	13.0	C only
P. damicornis Raivavae 9	KJ720219	AURV07	16.5	C only
P. damicornis Raivavae 10	KM975292	AURV07	15.6	C only
P. damicornis Raivavae 11	KJ720220	AURV07	17.0	C only
P. damicornis Raivavae 13	KJ720221	AURV08	14.5	C only
P. damicornis Raivavae 14	KJ790161	AURV08	14.5	ND
P. damicornis Raivavae 17	KJ720222	AURV08	14.0	C only
P. damicornis Raivavae 18	KJ720223	AURV08	14.0	C only
P. damicornis Tubuai 19	KJ790162	AUTB09	15.0	C only
P. damicornis Tubuai 20	KJ720224	AUTB09	14.5	C only
P. damicornis Tubuai 21	KJ720225	AUTB09	15.0	C only
P. damicornis Tubuai 23	KJ720226	AUTB10	15.5	C only
P. damicornis Tubuai 24	KJ790163	AUTB10	15.0	C only
P. damicornis Tubuai 25	KJ720227	AUTB10	15.5	C only
P. damicornis Tubuai 26	KJ720228	AUTB11	14.5	A + C
P. damicornis Tubuai 27	KJ720229	AUTB11	14.5	C only
P. damicornis Tubuai 28	KJ720230	AUTB11	14.5	C only
P. damicornis Tubuai 29	KJ720231	AUTB11	14.5	C only
P. damicornis Tubuai 30	KJ720232	AUTB11	14.0	C only
P. damicornis Tubuai 31	KJ720233	AUTB12	15.0	C only
P. damicornis Tubuai 33	KJ720234	AUTB13	14.5	C only
P. damicornis Tubuai 34	KJ720235	AUTB13	15.0	C only
P. damicornis Tubuai 35	KJ720236	AUTB13	14.5	C only
P. damicornis Tubuai 36	KJ790164	AUTB15	18.0	C only
P. damicornis Tubuai 37	KJ720237	AUTB16	18.0	C only
P. damicornis Rurutu 40	KJ720238	AURR18	11.0	C only
Pocillopora acuta (genotype β[a])				
P. acuta Raivavae 12	KJ720240	AURV06	13.0	ND
Pocillopora meandrina (genotype m[a])				
P. meandrina Tubuai 32	KJ720263	AUTB12	14.5	C only
P. meandrina Maria 54	KJ720264	AUMA28	14.5	C only
Pocillopora verrucosa (undescribed genotype)				
P. verrucosa Maria 46	KJ720271	AUMA26	15.0	C + D
P. verrucosa Maria 47	KJ720272	AUMA26	16.0	C only
P. verrucosa Maria 49	KJ720273	AUMA26	13.5	C only
P. verrucosa Maria 50	KJ720275	AUMA27	16.5	C only
P. verrucosa Maria 51	KJ720274	AUMA27	15.0	C only
P. verrucosa Maria 52	KJ790173	AUMA27	16.5	ND
P. verrucosa Maria 53	KJ720276	AUMA29	13.5	C only
P. verrucosa Maria 55	KJ720277	AUMA28	14.0	C only
P. verrucosa Maria 57	KJ720278	AUMA29	13.0	A + C
P. verrucosa Maria 58	KJ790174	AUMA29	13.5	C only
P. verrucosa Maria 59	KJ720279	AUMA30	14.0	C only
P. verrucosa Maria 60	KJ720280	AUMA30	9.0	C + D
P. verrucosa Maria 61	KJ720281	AUMA30	6.5	A + C
***Pocillopora* sp.** (haplotype 8a[b])				
Pocillopora sp. haplotype 8A Rurutu 45	KJ790172	AURR20	9.0	ND

[a]Nomenclature of Schmidt-Roach et al.(2014). [b]Nomenclature of Pinzon et al. (2013).

Table 4. **Cook Islands pocilloporid samples (*n*=41).** Additional site characteristics can be found in Table 2. "ND"=no data.

