



# Population connectivity and genetic diversity in brooding and broadcast spawning corals in the Kimberley

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# WAMSI Kimberley Marine Research Program Final Report

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#### WAMSI Kimberley Marine Research Program

Initiated with the support of the State Government as part of the Kimberley Science and Conservation Strategy, the Kimberley Marine Research Program is co-invested by the WAMSI partners to provide regional understanding and baseline knowledge about the Kimberley marine environment. The program has been created in response to the extraordinary, unspoilt wilderness value of the Kimberley and increasing pressure for development in this region. The purpose is to provide science based information to support decision making in relation to the Kimberley marine park network, other conservation activities and future development proposals.

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Image 1: Satellite image of the Kimberley coastline (Image: Landgate)

- Image 2: Pink Seriatopora hystrix nestled into Isopora brueggemanni; both brooding corals from the intertidal zone of Longitude Island in the Buccaneer Archipelago. (Image: Jim Underwood)
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# **Executive Summary**

This study utilised next generation sequencing to explore patterns of ecological connectivity and genetic diversity among populations of two species of staghorn coral in the Kimberley; the brooding *Isopora brueggemanni* and broadcast spawning *Acropora aspera*. These two species display the common reproductive modes in hard corals which create the crucial three dimensional structures that provide the habitat and protection that is the foundation of coral reef ecosystems. Both species are listed as "vulnerable" on the IUCN Red List of Threatened Species based on the general estimates of reef degradation within their range as well as their inherent susceptibility to bleaching and disease.

Samples of *I. brueggemanni* (n=612) and *A. aspera* (n=563) were collected across three hierarchical spatial scales. At broad (inter-regional) scales, sites were separated by 100s of kilometres and included the offshore site of Ashmore Reef, as well as inshore reefs of the Bonaparte Archipelago in the central Kimberley, and the Buccaneer Archipelago and Dampier Peninsula in the southern Kimberley. At the intermediate (inter-reef) scale, detailed sampling was undertaken at the Buccaneer Archipelago and Dampier Peninsular, where multiple sites were separated by distances of kilometres to tens of kilometres. At the local (within-reef) scale, sampling allowed for estimates of genetic structure over distances of tens to 100s of metres. Analyses revealed considerable genetic structure within both species at all three scales.

For the brooder, *I. brueggemanni*, 2,125 SNPs revealed three discrete genetic clusters over broad scales; Ashmore Reef in the north, Kooljaman in the far west of the Dampier Peninsula, and the southern inshore Kimberley. At the intermediate scale, the observed level of genetic structure in *I. brueggemanni* indicated that connectivity over more than 20 km is generally rare. More specifically, Dampier and Buccaneer corals formed two genetic groups, but with geographically intermediate Islands of Mermaid and Tide Rip sharing genetic affinities with both groups. Therefore, these two islands appear to be important stepping stones for maintaining occasional connectivity and genetic exchange across the Sunday Strait. At fine scales, significant differentiation was detected between subsites, and colonies separated by less than 500 metres were more closely related than those further apart, indicating that most brooded larvae recruit within a few hundred metres of their natal colony. A general attenuation of gene diversity was detected with increasing latitude, indicating that effective population sizes are larger, and genetic connections to exogenous sources are stronger, in populations in the central region compared with those in the south.

For the broadcast spawner, initial genetic analysis of the entire A. aspera collection using a subset of SNPs revealed the presence of four lineages that were genetically distinct but morphologically cryptic. The large magnitude of genetic differentiation among these lineages indicated these lineages are reproductively isolated, even though they look the same and live side by side. The subsequent analyses of population connectivity used 2,894 SNPs to focus on the most abundant and widespread lineage, Acropora asp-c (n = 322). Consistent with a greater propensity for widespread dispersal in the broadcast spawned larvae compared with the broaded larvae, the overall amount of genetic subdivision in the Acropora asp-c lineage ( $F_{ST} = 0.101$ ) was half that of I. brueggemanni (F<sub>ST</sub> = 0.230). Nevertheless, the pattern of geographic structure evident in Acropora asp-c was similar to I. brueggemanni, with four discrete genetic clusters detected over broad scales among Ashmore Reef, central Kimberley, Buccaneer Archipelago and Dampier Peninsula. At intermediate scales, genetic patterns in Acropora asp-c corals also matched those found in *I. brueggemanni*; spawned larvae rarely disperse more than 35 km while corals from Tide Rip and Mermaid Islands exhibited affinities to both the Dampier and Buccaneer clusters. Lastly, at fine scales, relatedness was relatively high among corals separated by less than 500 metres, indicating that many spawned larvae recruit back to their natal reef patch. Levels of gene diversity within the Acropora asp-c lineage appeared to be greater in the central Buccaneer Archipelago, and attenuated to west and east from this centre, suggesting that these are the largest and most well connected populations of this species in the region.

The oceanographic model supported the broad scale genetic patterns, with no evidence of any inter-regional connectivity via ocean currents between the offshore and inshore reefs. However, a more biologically realistic oceanographic model is required to properly capture the complex fine-scale hydrodynamics in this region.

# Implications for management

The key finding from this study is that ecological connectivity among populations of both the brooding coral and broadcast spawning coral is restricted to the scale of reef or reef patch, with few larvae dispersing more than 35 kilometres from their natal reef patch. This finding has important ramifications for the managers, policy makers and custodians of coral reefs of the Kimberley. Specifically, it implies that locally produced recruits are crucial to the persistence of coral populations, and recovery after disturbance will rarely be supplemented through the input of larvae from locations that are more than a few tens of kilometres away. Therefore, if the intention of Marine Protected Areas (MPA's) and Indigenous Protected Areas (IPA's) is to protect hard corals, they must consider the importance of local recruitment for population maintenance, recovery and adaptation to environmental change by ensuring the maintenance of connectivity networks among reef patches by positioning multiple sanctuaries over scales of less than a few tens of kilometres.

Further specific management considerations include:

- Exchange of genes between the inshore Kimberley and the offshore coral reefs is negligible meaning that inshore populations will rely on maintenance of standing genetic variation to recover from and adapt to natural and anthropogenic impacts.
- The Dampier Peninsula and Buccaneer Archipelago need to be managed as demographically independent systems, with the important consideration that Tide Rip and Mermaid Islands provide stepping stones of genetic exchange that likely augments population resilience and adaptation over multiple generations.
- For the brooding coral, the west coast of Dampier Peninsula appears to support a small, isolated, and genetically unique population that is demographically independent from populations east of the Dampier Peninsula.
- The high genetic diversity at the central Kimberley site of West Montalivet in the Bonaparte Archipelago indicates that these reefs are important reservoirs of genetic variation and have strong connections with other populations, making them priorities for conservation.
- The discovery of four genetically divergent lineages within *Acropora aspera* means that morphological assessments of biodiversity of hard corals in the Kimberley are likely substantial underestimates. Additionally, the effective population size of each lineage will be much smaller, and consequently more vulnerable to disturbance, than expected if assessments are based on distribution of the single morphospecies.

# 1 Introduction

The coral reef systems of the Kimberley in Northwest Australia (NWA) are diverse, unique, and understudied. They are also among the least impacted coral reefs on the planet (Halpern et al. 2008), and are characterised by extensive reef development, a diversity of reef forms of varied geological origin, and are regarded as a major center of coral biodiversity at the southern margin of the East Indies Coral Triangle (Wilson 2013). Macrotides (up to 12m) combine with complex geomorphology to create complicated hydrodynamics that could either be strong conduits, or barriers, to dispersal of larvae among coral populations. These reefs are also characterised by extreme physical conditions, including large variations in turbidity, nutrient concentrations and temperature. Further, physical evidence suggests that coral reefs of the inshore Kimberley appear to be relatively isolated from neighbouring coral systems; the major currents capable of transporting larvae long distances in this region occur on the continental shelf margin and do not intrude far into the shelf and inshore waters (D' Adamo et al. 2009). Theory predicts reproductive isolation of populations brought about by strong selection pressures from such environmental heterogeneity and physical isolation is conducive to the development of unique patterns of inter- and intra-specific genetic diversity and structure (Felsenstein 1976). A recent study in the Kimberley coast supports such expectations, recording seven new records for coral species in WA and expanding species distributions beyond expected zonation (Richards et al. 2015).

