Unravelling *Symbiodinium* diversity with Next Generation Sequencing from environmental extremes of Western Australia

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Aims

1) To document the diversity of *Symbiodinium* communities in dominant *Acropora* species from two contrasting marine environments at the latitudinal limits of the genus' distribution in Western Australia

2) To compare the power of detection using Sanger v. high-throughput approaches

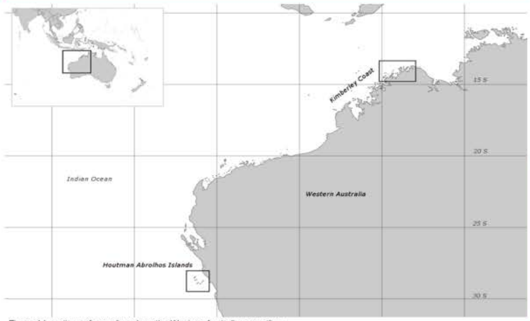

Figure 1 Locations of sampling along the Western Australian coastline.

Background

A single coral host may harbour a variety of *Symbiodinium* clades at any one point in time (see Baker 2003 for a review), which is thought to be a mechanism to facilitate acclimatization in a stochastic environment--during periods of prolonged thermal stress, corals have the ability to shuffle around their *Symbiodinium* so as to increase in proportion the most thermally tolerant strains, known as the *adaptive bleaching hypothesis* (Buddemeier and Fautin 1993).

Conventional molecular techniques used to evaluate *Symbiodinium* diversity in cnidarians are only capable of detecting the dominant clade from a single sample (Aprill and Gates 2007) and often fail to identify rare clades present at levels below 10%. Although perhaps not as ecologically or physiologically important as the dominant clades, background clades may prove to be major players influencing the acclimating potential of coral communities to stressors associated with climate change. In turn, a comprehensive and accurate evaluation of *Symbiodinium* diversity is central to predicting how a coral reef system might respond to climatic fluctuation.

Table 1 List of the dominant *Acropora* species sampled from each region. *Symbiodinium* chloroplast 23s sequences identified using Sanger sequencing are also provided. Numbers superscript and in parenthesis indicate the frequency of that sequence recovered from each species.

Sampling location	Acropora species	N	23s Signature
Kimberley Coast	A. cytherea	6	Cp4(6)
Latitude: 13.80° - 14.10° S	A. divaricata	5	Cp4(5)
	A. millepora	4	Cp4(4)
	A. muricata	5	Cp4(5)
	A. spicifera	4	Cp4(4)
	A. subulata	4	Cp4(4)
	A. tenuis	7	Cp4(6) Dp1(1)
Houtman Abrolhos Islands	A. aspera	3	Cp4 (3)
Latitude: 28.20° - 28.90° S	A. intermedia	6	Cp4 (6)
	A. loisetteae	7	Cp4 (7)
	A. muricata	7	Cp4 (7)
	A. pulchra	6	Cp4 (6)
	A. spicifera	6	Cp4 (6)

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References: Apprill, A. M., & Gates, R. D. (2007). Recognizing diversity in coral symbiotic dinoflagellate communities. Molecular ecology, 16(6), 1127-34. Baker, A. C. (2003). Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. Annual Review of Ecology and Systematics, 34(2003), 661-689. Buddemeier, R.W. & Fautin, D.G. (1993) Coral bleaching as an adaptive mechanism: a testable hy- pothesis. BioScience 43:320–326

Methods

Sampling: Thirty-five samples of the most dominant *Acropora* species were collected from the HAI (28° S) in October of 2012 and from offshore reefs of the Kimberley Region (14° S) in October of 2010.

Sanger Sequencing: The 23s-rDNA Domain V region of the *Symbiodinium* chloroplast was amplified in PCR using primers 23s1 (F: 5'-GGCTGTAACTATAACGGTCC-3') and 23s2 (R: 5'-CCATCGTATTGA ACCCAGC-3')

NGS: The 23s-rDNA hypervariable region of the symbiodinium chloroplast was amplified in PCR using barcoded fusion primers that contained the Roche-454 sequencing adapter, a template specific sequence, and a unique barcode tag which allowed analysis at the individual colony level.

Results

Sanger Sequencing: A single strain of clade C (Cp4) represented 100 % and 97 % of the sequences recovered from HAI and KIM, respectively (Table 1). One sample from the KIM was shown to associate with clade D (Dp1).

NGS: Pyrosequencing returned 212,135 sequences (2,411 +/- 1128 SD sequences per sample). Following quality control and clustering analysis (similarity cut-off of 0.02), 282 and 223 unique sequence types were recovered from the HAI and KIM, respectively. These sequences represented three clades from HAI (C, G, F) and three from KIM (C, D, F). A single strain of Clade G was the second most dominant sequence recovered from HAI colonies, but only represented <2 % of sequences. This is the first evidence of clade G associating with *Acropora spp.* Clade F appeared at very low background levels in both regions. Please note that these are all just preliminary results. Analysis down to the colony level is next!

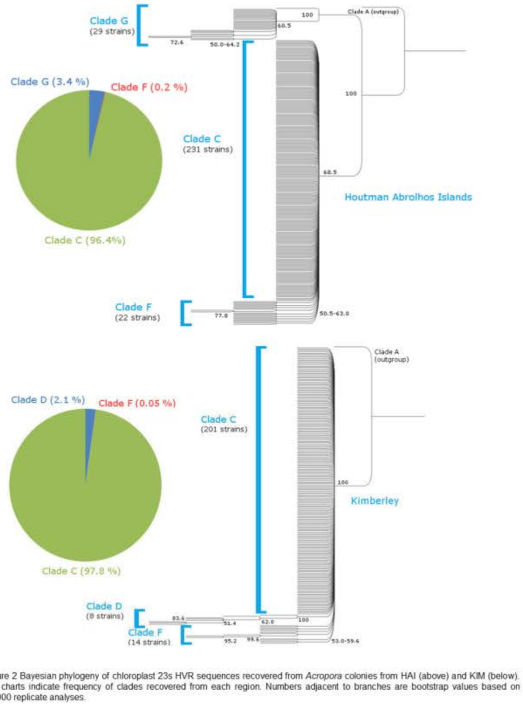

Figure 2 Bayesian phylogeny of chloroplast 23s HVR sequences recovered from *Acropora* colonies from HAI (above) and KIM (below). Pie charts indicate frequency of clades recovered from each region. Numbers adjacent to branches are bootstrap values based on 10,000 replicate analyses.

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