Sample (locus) name	NCBI accession	Site	Depth (m)	*Symbiodinium* clade(s)
Pocillopora damicornis (genotype α[a])				
P. damicornis Rarotonga 81	KJ720239	CIRR06	16.0	C only
P. damicornis Rarotonga 86	KJ790165	CIRR08	16.0	C only
Pocillopora acuta (genotype β[a])				
P. acuta Rarotonga 62	KJ790168	CIRR01	15.0	C only
P. acuta Rarotonga 64	KJ790169	CIRR01	16.0	ND
P. acuta Rarotonga 73	KJ720241	CIRR04	14.0	C only
P. acuta Rarotonga 76	KJ720242	CIRR05	14.5	C only
P. acuta Rarotonga 78	KJ720243	CIRR05	15.5	C only
P. acuta Rarotonga 82	KJ720244	CIRR07	16.0	C only
P. acuta Rarotonga 83	KJ720245	CIRR07	17.0	C only
P. acuta Rarotonga 84	KJ720246	CIRR07	14.5	C only
P. acuta Rarotonga 88	KJ720247	CIRR08	9.0	C only
P. acuta Aitutaki 96	KJ720248	CIAT13	11.5	C only
P. acuta Aitutaki 97	KJ720249	CIAT13	10.0	C + D
P. acuta Aitutaki 98	KJ720250	CIAT13	10.5	C only
P. acuta Aitutaki 99	KJ720251	CIAT15	5.0	C only
P. acuta Aitutaki 100	KJ720252	CIAT16	10.0	C only
P. acuta Aitutaki 101	KJ880801	CIAT16	6.5	C only
P. acuta Aitutaki 102	KJ720253	CIAT17	15.0	C only
P. acuta Aitutaki 103	KJ720254	CIAT17	15.0	C only
P. acuta Aitutaki 104	KJ790170	CIAT18	14.5	ND
P. acuta Aitutaki 105	KJ720255	CIAT19	12.0	C + D
P. acuta Aitutaki 107	KJ720256	CIAT20	8.0	C only
P. acuta Palmerston 109	KJ720257	CIPA23	8.0	C only
P. acuta Palmerston 110	KJ790171	CIPA23	6.5	C only
P. acuta Palmerston 112	KJ720258	CIPA25	11.0	C only
P. acuta Palmerston 119	KJ880803	CIPA28	10.0	C only
P. acuta Palmerston 120	KJ720259	CIPA28	9.0	C only
P. acuta Palmerston 121	KM975293	CIPA28	9.0	C only
P. acuta Palmerston 123	KJ880804	CIPA29	7.0	C only
Pocillopora meandrina (genotype m[a])				
P. meandrina Rarotonga 65	KJ720265	CIRR02	18.5	C only
P. meandrina Rarotonga 66	KJ720266	CIRR02	18.5	C only
P. meandrina Rarotonga 71	KJ720267	CIRR01	16.0	C only
P. meandrina Rarotonga 72	KJ720268	CIRR01	16.0	A + C
P. meandrina Aitutaki 106	KJ720269	CIAT20	11.0	C only
P. meandrina Palmerston 113	KJ720270	CIPA25	12.0	C only
P. meandrina Palmerston 117	KJ880802	CIPA27	13.5	ND
P. meandrina Palmerston 118	KJ790166	CIPA27	6.5	C only
P. meandrina Palmerston 122	KJ790167	CIPA29	7.0	C only
***Pocillopora* sp.** (haplotype 8a[b])				
Pocillopora sp. haplotype 8A Palmerston 111	KJ720260	CIPA24	14.0	C only
Pocillopora sp. haplotype 8A Palmerston 114	KJ720261	CIPA26	12.0	C only
Pocillopora sp. haplotype 8A Palmerston 115	KJ720262	CIPA26	10.0	C only

[a]Nomenclature of Schmidt-Roach et al. (2014). [b]Nomenclature of Pinzon et al. (2013).