Coral reefs throughout the world are declining rapidly due to increases in sea-water temperatures, ocean acidification, and local anthropogenic disturbances (Hoegh-Guldberg et al. 2007, Hoey et al. 2016), with one third of all reef building corals facing a high risk of extinction (Carpenter et al. 2008). The recent mass bleaching event at Scott Reef (offshore Kimberley) and the inshore reefs of the southern Kimberley in 2016 (Gilmour pers *comm*), highlights that the worldwide call to conserve coral ecosystems in the face of climate change (see Jones et al. 2016) is especially pertinent in NWA. To manage coral reefs and enhance their resilience, a range of strategies must be employed in tandem, and includes effective spatial planning of Marine Protected Areas (MPAs) and/or Indigenous Protected Areas (IPAs) (McCook et al. 2010, Lamb et al. 2015, Mellin et al. 2016). To provide an empirical basis for a regional network of Kimberley marine parks and reserves, and to inform risk assessments and impact assessments, it is crucial to know where new recruits in a reserve originate from and where larvae from that area disperse to (Kendrick et al. 2016). These patterns of immigration and emigration, or "population connectivity", are key ecological drivers of population maintenance and recovery after disturbance. Indeed, the current lack of knowledge about the way populations are connected, along with the inability of management agencies to effectively implement the knowledge that is available, is a major impediment to management of marine systems throughout the world (Magris et al. 2014). Because genetic divergence among individuals and populations arises when interbreeding is restricted, a spatial analysis of genetic structure is a pivotal method that provides insights into the degree of connectivity among those individuals and populations over generation by generation time scales.

Patterns of genetic diversity and structure are also required to understand the stability of populations over multigenerational time scales, because adaptation to a changing environment depends on the amount and configuration of standing genetic variation that is available. Further, populations, communities and ecosystems with high genetic diversity are generally resilient to disturbance (Hughes et al. 2008). Therefore, genetic studies that accurately describe evolutionary significant units (reproductively isolated lineages) and how genetic diversity is partitioned among populations, are crucial to management efforts that are intended to protect a representative range of the biodiversity and understand the processes that sustain that diversity (Moritz 2002, Bowen and Roman 2005, Pante et al. 2015). For corals, it is notoriously difficult to resolve species boundaries due to a combination of morphological plasticity and interbreeding (Willis 1990, Miller and Benzie 1997, van Oppen et al. 2001, Willis et al. 2006, Ladner and Palumbi 2012, Ohki et al. 2015, Richards and Hobbs 2015, Rosser 2015, Gilmour et al. 2016b, Richards et al. 2016), and the relationship between morphology and genetics is certainly less well understood for corals of the Kimberley coast than in other reef systems, with concomitant effects for management (Underwood et al. 2013).

Hard corals create the crucial three dimensional structures that provide habitat and protection for many other coral reef species, and research is required to elucidate patterns of genetic diversity and population connectivity within species in these crucial habitat forming organisms. This study utilises single nucleotide polymorphisms (SNPS), isolated across the coral genome to assess the genetic structure and diversity for the brooding hard coral, *Isopora brueggemanni*, and the broadcast spawning coral, *Acropora aspera*, over broad scales (inter-region; tens to hundreds of kilometres), intermediate scales (inter-reef; kilometres to tens of kilometres) and fine scales (within-reef; hundreds of metres) in the Kimberley. The genetic patterns are compared to oceanographic models to elucidate more general biophysical processes influencing patterns of connectivity for coral reef species in this region.

# 2 Materials and Methods

#### 2.1 Study species

Isopora brueggemanni is one of the most easily identifiable corals within the family Acroporiidae. In the Kimberley, it occurs in shallow (< 20 m) water, especially on exposed upper reef slopes and sand flats, and is abundant and widespread on inshore and offshore reefs. Unlike the majority of species on NWA coral reefs, I. brueggemanni's sexual reproductive mode involves release of sperm into the water column and fertilisation of eggs within the polyp, although self-fertilisation has also been reported (Okubo et al. 2007). The resulting larvae are brooded within the polyp and then released at an advanced developmental stage. Planula release is likely to be extended over several months through spring to autumn in the Kimberley (Gilmour et al. 2016a), but the exact timing is not currently known in this region, as in other parts of the world (Okubo et al. 2007). Similar to other brooders, larvae are probably capable of settling within hours of release (minimum competency period) (Harrison and Wallace 1990). Brooding hard corals are generally characterised by strong levels of genetic subdivision, and self-seeding is well established (Ayre and Dufty 1994, Ayre and Hughes 2000, Underwood et al. 2007, 2009, van Oppen et al. 2011, Starger et al. 2013). However, brooded planulae are relatively large when released into the water column and some contain maternal zooxanthellae, and therefore appear to be provisioned for a long maximum competency period (Harrison and Wallace 1990). Thus, dispersal is likely to be bimodal, with long-distance dispersal (teleplanic) occasionally supplementing more routine, philopatric dispersal or self-seeding. The success of either strategy is likely to depend on environmental and demographic conditions: stable healthy populations are expected to be maintained by locally derived recruits, while recovery after disturbance is expected to be initiated from input of exogenous larvae followed by local recruitment. Both strategies can also be supplemented by asexual reproduction, via vegetative fragmentation in branching corals, which is likely to be more common in exposed, platform habitats. I. brueggemanni is listed as "vulnerable" on the IUCN Red List of Threatened Species (Richards et al. 2008).

Acropora aspera is widely distributed, and is found in the northern Indian Ocean, the central Indo-Pacific, Australia, Japan and the East China Sea, and the oceanic west Pacific. In Western Australia, Acropora aspera occurs from the oceanic shoals in the north to the Abrolhos Islands in the south, but is most abundant at inshore reefs in the Kimberley and Pilbara. Acropora aspera is a broadcast spawner, whereby eggs and sperm are released during mass spawning events, after which fertilisation and larval development occur in the plankton. Data on spawning time of *A. aspera* in the Kimberley is sparse, but a primary spawning most likely occurs in the Austral autumn, and there may be a secondary spawning in spring (Gilmour et al. 2016a). In contrast to brooded larvae, broadcast spawned larvae must spend a few days in the plankton before they are competent to settle, and thus have weaker potential for self-seeding compared with brooding corals. However, if suitable substrate is available, the majority of larvae probably settle as soon as they are competent, as they are less well provisioned for teleplanic dispersal than brooded larvae and their probability of settling and surviving drops rapidly the longer they spend in the plankton (Harrison and Wallace 1990, Baird 2004). Like *I. brueggemanni*, asexual reproduction via vegetative fragmentation may well supplement the broadcast spawning reproduction particularly in exposed, platform habitats. *A. aspera* is considered to be relatively easily identified in the field although at some locations it can be confused with *Acropora pulchra* (Richards unpublished). A. aspera is listed as "vulnerable" on the IUCN Red List of Threatened Species (Aeby et al. 2014).

## 2.2 Sampling design

A total of 612 I. brueggemanni corals were sampled from 18 sites (between 20 and 60 samples per site) along the Kimberley coast, including the reef systems of the Dampier Peninsula, Buccaneer Archipelago, the central Kimberley, and Ashmore Reef (Fig. 1). A total of 563 corals identified in the field as A. aspera were sampled from 16 sites (between 24 and 83 colonies per site) along the Kimberley coast and Ashmore Reef (Fig. 2). Both species were sampled at ten of these sites. Samples were collected by walking on exposed platforms at spring low tides and removing one centimetre fragments from coral colonies. Fragments were placed in 100% ethanol prior to DNA extraction in the laboratory. Photographs were taken of colonies representing common morphologies, along with the collection of voucher specimens for taxonomic identification. Colonies were separated by at least 1.5 metres to reduce the likelihood of collecting clone mates that were the product of fragmentation, and each site spanned no more than 500 metres along the reef flat. In addition to the broad scale collection, we sampled also at an intermediate and fine scale. The intermediate scale sampled multiple reefs separated by kilometres to tens of kilometres through detailed collections from the Buccaneer Archipelago and the Dampier Peninsula in the west of the Kimberley. The fine scale sampling within reefs recorded the location of each colony with GPS for both species, and for the brooding coral, we also sampled a second subsite (separated from the first subsite by ~ 500 metres) within the Buccaneer Archipelago at three sites (Fig. 1).



Fig. 1 Map of *Isopora brueggemanni* collections from the inshore Kimberley and Ashmore Reef in North West Australia. Insert shows the detailed collections from the Dampier Peninsula (black text) and the Buccaneer Archipelago (blue text).



Fig. 2 Map of *Acropora aspera* collections from the inshore Kimberley and Ashmore Reef in North West Australia. Insert shows the detailed collections form the Dampier Peninsula (black text) and the Buccaneer Archipelago (blue text).

# 2.3 DNA extractions and DArTseq development of SNPS

Genomic DNA was extracted using a salting out protocol modified from Cawthorn et al. (2011) and purified with Zymo Plate filter plates (Zymo-Spin I-96). Genome-wide single nucleotide polymorphism (SNP) data were generated using a next generation sequencing platform following the DArTseq protocol (Diversity Arrays Technology; Appendix 1), 195 *Isopora* samples and 126 *Acropora* samples were genotyped twice as technical replicates and scoring consistency was used as the main selection criteria for high quality/low error rate markers, and loci with reproducibility less than 0.94 were excluded. The call quality of the initial SNP data set was further assured by setting a cut-off of read depth per locus (coverage) < 7, call rate >0.35, minimum allele frequency >0.00075 for *Isopora* and >0.0017 for *Acropora*. Sequences were blasted on GenBank to check for general contamination and endosymbionts including genomes and transcriptomes of the symbiotic zooxanthellae, *Symbiodinium*, which lives in coral host tissue. No sequences aligned to the *Symbiodinium* genome with E-values between 1.36E<sup>-19</sup> and 2.73E<sup>-27</sup> and these were removed form downstream analysis. The primary data set comprised 23,165 SNPS for *I. brueggemanni* and 34,304 SNPS for *A. aspera*.

# 2.4 Initial quality control, identification of clone and genomic summary statistics

For *I. brueggemanni*, we used adegenet (Jombart 2008) and a custom R script to filter the primary data set at the following levels; call rate > 0.95, coverage > 20, minimum allele frequency >0.05, max heterozygosity <0.75 (graphical summaries in Appendix 2, Fig. A1). In addition, we utilised the reproducibility statistic (calculated from the 195 technical replicates) to filter out all loci with < 0.99 correct calls across individuals. This filtering resulted in 2,946 loci, to which we then filtered out loci that exhibited significant Hardy Weinberg and linkage disequilibrium using custom R scripts and the R packages SNPassoc (Gonzalez et al. 2007), adegenet and pegas (Gonzalez et al. 2007, Paradis 2010). Both Hardy-Weinberg and linkage disequilibrium tests were carried out

separately for each sampling site (n=21). For Hardy-Weinberg disequilibrium, we removed 133 loci that showed departures from expectations at P < 0.05 in five or more of the 21 sites. For linkage disequilibrium we removed 681 loci with *r* values > 0.8 among five or more sites. Six individuals with greater than 15% missing data were removed. 2,132 SNPS remained, to which we identified loci under directional selection with OutFLANK v0.1 (Whitlock and Lotterhos 2015) using with 5% left and right trim for the null distribution of  $F_{ST}$ , minimum heterozygosity for loci of 0.1, and a 5% false discovery rate (q value). Initial analysis using the entire data set did not detect any outliers, but when OutFLANK v0.1 (Whitlock and Lotterhos 2015) was applied to the inshore data only, seven loci were identified as outliers and were removed from subsequent analyses.

For *A. aspera*, initial investigations during the SNP development indicated the presence of multiple genetic lineages which were characterised by morphologically cryptic but major genetic divergences among colonies living in sympatry. From the primary data set of 34,304 SNPS, we filtered the data at a highly stringent level (call rate > 0.95, coverage > 20, minimum allele frequency >0.05, max heterozygosity <0.75). In addition, we utilised the reproducibility statistic (calculated from the 126 technical replicates) to filter out all loci with < 0.999 correct calls across individuals (APPENDIX 3, Fig. A1). This extremely stringent filter provided very reliable calls for all samples, and mitigated interference of genotyping (e.g. null alleles) brought about by differences in the target sequences among genetic groups. The result was 585 SNPS that could accurately ascertain the levels of divergence among genetic lineages. We did not filter for Hardy Weinberg or gametic-phase disequilibrium at this stage of the analysis because large (potentially interspecific) divergence would be associated with such disequilibrium, and removal of such markers would likely limit power of the analyses. Seven individuals with greater than 15% missing data were removed.

Four highly divergent lineages were identified in the *A. aspera* collection that occurred in sympatry and exhibited genetic cohesion among geographically distant populations. Therefore, a targeted re-analysis based on the most common and widespread lineage (*Acropora* asp-c) was required for population genetic analysis at the intra-specific level. To this end, we re-calculated the descriptive statistics across all SNP loci for those samples identified as *Acropora* asp-c with the same filters and methods as for the entire *A. aspera* collection except we relaxed the reproducibility (> 0.98) and call rate (> 0.90) thresholds (Appendix 3, Fig. A2). This filtering resulted in 3,472 loci, to which we then filtered out loci that exhibited significant departures from Hardy Weinberg equilibrium gametic-phase equilibrium as in the *I. brueggemanni* QC analysis, but because of smaller sample sizes, disequilibrium testing was carried out separately for each sampling site only for those sites with more than 15 samples (N = 5). For Hardy-Weinberg testing, we removed 343 loci that showed departures from expectations at P < 0.05 in three or more (out of five) sites. For gametic-phase disequilibrium, we removed 294 loci with r values > 0.8 among three or more sites. 2,898 SNPS remained, to which we identified loci under directional selection with OutFLANK using the same parameters as for the *I. brueggemanni* analysis. Four loci were identified as outliers and these were removed from subsequent analyses.

To establish whether colonies were clone mates, we used the technical replicates (n = 195 for *I. brueggemanni*, and n = 126 for *A. aspera*) to determine a threshold of maximum genetic distance (based on hamming distance) between the two genotypes of each repeat pair, and identified clones (ramets) as samples with genotypic distance below this threshold. For colonies that were identified as ramets, all but one individual was removed from the data, yielding a final data set comprising individual genets. Genotypic richness was calculated as the ratio of number of genets to total number of samples (ramets). Summary statistics of the final data sets were calculated in GenAlEx v6.5 (Peakall and Smouse 2006) and included; number of positive calls (*N*), number of alleles (*N*<sub>A</sub>), observed heterozygosity (*H*<sub>o</sub>), unbiased expected heterozygosity (*H*<sub>E</sub>), and fixation index (*F*<sub>IS</sub>) at each site and averaged across sites (± SE).

#### 2.5 Broad and intermediate scale genetic structure

To explore the broad scale of geographic structure in *I. brueggemanni*, we used the Bayesian software STRUCTURE v2.3 (Pritchard et al. 2000) to estimate membership coefficients (q) of each individual colony to each cluster for a range of populations and identify the optimal number of genetic clusters (K). Initial

exploration of the data used the correlated and independent allele frequency model, both without (NOPRIOR) and with (LOCPRIOR) information on sampling location of colonies. The results of the correlated and independent allele frequency models were extremely congruent in identifying the most appropriate K as well as individual membership to clusters, but correlated allele frequency model and the LOCPRIOR model resolved the data with the most clarity and produced the highest  $\Delta K$  values, so we only present those results. Mean and variance of log likelihoods and posterior probabilities of the number of clusters from K = 1 to 10 were inferred from 20 independent runs using the admixture models with burn in of 100,000 and then 500,000 MCMC repetitions. All other parameters were default values. Convergence of algorithms was checked by assessing the variability in individual assignment proportions across runs, and the similarity score calculated with the online program CLUMPAK (Kopelman et al. 2015). STRUCTURE runs were performed on the CSIRO Accelerator Cluster "Bragg", which consists of 128 Dual Xeon 8-core E5-2650 computer nodes. CLUMPAK was used to summarise and graphically present the STRUCTURE results as well as to calculate the most appropriate K using the  $\Delta K$ method of Evanno et al. (2005). When deciding on the most appropriate K, we considered biological interpretations for a range of K values, and chose the K which best addressed our a priori questions and expectations (see Pritchard and Wen 2003, Meirmans 2015). Further, when highly divergent samples were detected, we ran STRUCTURE excluding those samples to ascertain whether they interfered with clustering among the more genetically coherent samples, and thus our ability to describe patterns of genetic structure at intermediate (inter-reef) scales.