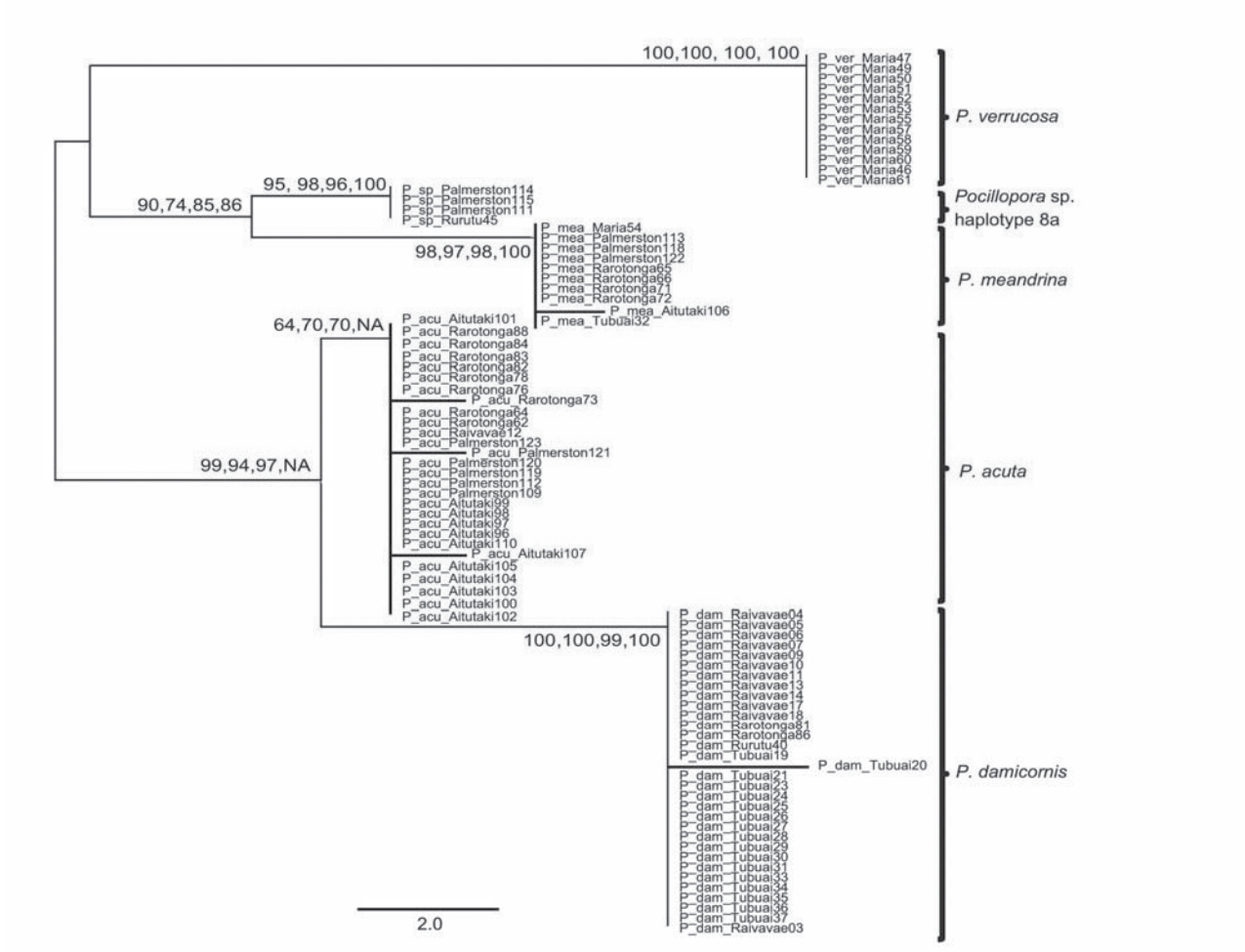

Fig. 5. An un-rooted phylogenic tree of 88 pocilloporid corals of the South Pacific based on sequence analysis of an ~800-bp portion of the partial mitochondrial *atp6* and the putative control region (*pmapcr*). Bootstrap values of 500 replicates based on the neighbor jointing, maximum parsimony (MP), and maximum likelihood methods, as well as posterior probabilities of the Bayesian analyses greater than 60%, are shown on the branches in the respective order; only the MP tree has been depicted due to the others yielding the same tree topology. Scale bar: substitution per site.