To provide an alternative measure of number and membership of major clusters to the Bayesian analyses, we also explored the genetic relationships among all *I. brueggemanni* individuals with a principal coordinate analysis (PCoA) in GenAlEx. PCoA uses is a simple multi-ordination calculated from a codominant genotypic distance among pairs of samples and does not take into account any *a priori* information such as sampling location or assumptions of equilibrium, and thus provides a complimentary analysis to the sophisticated Bayesian approach of STRUCTURE. As with the STRUCTURE analysis, to tease out genetic relationships among corals at intermediate and fine scales which were obscured by highly divergent samples, PCoA was repeated excluding those samples. The PCoA was performed using the standardised distance option in GenAlEx.

To estimate the amount of genetic variation that was partitioned among geographic locations in the *I. brueggemanni* collection at a hierarchy of scales, we conducted an hierarchical AMOVA in GenAlEx with the traditional fixation index of genetic subdivision ( $F_{ST}$ ). This analysis measured variation among the four systems of Ashmore, central Kimberley, Buccaneer Archipelago and Dampier Peninsula ( $F_{RT}$ ), among sites within systems ( $F_{SR}$ ), and among all sites ( $F_{ST}$ ). As part of this AMOVA, pairwise  $F_{ST}$  between all sites was calculated, and tests for statistical significance of all analyses were based on 999 random permutations.

For *A. aspera*, an initial cluster analysis was performed with STRUCTURE and PCoA (model conditions were the same as for *I. brueggemanni* but with no *a priori* information on sampling location in the STRUCTURE runs). This analysis identified four divergent lineages (see results). To gauge the magnitude of divergence among these lineages, we calculated pairwise  $F_{ST}$  between lineages in GenAlEx, testing for statistical significance with 999 random permutations. Lineages were subsequently deemed reproductively isolated, which meant that a reanalysis of the most common and widespread lineage (*Acropora* asp-c) was required for inference of intraspecific population connectivity in the broadcast spawning coral.

To this end, we applied the same methods using STRUCTURE, PCoA and AMOVA as for *I. brueggemanni* to describe genetic structure over multiple geographic scales within the *Acropora* asp-c lineage. Model runs with and without prior information of sampling location were highly congruent, as were runs using correlated and independent allele frequencies, but correlated allele frequency model and the LOCRIOR model resolved the data with the most clarity and produced the highest  $\Delta K$  values so we only present those results. For the AMOVA, some sites had small sample sizes, but because we employed thousands of SNPs, estimation of  $F_{ST}$  for samples sizes > 4 is likely to be robust (Willing et al. 2012). However, to substantiate this expectation, we also calculated an AMOVA only using those sites where n ≥ 9.

#### 2.6 Fine scale patterns of genetic structure

To explore patterns of fine scale genetic structure and infer routine dispersal distances in *I. brueggemanni* and within the Acropora Asp-c lineage, we performed a spatial autocorrelation analysis on the Dampier Peninsula and Buccaneer Archipelago collections that were sampled in most detail in the inshore Kimberley. Spatial autocorrelation utilises the spatial position and genetic identity of each individual coral, and is well suited to establishing the finest scale of genetic structure that is sensitive to recent dispersal processes (Double et al. 2005, Epperson 2005). An autocorrelation was calculated between the genetic distance (codominant genotypic) and geographic (Euclidean) distance of all pairs of individuals that fell within a given distance class, and each autocorrelation coefficient, r, was plotted with respect to its given distance class in GenAlEx. Under conditions of restricted gene flow, the autocorrelation coefficient is expected to be positive at short distance classes, and will subsequently decline through zero and become negative at larger distance classes (Sokal and Wartenberg 1983, Epperson and Li 1996, Smouse and Peakall 1999). For I. brueggemanni, initial analysis showed that Kooljaman was a clear outlier to the general patterns of spatial genetic structure, and so was excluded from this analysis, which also provided comparability with the study of A. aspera. To test for statistical significance of r at each distance class, a 95% confidence interval about r was generated via 1000 bootstrap trials by drawing (with replacement) from within the set of pairwise comparisons for a specific distance class, and when this interval did not straddle r = 0, significant spatial genetic structure was inferred.

In addition to spatial autocorrelation, because we sampled *I. brueggemanni* at level of sub-site at three sites (Bathurst, Pope and Mermaid Islands), we estimated genetic variation among these sites for this species ( $F_{RT\_SITES}$ ), between sub-sites within sites ( $F_{SR\_SUBSITES}$ ), and among all sub-sites ( $F_{ST\_SUBSITES}$ ). As part of this AMOVA, pairwise  $F_{ST}$  between all sub-sites was calculated. Tests for statistical significance were based on 999 random permutations.

#### 2.7 Oceanographic modelling

To estimate the potential for transport of larvae via oceanographic currents among inshore and offshore Kimberley sites, we used a biophysical dispersal model based on Regional Ocean Modelling System with 2 km resolution. The model was nested within the Ocean Forecasting for Australia Model 3 (OFAM3) simulation (Feng et al. 2016) and forced by 3-hourly meteorological measures derived from Kobayashi et al. (2015). The model simulation was based on data from 2011. Hourly sea surface current velocities (0-5 m) were extracted from the model output and used for particle tracking modelling. A 4th-order Runga-Kutta sub-time-stepping scheme was used to update the particle locations every hour (Feng et al. 2010). For I. brueggemanni, total of 100 particles were seeded in sampling sites during austral spring-summer-autumn period (September-May), at 3-day intervals. This particle release period represents the season of planulation of *I. brueggemanni* based on field observations that identified gametes and planula larvae in all stages of development during these months (Gilmour et al. 2016a). Using the random walk effect of 1 m2s-1, particles were tracked for two time periods that represented our best estimates of the common competency window for brooding corals (zero to eight days). For A. aspera, a total of 100 particles were seeded in sampling sites during predicted time of the main mass spawning in the austral autumn of 2011, which most likely occurred on March 28 (seven to nine nights after the full moon). Because the exact timing of spawning of Acropora corals is not known in the Kimberley region, and spawning varies on nearby oceanic atolls by several days (Gilmour et al. 2016a), we also released particles three days before, and three days after the 28<sup>th</sup> of March. Particles were tracked from day three to day eight. These days represented our best estimates of the common competency window of the majority of larvae based on field observations of Acroporas on offshore NWA reefs (Gilmour et al. 2009) and from laboratory studies on the closely related Acropora pulchra (Baird 2004). The grid size for tracking the particles from each sampling site was set to 500m x 500m. Connectivity among sampling sites was estimated as the average number of particles released from site i that were tracked to be in site j during the competency period. To make this matrix symmetric we summed connectivity between i and j and j and i. Oceanographic connectivity was calculated as the proportion of released particles from i and j that settled at i and j. This value was converted to an oceanic resistance as 1 - oceanographic connectivity. Values were arcsine transformed

before further analysis. To graphically depict the oceanographic results at the regional scale, we used custom R scripts to produce plots of particle tracks run over eight and 40 days in each austral season of 2011 and incorporated a broader range of sites compared with the genetic collections for corals including several inshore sites and also Scott Reef.

# 2.8 Oceanographic, geographic and genetic distance

To explore whether genetic structure can be explained by oceanographic and/or geographic distance, we compared the pairwise  $F_{ST}$  (linearised) matrix to the oceanographic resistance matrix and geographic distance (Euclidean) matrix using a simple paired Mantel test in GenAlEx. For *I. brueggemanni*, initial analysis showed that Kooljaman was a clear outlier to the general patterns, and so was excluded from this analysis, which also provided comparability with the study of *A. aspera*. Tests for statistical significance of correlation coefficient were based on 999 random permutations.

# 3 Results: Isopora brueggemanni

# 3.1 Identification of clones, genotypic richness and gene diversity in I. brueggemanni

For *I. brueggemanni*, all technical replicates exhibited a hamming distance of  $\leq$  0.02, and thus pairs of samples in the complete data set that fell below this threshold were deemed clones (ramets). All ramets of each nearidentical genotype were collected at the same site, and the majority were sampled within a few metres of each other reinforcing their clonal origin through fragmentation. Only one genotype of each ramet was retained in the final data set, resulting in the removal of 45 samples.

Genotypic richness (the ratio of number of genets to ramets) was generally high, with an average of 0.93 across all sites (Table 1). Nine sites were comprised entirely of unique genets, although one site (Hedley Island) had a much lower genotypic richness than all other sites (0.39; Table 1). Average observed heterozygosity was 0.176, average expected heterozygosity was 0.173, and average  $F_{IS}$  was -0.069 across all loci (genotypes and summary statistics are given digital data repository; http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7). Gene diversity, as measured by expected heterozygosity, was relatively constant over most of

220424043537). Gene diversity, as measured by expected neterozygosity, was relatively constant over most of the sampling sites with two exceptions; West Montalivet in the far east had the highest gene diversity and Kooljaman in the far west had the lowest diversity (Fig. 2). These two sites created a general trend of declining gene diversity with increasing latitude ( $r^2 = 0.213$ ).

# 3.2 Genetic structure among regions, systems and reefs in I. brueggemanni

The STRUCTURE analysis of the *l. brueggemanni* collection revealed distinct genetic clusters among geographically separate (allopatric) populations at a hierarchy of spatial scales and no evidence of cryptic divergence among sympatric corals. The Evanno et al. method indicated highest level of structure at K = 2 (APPENDIX 2 Fig. A2), with membership coefficients (q) of 100% of colonies to either the Ashmore cluster, or the inshore Kimberley cluster at all sites, with the exception of the central Kimberley site of West Montalivet which was admixed (with the majority of q~ 50% to either cluster; Fig. 3). However, at K > 2, clusters continued to segregate according to geography with an additional cluster formed by Kooljaman (with q= 100%) at K = 3, and a fourth cluster formed by the Dampier Peninsula sites at K = 4 (with q > 70%; Fig. 3). Furthermore, at K = 4, the sites of Tide Rip and Mermaid Island\_1 exhibited admixed membership to the Dampier and the Buccaneer clusters either within the individual (at Tide Rip, q ~ 50% in all individuals), or among individuals (at Mermaid Island\_1, q ~ 75% to Dampier cluster in 20 individuals, and q > 80% to the Buccaneer cluster in nine out of the remaining 10 individuals; Fig. 3). At K = 5, a cluster at Pope Island segregated, and the sites of Irvine and Bathurst\_W segregated at K = 6, while at K > 6, the corals from West Montalivet formed their own cluster separate from the Ashmore cluster (APPENDIX 2 Fig. A3).

Region	SITE	N	Ng	Ng:N
Ashmore	Ashmore Reef	29	29	1.00
Central Kimberley	West_Montalivet	32	25	0.78
	Hedley_I.	28	11	0.39
	Irvine_I.	27	27	1.00
Buccaneer Archipelago	Bathhurst_W_1	28	28	1.00
	Bathhurst_W_2	20	20	1.00
	Longitude_I.	29	29	1.00
	Frazer_I.	31	30	0.97
	Barret_Rock	29	27	0.93
	Asshlyn_I.	31	30	0.97
	Pope_I1	31	30	0.97
	Pope_I2	31	30	0.97
	Tide_Rip_I.	31	29	0.94
	Mermaid_I1	30	30	1.00
	Mermaid_I2	30	26	0.87
Dampier Peninsula	Janinko	29	26	0.90
	Ngoorroodool	20	20	1.00
	Jalan	30	30	1.00
	Noyon	30	28	0.93
	Ardinoogoon	30	25	0.83
	Kooljaman	31	31	1.00
	TOTAL	606	561	0.93

Table 1 Numbers of samples minus missing data (N), number of genets (Ng) and genotypic richness (Ng:N) in *Isopora brueggemanni* collected from Ashmore reef and sites throughout the inshore Kimberley coast in North West Australia.



Fig. 2 Estimates of gene diversity based on expected heterozygosity ( $\pm$  standard errors) averaged across loci at all sites for *I. brueggemanni*. Sites are colour coded with Ashmore in orange, central Kimberley in light orange, the Buccaneer Archipelago in blue, and the Dampier Peninsula sites in green. A trend line with r<sup>2</sup> = 0.213 is shown.

The results of the PCoA of *I. brueggemanni* were congruent with the STRUCTURE analysis in which the geographic structure was evident over multiple scales. At the broad scale, three separate clusters were associated with samples from Ashmore Reef, a main inshore cluster and Kooljaman, with West Montalivet samples intermediate to Ashmore and main inshore cluster (Fig. 4). Further, the PCoA that focused on the intermediate scale at the southern inshore sites corresponded closely with STRUCTURE results for K = 4 (Fig. 3) in which two clusters segregated into the Buccaneer Archipelago and Dampier Peninsula sites, but with most of the samples from Tide-Rip and Mermaid Island intermediate to the Buccaneer and Dampier clusters. Lastly, consistent fine scale patterns were identified in the PCoA and STRUCTURE analysis, with the same nine colonies from Mermaid Island\_2 exhibiting strong genetic affinities with the Pope Island corals (Fig. 3 and 5).

The AMOVA also revealed strong geographic structure across broad and intermediate scales. Large and significant variation was attributed to differences among systems ( $F_{RT} = 0.151$ , P < 0.001) and among sites within regions ( $F_{SR} = 0.092$ , P < 0.001), yielding a large overall level subdivision among all sites ( $F_{ST} = 0.230$ , P < 0.001). Average pairwise  $F_{ST}$  between Ashmore Reef and West Montalivet was 0.227, but averaged 0.450 (±SE 0.015) with all inshore reefs, and between Kooljaman and the other inshore sites was 0.241 (±SE 0.019) (Appendix 2; Table A1).



Fig. 3 Barplots of membership coefficients of individual corals from *I. brueggemanni* to different clusters calculated in STRUCTURE with the LOCPRIOR model for K = 2 to 4. These are the major mode plot produced by CLUMPAK calculated from 20/20 runs for K = 2 to 5, and 17/20 runs for K = 6. Similarity score = 0.999 and mean (LnProb) = -710322.065 for K = 2, similarity score = 0.991 and mean (LnProb) = -694772.740 for K = 3, similarity score = 0.985 and mean (LnProb) = -692167.990 for K = 4.



Fig. 4 Principal Coordinates Analysis (PCoA) calculated from individual pairwise genotypic distance of the entire *I. brueggemanni* collection. Individuals are colour coded according to location, with Ashmore Reef represented by diamonds, central Kimberley by squares, the Buccaneer Archipelago by circles and Dampier Peninsula by triangles. Percentage of variation explained by each axis is given in brackets.



Fig. 5 Principal Coordinates Analysis (PCoA) calculated from individual pairwise genotypic distance of *I. brueggemanni* samples collected from the Dampier Peninsula and the Buccaneer Archipelago. Individuals are colour coded according to location, and the Buccaneer Archipelago samples are represented by circles and Dampier Peninsula samples by triangles. Note that Kooljaman was excluded to elucidate local scale patterns of genetic structure. Percentage of variation explained by each axis is given in brackets.

## 3.3 Genetic structure within reefs in I. brueggemanni

Results from the analysis that focused on the patterns of genetic structure within the Dampier Peninsula and Buccaneer Archipelago showed significant structure over fine scales. The spatial autocorrelation analysis yielded a large and significant autocorrelation coefficient ( $r \sim 0.15$ ) and relatively constant up to distances of 500 m, and then decreased with distance (Fig. 6). This distance indicates the extent of the genetic patch in which the homogenising influence of localised recruitment on relatedness among individuals first becomes limited. The autocorrelation coefficient crossed the x-axis and becomes negative at just over 20 km, which is the distance at which the random effects of genetic drift, not gene flow, drive genetic relatedness. Results of the AMOVA of the three sites that included replicate subsites that were separated by over 20 km showed that the majority of variation was altributed to significant subdivision among sites ( $F_{RT_SITES} = 0.085$ , P  $\leq 0.001$ ), but significant subdivision was also detected between subsites within sites ( $F_{SR_SUBSITES} = 0.010$ , P  $\leq 0.01$ ) over distances of about 500m. Pairwise  $F_{ST}$  comparisons indicated the significant subdivision between sub-sites was driven by Pope Island ( $F_{ST} = 0.013$ , P  $\leq 0.030$ ) and Mermaid Island ( $F_{ST} = 0.014$ , P  $\leq 0.020$ ), but not Bathurst West ( $F_{ST} = 0.002$ , P  $\leq 0.226$ ).



Fig. 6 Spatial autocorrelation analyses of the genetic correlation coefficient (*r*) as a function of distance for the *l. brueggemanni* corals sampled from the Dampier Peninsula and the Buccaneer Archipelago. The bootstrapped 95% confidence intervals were generated by 1000 bootstrap trials.