Table 5. **Estimation of evolutionary divergence over sequence pairs among different pocilloporid lineages/species using the maximum composite likelihood model.** The number of base substitutions per site (averaged over all sequence pairs between groups) between lineages is shown below the diagonal. The associated standard error for the respective comparison is shown above the diagonal.

	P. acuta	*P. damicornis*	*P. meandrina*	*P. verrucosa*	*Pocillopora* sp. haplotype 8a
P. acuta		0.0029	0.0036	0.0048	0.0036
P. damicornis	0.0079		0.0048	0.0054	0.0044
P. meandrina	0.0119	0.0196		0.0052	0.0031
P. verrucosa	0.0196	0.0248	0.0209		0.0047
Pocillopora sp. haplotype 8a	0.0117	0.0168	0.0078	0.0181	

sp. haplotype 8a was only found on windward reefs (Fisher's exact test, $p<0.05$ in both cases).

There was also a depth (Fig. 6A) effect on species distribution ($X^2=25$, $p<0.05$). *P. acuta* colonies were more likely to be found at 5-10 m (69% of the colonies sampled in this depth range were *P. acuta*.), whereas only 24% of the sampled *P. acuta* colonies were found at depths greater than 15 m. *P. damicornis* showed the opposite trend; no *P. damicornis* colonies were found shallower than 10 m, whereas 48% of the colonies sampled at depths >15 m were *P. damicornis*. In the 10-15 m depth range, both *P. acuta* and *P. damicornis* could be found, though there was a significantly higher percentage of the latter species (45%; Fisher's exact test, $p<0.05$). The distribution of the five documented coral genotypes was also significantly affected by coral cover ($X^2=55$, $p<0.0001$; Fig. 6B [*P. damicornis* and *P. acuta* only]). When ALCC was between 15 and 30%, a significantly higher proportion (Fisher's exact test, $p<0.01$) of colonies were of the *P. acuta* genotype (80%) compared to the *P. damicornis* genotype (7%). In contrast, when coral cover was between 30 and 45%, a significantly higher percentage (Fisher's exact test, $p<0.05$) of the sampled colonies was found to be *P. damicornis* (62%), rather than *P. acuta* (16%).

Discussion

Only 3 of the 60 reefs (5%) surveyed (1 at Raivavae and 2 at Rarotonga) housed both *P. damicornis* and *P. acuta*. Although the fact that these reproductively isolated (Torda et al., 2013) species were found simultaneously on such a small portion of the reefs could be due to competition, the

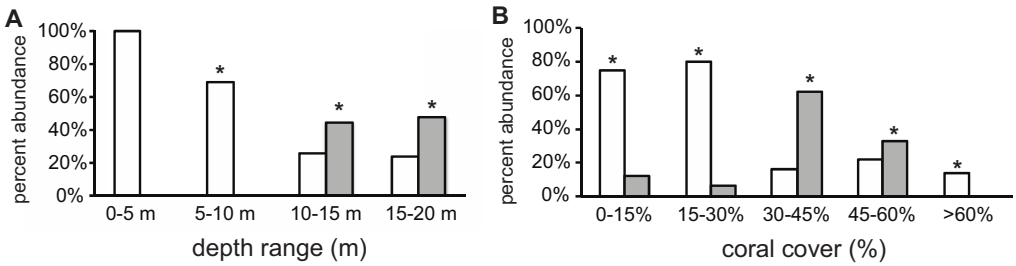

Fig. 6. Niche partitioning of *Pocillopora damicornis* and *P. acuta*. (A) The percent abundance of *P. damicornis* (grey columns) and *P. acuta* (white columns) at different depth ranges. (B) The percent abundance of *P. damicornis* (grey columns) and *P. acuta* (white columns) at different coral cover ranges. When a significant difference in the percent abundance was determined by Fisher's exact test ($p<0.05$) between species at each depth (A) or coral cover (B) range, an asterisk (*) has been placed above the column depicting the higher percent abundance.

niche partitioning data appear to suggest that they prefer different depths and coral cover areas; *P. damicornis* was more likely to be found at depths greater than 15 m and in higher coral cover areas. Such niche partitioning may have allowed these species to co-localize to these three reefs. Therefore, they do not necessarily compete to the point of exclusion, though this may indeed have occurred on certain reefs around Aitutaki and Palmerston, in particular, and could explain the absence of *P. damicornis* at these atolls. It could also simply be that *P. damicornis* larvae were unable to travel the extensive distances needed to reach these remote, isolated regions of the South Pacific Ocean.

Upon a collective assessment of the 88 samples genotyped herein (Table 6), many of which looked quite similar in the field, it appears that it is more difficult than previously thought to identify the α genotype of *P. damicornis in situ*. The cryptic sister species *P. acuta*, which showed distinct habitat preferences, was just as likely to be sampled across the ~2,000-km transect made during the research cruise. The possession of a mix of closely related pocilloporids harboring predominantly clade C *Symbiodinium* populations may allow for the ability to understand the genetic basis for intra-generic differences in coral holobiont stress tolerance and resilience. Future work with this same sample set will also attempt to use molecular biomarkers to assess the health of the resident corals and their *Symbiodinium* populations, and this may allow for an understanding of which pocilloporid species/morphologies and *Symbiodinium* assemblages are most sensitive to environmental change.

Table 6. Pocilloporids sampled in the Austral (Raivavae, Tubuai, Rurutu, and Maria) and Cook (Rarotonga, Aitutaki, and Palmerston) Islands. The corresponding percent abundance value for each genotype at each island data are summarized in pie graphs in Fig. 2. An image of a typical haplotype 8a colony can be seen in Fig. 7.

Island	*P. damicornis* (genotype α)	*P. acuta* (genotype β)	*P. verrucosa* (undescribed genotype)	*Pocillopora* sp. (haplotype 8a)	*P. meandrina* (genotype m)	Total	%
Raivavae	12	1	0	0	0	13	14.9
Tubuai	17	0	0	0	1	18	20.7
Rurutu	1	0	0	1	0	2	2.3
Maria	0	0	13	0	1	14	16.1
Maria	0	0	13	0	1	14	16.1
Rarotonga	2	9	0	0	4	15	17.2
Aitutaki	0	11	0	0	1	12	13.8
Palmerston	0	7	0	3	3	13	14.9
Total	32	28	13	4	10	87	100
%	36.8	32.2	14.9	4.6	11.5	100	