# 4 Results: Acropora aspera

#### 4.1 Clonality, gene diversity and major genetic lineages in the entire A. aspera collection

All technical replicates from the entire *Acropora aspera* collection exhibited a hamming distance of  $\leq 0.005$ , and therefore individuals with a hamming distance of less than this were deemed clones (ramets), and resulted in the removal of about one third (n = 207) of these samples. All ramets of each genotype were collected at the same site, the majority of which were sampled within ten metres of each other. Genotypes and summary statistics of the entire *Acropora aspera* collection are given digital data repository (http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7).

The results of the cluster analysis of the remaining 349 genets revealed major genetic lineages that were living side by side and only weakly associated with geography. Using the Evanno et al. (2005) method indicated that K = 3 was the most appropriate number of discrete populations for this data, but there was also evidence (i.e. high  $\Delta$ K) for four clusters at K= 4 (Appendix 3 Fig A3). Moreover, at K = 3, the barplots indicated a fourth group with intermediate ancestry (membership coefficients, q ~ 50% to the blue and green clusters) while at K = 4, this group segregated into a separate cluster with high membership coefficients (q > 0.90; Fig. 7), suggesting this is the most appropriate K. Congruently, the results of the PCoA revealed four distinct genetic clusters within the entire collection of *A. aspera* (Fig. 8). Some geographic pattern in the distribution of the clusters was apparent with the mainland sites on the Dampier Peninsula of Noyon and Ardinoogoon dominated by *Acropora* asp-b, the island sites of the Buccaneer Archipelago dominated by *Acropora* asp-c and the central Kimberley sites of White and Condilac Islands dominated by *Acropora* asp-d (Fig. 7). However at almost all sites, multiple clusters occur at the same site; for example, all four clusters occur at the central Kimberley site of Condilac Island, while at Ashmore Reef, the three clusters of asp-a, asp-b and asp-c all co-occur (Fig. 7).



Fig. 7. Barplot of membership coefficients of individual corals from the entire *Acropora aspera* collection calculated in STRUCTURE v2.3 with no prior information for K = 3 and 4. Orange denotes membership to asp-a, purple to asp-b, blue to Acropora asp-c and green to asp-d (four K = 4). CLUMPAK calculated both plots from 20/20 runs and a similarity score = 0.999 and mean (LnProb) = -62402.450 for K = 3, and a similarity score = 0.999 and mean (LnProb) = -71462.630 for K = 4. For comparison at other K's, barplots for K = 2 to 8 are given in Appendix 3 Fig. A4.



Fig. 8 Principal Coordinates Analysis (PCoA) calculated from individual pairwise genotypic distance of the entire *Acropora aspera* collection. Individuals are colour coded according to the clusters assigned by the STRUCTURE analysis. Percentage of variation explained by each axis is given in brackets.

The magnitude of this differentiation among the four *A. aspera* clusters was very large (overall  $F_{ST} = 0.587$ ), with pairwise  $F_{ST}$  ranging from 0.469 to 0.705 (Table 2). Morphological assessments in the field, along with preliminary macro-morphological assessments of skeletal material and photos, indicate that variation in macro-morphology between lineages is similar to variation within lineages (see Fig. 9). We conclude that *A. aspera* in the Kimberley represents a cryptic species complex which comprises four genetic lineages that are reproductively isolated and living in sympatry.

Table 2 Pairwise values of  $F_{ST}$  among clusters of corals identified from the entire Acropora aspera collection with STRUCTURE and PCoA. Values above diagonal are P-values from 999 permutations.

	asp-a	asp-b	asp-c	asp-d
asp-a	0.000	0.001	0.001	0.001
asp-b	0.643	0.000	0.001	0.001
asp-c	0.705	0.498	0.000	0.001
asp-d	0.643	0.478	0.469	0.000



Fig. 9 Colonies belonging to each of the four A. aspera genetic lineages. A. Acropora asp-a collected from Ardinoogoon (Shenton Bluff); B. Acropora aspa collected from Aloon (Jackson Island); C. Acropora asp-b collected from Noyon; D. Acropora asp-b collected form Ardinoogoon (Shenton Bluff); E. Acropora asp-c collected from Janinko (Sunday Island); F. Acropora asp-c collected from Mermaid Island; G. Acropora asp-d collected from Janinko (Sunday Island); H. Acropora aspd collected form Aloon (Jackson Island).

Gene diversity, as measured by unbiased expected heterozygosity, varied considerably among the lineages and was highest in *Acropora* asp-a, and lowest in *Acropora* asp-d (Fig. 10). Further, 16 loci were fixed among the different lineages, lending support to species-level, or evolutionary significant, divergence among lineages.

Consequently, heterozygosities were zero and fixation indices could not be calculated at these loci (<u>http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7</u>).



Fig. 10 Estimates of gene diversity of the four *Acropora aspera* clusters based on expected heterozygosity (± standard errors).

# 4.2 Clonality and gene diversity in Acropora asp-c

Of the 322 ramets that belonged to the Acropora asp-c lineage, 145 were identified as clones and excluded from further population-level analysis, leaving a total of 177 genets belonging to the Acropora asp-c lineage. Genotypic richness was relatively low, averaging 0.61 across all sites but varied from 0.25 to 1.00 (Table 3). Clones were particularly abundant at White Island, Bathurst N Sat, Bathurst E Sat, Bowles Rock and Pope Island where 60% - 70% of all samples were clones (Table 3). At Bowles Rock and Pope Island, there were lots of clones that represented a few individuals, while the collection at Bathurst N sat was dominated by one common clone. Average observed heterozygosity across loci was 0.205, average expected heterozygosity was 0.249, and average  $F_{\rm IS}$  was 0.115 suggesting a general deficiency in heterozygotes that would be expected under Hardy Weinberg equilibrium (genotypes and summary statistics of the Acropora asp-c collection are given digital data repository (http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7). Gene diversity measured by expected heterozygosity was higher in the center of the sampling area at the Buccaneer Archipelago sites compared with Dampier Peninsula sites (Janinko and Aloon) and the Central Kimberley sites (Condillac and White Islands; Fig. 11), and was very low among the Ashmore Reef samples ( $H_E$  = 0.045). The low diversity at Ashmore Reef created a general trend of increasing gene diversity with decreasing latitude, but the low sample size at Ashmore (n = 5) along with a low correlation coefficient ( $r^2 = 0.126$ ) indicate this trend is well supported.

Table 3 Numbers of samples in entire collection of *Acropora aspera* less those excluded due to missing data [*N*(all)], total number of samples identified as Acropora asp-c [*N*(*Acropora* asp-c)], number of genets of *Acropora* asp-c [*N*g(*Acropora* asp-c)] onliceted from sites in the Kimberley coast and at Ashmore Reef in North West Australia.

Region	Site	N (all)	N (asp-c)	<i>Ng (</i> asp-c)	Ng:N(asp-c)
Ashmore	Ashmore_Reef	34	7	5	0.71
Central Kimberley	Condillac_I.	32	10	10	1.00
	White_I.	29	20	8	0.40
Buccaneer Archipelago	Bathurst_N_Sat	27	12	3	0.25
	Bathurst_E_Sat	30	30	12	0.40
	Bowles_Rock	30	30	10	0.33
	Barret_Rock	31	30	19	0.63
	Asshlyn_I.	61	58	36	0.62
	Pope_I.	30	30	10	0.33
	Tide_Rip	31	27	20	0.74
	Mermaid_I.	30	29	15	0.52
Dampier Peninsula	Janinko	31	28	18	0.64
	Ngoorroodool	32	2	2	1.00
	Aloon	24	9	9	1.00
	Noyon	28	0	-	-
	Ardinoogoon	83	0	-	-
	TOTAL	563	322	177	0.61



Fig. 11 Estimates of gene diversity at sites of the *Acropora* asp-c lineage based on expected heterozygosity ( $\pm$  standard errors). Sites are colour coded with Ashmore in orange, central Kimberley in green, the Buccaneer Archipelago in blue, and the Dampier Peninsula sites in purple. A trend line with r<sup>2</sup> = 0.126 is shown.

#### 4.3 Genetic structure among regions, systems and reefs in Acropora asp-c

In contrast to cluster analysis of the entire Acropora aspera collection, results of the STRUCTURE and PCoA analysis within Acropora asp-c lineage identified four genetic clusters that corresponded to geographic location and thus provided evidence that this subsample is one interbreeding metapopulation. The Evanno et al. (2005) indicated that K = 4 is most appropriate number of discrete populations (Appendix 3, Fig. A5) with membership coefficients greater than 90% to one of the four clusters (Fig. 12, for barplots at K=2 to 8 see Appendix 3, Fig. A6). The four separate groups were associated with Ashmore Reef, the central Kimberley sites (Condillac and White Island), the Buccaneer Archipelago (Bathurst E Sat, Bathurst N Sat, Bowles Reef, Barret Rock, Asshlyn Islands and Pope Island), and the Dampier Peninsula (Janinko, Ngooroodool and Aloon). The sites of Tide-Rip and Mermaid Island were admixed between the Buccaneer and Dampier clusters; half of the colonies from these two sites had strong affinities with Buccaneer cluster (the majority q > 85%), while the other half exhibited evidence of mixed ancestry with q's between 50 and 65% to either cluster (Fig. 12). This geographic structuring into four major clusters is also obvious in the PCoA, with Tide-Rip and Mermaid Islands formed a fifth group intermediate to the Dampier and Buccaneer clusters (Fig. 13). Analysis of only the inshore Kimberley populations revealed identical patterns to the analysis of the entire collection, showing that the divergent Ashmore Reef samples did not influence clustering resolution in the STRUCTURE analysis (data not shown). However, in the PCoA, the clustering of three genetic groups was more distinct compared with the analysis that included Ashmore Reef, corresponding to the central Kimberley, Buccaneer Archipelago and Dampier Peninsula, with sites of Tide-Rip and Mermaid Island intermediate to Buccaneer Archipelago and Dampier Peninsula (Fig. 14).

The AMOVA showed that the majority of the geographic variation was attributed to differences among systems ( $F_{RT} = 0.094$ , P < 0.001), with small but significant differences among sites within systems ( $F_{SR} = 0.008$ , P < 0.05), revealing a moderate but highly significant level subdivision among all sites ( $F_{ST} = 0.101$ , P < 0.001) (Table 4). Large differences were detected between Ashmore Reef and all the other sites, with average pairwise  $F_{ST}$  of 0.380 (± 0.011) (Appendix 3; Table A1). Therefore, levels of subdivision were weaker when Ashmore and other sites with sample sizes <10 were excluded in the AMOVA, but general patterns and significance were the same (Table 4).



Fig. 12 Barplots of membership coefficients of individual corals from the *Acropora* asp-c lineage calculated in STRUCTURE v2.3 with the LCOPRIOR model for K = 4. This is the major mode plot produced by CLUMPAK calculated from 12/20 runs and a similarity score = 0.986, and a mean (LnProb) = -423687.042. The minor mode plot was almost identical.



Fig. 13 Principal Coordinates Analysis (PCoA) calculated from individual pairwise genotypic distance of corals from the *Acropora* asp-c lineage. Percentage of variation explained by each axis is given in brackets.

Table	4 Results of AMOVA	that partitioned	genetic variation	among systems,	among sites	within systems	and among all
sites.	Analysis involved all sit	tes, and only tho	se sites with $n \ge 9$	. All estimates of	differentiatior	n were significan	t at P < 0.05.

	all sites	sites (n≥9)
F <sub>RT</sub> : among systems	0.094	0.050
F <sub>SR</sub> : within systems	0.008	0.006
F <sub>ST</sub> : among all sites	0.101	0.056



Fig. 14 Principal Coordinates Analysis (PCoA) calculated from individual pairwise genotypic distance of corals from the *Acropora* asp-c lineage from the inshore Kimberley only (i.e. Ashmore Reef samples are excluded). Percentage of variation explained by each axis is given in brackets.

#### 4.4 Genetic structure within-reefs in Acropora asp-c

Within *Acropora* asp-c, there was evidence of spatial autocorrelation, with small but significant positive correlation coefficient that remained relatively constant up to 500 m, and then dropped away after this initial plateau (Fig. 15). This distance indicates the extent of the genetic patch and the spatial limit of completely mixed genotypes. Additionally, *r* first crossed the x-intercept at 35 km, revealing the distance at which the random effects of genetic drift, not the homogenising influence of gene flow, drive genetic relatedness.



Fig. 15 Spatial autocorrelation analyses of the genetic correlation coefficient (r) as a function of distance for the *Acropora* asp-c lineage at the Dampier Peninsula and the Buccaneer Archipelago. The bootstrapped 95% confidence error bars generated via 1000 bootstrap trials are shown.

# 5 Results: Oceanographic modelling and tests for isolation by distance and oceanographic resistance

The mantel tests for correlation between genetic distance, geographic distance, and oceanographic resistance showed that geographic distance explained more (for *I. brueggemanni* R = 0.634, for *Acropora* asp-c R = 0.681) of the genetic structure among sites from the Buccaneer Archipelago and Dampier Peninsula than the oceanographic model (for *I. brueggemanni* R = 0.460, for *Acropora* asp-c R = 0.477; Table 5). However, there was also a strong correlation between oceanic resistance and geographic distance (for *I. brueggemanni* R = 0. 720, for *Acropora* asp-c R = 0.517). The outputs of the oceanographic model that was run for eight or 40 days in the different austral seasons showed a lack of cross shelf connectivity of passive particles between the offshore and inshore reefs in any season (Appendix 4 Fig. A1 and Fig. A2).

Table 5 Results of mantel test for correlation between genetic distance (pairwise *F*<sub>ST</sub>) between sites in the Buccaneer Archipelago and Dampier Peninsula, geographic distance (GGD) and oceanographic resistance (OR) in *I. brueggemanni* and *Acropora* asp-c. The outlying site of Kooljaman was not included in the analysis.

	OR vs FST		GGD vs FST		OR VS GGD	
	R	Р	R	Р	R	Р
I. brueggemanni	0.460	0.001	0.634	0.001	0.720	0.001
Acropora asp-c	0.477	0.001	0.681	0.001	0.517	0.001

# 6 Discussion

This first-ever population genomic study of connectivity in hard corals of the inshore Kimberley shows that most recruits to populations of both the brooding coral (*Isopora brueggemanni*) and broadcast spawning coral (*Acropora aspera*) originate from their natal reef or reef patch, with few larvae dispersing and recruiting successfully between reefs over distances of more than 35 kilometres. Further, cross-shelf connectivity between the offshore and inshore reefs appears to be negligible, even over multiple generations. Finally, in the *A. aspera* collection, we identified four distinct genetic lineages that are morphologically cryptic, but reproductively isolated despite coexisting on the same reefs.

# 6.1 Patterns of ecological connectivity

Over the broadest, inter-regional, scale of the study (10s – 100s of kilometres), the greatest level of divergence among populations of *I. brueggemanni* and the major *A. aspera* lineage (*Acropora* asp-c) was detected between offshore reef at Ashmore Reef and the inshore reefs of the Kimberley. These results indicate that cross shelf connectivity for both the brooding and spawning coral occurs rarely, even over evolutionary time scales. This conclusion is well supported by the oceanographic results which exhibited an absence of cross shelf connectivity even when the model was run over the upper competency of corals of 40 days.

There was only one exception to this regional scale divergence; the cluster analysis revealed that *l.* brueggemanni corals from the most northern of our inshore Kimberley sites - West Montalivet Island in the Bonaparte Archipelago, exhibited genetic affinities with Ashmore Reef. However, several lines of indirect evidence indicate that this relationship most likely reflects the limited sampling in the northern and central Kimberley in which unsampled "ghost" populations may have created the appearance of cross shelf migration between Ashmore and West Montalivet but they may not actually exchange migrants (Slatkin 2005). First, genetic differentiation between Ashmore Reef and West Montalivet as measured by  $F_{ST}$  was large ( $F_{ST} = 0.227$ ). Second, gene diversity was very high at West Montalivet, suggesting that this site is part of a large population that has been well connected to exogenous sources of genetic variation. Third, in the STRUCTURE analysis for K > 6, *I. brueggemanni* corals from West Montalivet formed a coherent cluster with high membership coefficients that was separate from corals at Ashmore Reef. Thus, we conclude that *I. brueggemanni* corals on the offshore and inshore reefs are separate evolutionary significant units (*sensu* Moritz 2002), while also recognising the need for future research that targets sites in the central Kimberley to clarify origins of the striking genetic diversity at West Montalivet.