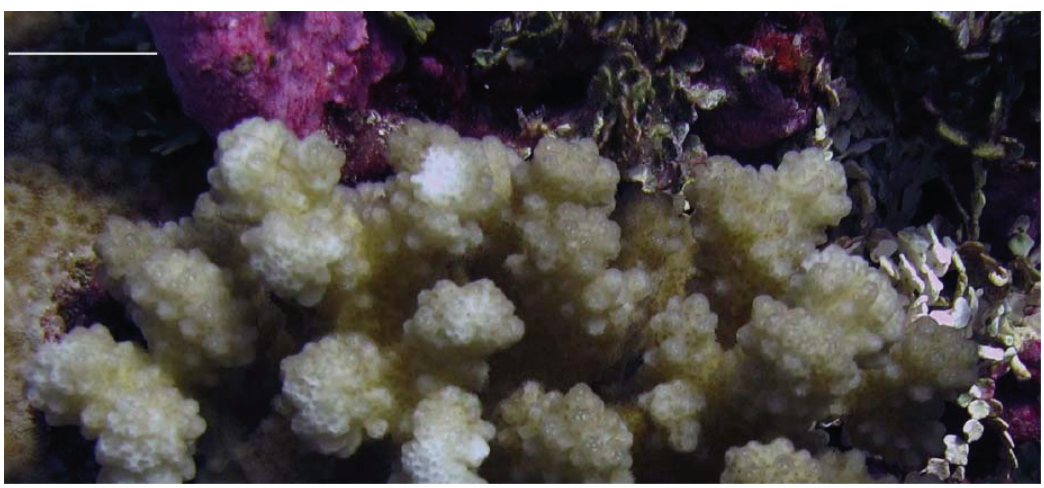

Fig. 7. A *Pocillopora* sp. haplotype 8a colony from Palmerston Atoll in the Cook Islands. The scale bar represents 1.5 cm.

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References

Chen, C., C.Y. Chiou, C.F. Dai & C.A. Chen. 2008a. Unique mitogenomic features in the scleractinian family Pocilloporidae (Scleractinia: Astrocoeniina). Marine Biotechnology, 10: 538-553.

Chen, C., C.F. Dai, S. Plathong, C.Y. Chiou & C.A. Chen. 2008b. The complete mitochondrial genomes of needle corals, *Seriatopora* spp. (Scleractinia: Pocilloporidae): an idiosyncratic *atp8*, duplicated *trnW* gene, and hypervariable regions used to determine species phylogenies and recently diverged populations. Molecular Phylogenetics and Evolution, 46: 19-33.

Flot, J.-F., A. Couloux & S. Tillier. 2010. Haplowebs as a graphical tool for delimiting species: a revival of Doyle's "field for recombination" approach and its application to the coral genus *Pocillopora* in Clipperton. BMC Evolutionary Biology, 10: 372.

Marti-Puig, P., Z.H. Forsman, R.D. Haverkort-Yeh, I.S.S. Knapp, J.E. Maragos & R.J. Toonen. 2014. Extreme phenotypic polymorphism in the coral genus *Pocillopora*: micro-morphology corresponds to mitochondrial groups, while colony morphology does not. Bulletin of Marine Science, 90: 211-231.

Mayfield, A.B., T.Y. Fan & C.S. Chen. 2013a. Physiological acclimation to elevated temperature in a reef-building coral from an upwelling environment. Coral Reefs, 32: 909-921.

Mayfield, A.B., T.Y. Fan & C.S. Chen. 2013b. The physiological impact of *ex situ* transplantation on the Taiwanese reef-building coral *Seriatopora hystrix*. Journal of Marine Biology, Article ID 569369.

Pinzón, J.H., E. Sampayo, E. Cox, L.J. Chauka, C.A. Chen, C.R. Voolstra & T.C. LaJeunesse. 2013. Blind to morphology: genetics identifies several widespread ecologically common species and few endemics among Indo-Pacific cauliflower corals (*Pocillopora*, Scleractinia). Journal of Biogeography, 40: 1595-1608.

Schmidt-Roach, S., K.J. Miller, P. Lundgren & N. Andreakis. 2014. With eyes wide open: a revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae) using morphology and genetics. Zoological Journal of the Linnean Society, 170: 1-33.

Torda, G., S. Schmidt-Roach, L.M. Peplow, P. Lundgren & M.J.H. van Oppen. 2013. A rapid genetic assay for the identification of the most common *Pocillopora damicornis* genetic lineages on the Great Barrier Reef. PLoS ONE, e58447.

Veron, J.E.N. 2000. Corals of the World, vols 1-3. Townsville, Australia: Odyssey Publishing. 1382 p.