At the intermediate, inter-reef scale of the study (kilometres – 10s of kilometres), there was a clear association between genotype and geography for both corals, with three distinct genetic groups identified in the inshore systems of the Central Kimberley, the Buccaneer Archipelago, and the Dampier Peninsula. The pattern of divergence of these two latter systems in the southern Kimberley identified the Sunday Strait as a semipermeable barrier to gene flow between the Buccaneer and Dampier reefs, a pattern that was common to both species. The Sunday Strait is a relatively deep water channel, through which majority of water that fills and drains King Sound funnels at extreme velocities. Putative restrictions to dispersal of brooded and broadcast spawned larvae between of the Buccaneer and Dampier systems across this barrier explains the genetic divergence between the two systems, with occasional dispersal across the barrier via the stepping stones of Tide Rip and Mermaid Islands likely facilitating the evolutionary important exchange of genes. In addition, within the Dampier peninsula, I. brueggemanni corals from the mainland site of Kooljaman in the far west of the sample area were very divergent from the other inshore Kimberley corals and was characterised by the lowest gene diversity of all sites, suggesting that it is a small and isolated population that may well be end of its range at least within the Kimberley region. This explanation is consistent with observations in the field; we did not find populations of *I. brueggemanni* along the west coast of the Dampier Peninsula further south than Kooljaman, which is the southernmost extent of the Kimberley. These results indicate that the population of Kooljaman may be reliant on its own genetic variation to adapt to environmental change in coming decades.

At the fine, within-reef scale of the study (100s metres), colonies of both species within 500 m of each other are genetically more related than colonies further apart, indicating the general size of the genetic patch. Congruently, significant differentiation was detected in *I. brueggemanni* between subsites separated by 500 metres, although the magnitude of differentiation was relatively small compared with that among sites. More importantly, colonies separated by more than 20 km for *I. brueggemanni* and 35 km for *A. aspera* were not positively related, revealing the distance where the random effects of genetic drift, not homogenising influence of gene flow mediated by dispersal, are the primary determinants of genetic composition and thus providing inference of the general scale of demographic independence.

Finally, geographic distance was a better predictor of genetic structure for both corals than the oceanographic model, indicating the simple pattern of isolation by distance and a need for more biologically relevant and finer scale modelling. Outputs of the model that was run over eight days also show that there is no distinct directional current in operation along the inshore Kimberley, but large complex tidal flows and wind driven currents prone to reversals and multidirectionality are the primary drivers of connectivity for species with pelagic larvae in this region.

## 6.2 Patterns of clonality and genetic diversity

The results presented here indicate that in the branching coral *Acropora* asp-c, clonal propagation is an important mode of reproduction at some sites in the Kimberley. In particular, genotypic richness was particularly low at the four sites in the Buccaneer Archipelago (Bathurst\_N\_Sat, Bathurst\_E\_Sat, Bowles\_Rock and Pope\_Is) as well as at White Island. This conclusion is congruent with other work in NWA, that indicates the establishment of vegetative fragments that aid recovery after tropical storms on the inshore reefs with shallow depth gradients is common (Underwood 2009). The relatively high levels of clonality were not associated with reduced gene diversity, with high expected heterozygosity detected at all these sites (Fig. 11). Therefore, even for those reefs where vegetative fragmentation dominates, sexual reproduction continues to be important for maintenance of genetic variation. In contrast, vegetative fragmentation appears to be much less common in the more robust branching growth form of I. *brueggemanni*, with high genotypic richness at all the sites except one, suggesting that sexually produced larvae dominate reproduction in these populations.

The presence of a either inter or intra-specific declining diversity gradient from north to south along the North West Shelf is often alluded to, but empirical supporting data has been lacking (Wilson 2013). Our results suggest such a pattern does exist for the brooding coral, but sampling further south in the Pilbara is required to verify whether the regional scale pattern holds over broader scales. In contrast, for the spawner, there was little evidence of a diversity gradient in the inshore Kimberley, suggesting that the diversification of the Acropora aspera species in the Kimberley has had a dominant influence on distribution of genetic diversity of the Acropora asp-c lineage. In particular, our results indicate that at the regional scale, the centre of genetic diversity within Acropora asp-c is in Buccaneer Archipelago in the Kimberley, with expected heterozygosity attenuating to the west and east from this centre. Furthermore, Acropora asp-c was rare or non-existent in our collections from the Dampier Peninsula, and much less abundant in the central Kimberley and Ashmore Reef collections. Therefore, the largest and most well connected populations of this lineage seem to occur in the central Buccaneer Archipelago. However, the sampling scale of this study needs to be extended to the central and northern Kimberley, the offshore atolls and the Pilbara to test these hypotheses, along with a consideration of distribution of diversity of the other Acropora aspera lineages. Nevertheless, results from our oceanographic model support the prediction that, in contrast to the offshore reefs on the shelf margin, there is no distinct north south current along the inner shelf, but complex tidal flows and wind driven currents prone to reversals are the primary drivers of connectivity for species with pelagic larvae.

#### 6.3 Cryptic diversity in Acropora aspera

In the marine realm, it is estimated that tens of thousands of cryptic species are undescribed (Appeltans et al. 2012), and such cryptic diversity appears to be particularly prevalent among coral reef taxa (Rocha et al. 2007). Here, the detection of four distinct genetic lineages that are morphologically cryptic is consistent with numerous genetic studies in hard corals throughout the world (Wallace and Willis 1994, Miller and Benzie 1997, van Oppen et al. 2001, Willis et al. 2006, Ladner and Palumbi 2012, Pinzon et al. 2013, Prada and Hellberg 2013, Schmidt-Roach et al. 2014, Combosch and Vollmer 2015, Ohki et al. 2015, Warner et al. 2015), and particularly in NWA (Richards et al. 2013, Rosser 2015, Gilmour et al. 2016b, Richards et al. 2016, Rosser 2016). The delimitation of four clusters by 100% agreement with two alternative methods (PCoA and Bayesian clustering) with large fixation indices greater than 0.469 show that these corals living in sympatry represent unique evolutionary significant units. However, morphological assessments in the field, along with preliminary macro-morphological assessments of skeletal material and photos, indicate that no clear macro-morphological differences exist among the lineages (see Fig. 9). This pattern contrasts to that observed on the Great Barrier Reef in Eastern Australia of where A. aspera was the only morphospecies among five sister species which was genetically distinct (Van Oppen et al. 2002). However, Van Oppen et al. (2002) also showed evidence of successful hybridization between A. aspera and one of these sister species and it thus appears that reproductive barriers in this species group are semi-permeable, the strength of which varies between species and over time. A major revision of the taxonomic status of Acropora aspera is thus warranted.

Coral reefs of the Kimberley are recent phenomena: during the low sea levels of the last interglacial period the coastline occurred along the continental shelf margin many hundreds of kilometres to the north and west of today's coast line (Wilson 2013), with the formation of coral reefs in the inshore Kimberley commencing only 8,000 years ago (Solihuddin et al. 2016). Indeed, such transgressions and regressions have occurred many times during the late quaternary due to eustatic sea level change, and these processes likely underlie the current divergence of *Acropora aspera* lineages. Further, the patchy distribution and habitat heterogeneity of present day reefs provide fertile conditions for strong local selection, and thus processes of ecological speciation appear to have played an important role along with the vicariant processes and founder effects in the evolution of corals in this region. One documented mechanism that appears to be central to the diversification of sympatric broadcast spawners in NWA involves differences in timing of spawning, with direct evidence that distinct genetic lineages within morphologically cryptic *Acropora* "species" exist between corals that spawn in Spring and Autumn (Rosser 2015, Gilmour et al. 2016b, Rosser 2016). Such temporal reproductive barriers are the most likely explanation for diversification of the *A. aspera* lineages detected here.

The discovery of cryptic diversity has important implications for management of coral reefs in the region that concern estimates of biodiversity and effective population size. The most concerted biodiversity study based on morphological identifications of museum-registered specimens published so far for the Kimberley coast reported seven new coral records for WA (Richards et al. 2015). Additionally, many coral species that had previously been recorded in clear offshore habitats were found to occur on inshore reefs, and 34 species were found in the intertidal zone that have only been recorded to occur in the subtidal zone. Richards et al. (2015) undertook their study in a relatively small sample area in the Bonaparte Archipelago, central Kimberley, in tandem with an analysis of historical specimen-based records in Australian Museums (Richards et al. 2014), and show a total of 338 species of coral have been recorded from the Kimberley. If cryptic speciation is common throughout the Kimberley, then this estimate of coral biodiversity may be a substantial underestimate. Additionally, given these A. aspera lineages are likely reproductively isolated, the effective population size of each is much smaller than expected, which has implications for their ecological and evolutionary capacity to recover after disturbance. In particular, lineages may have different susceptibility to environmental change, such as fluctuations in water temperature, acidity, or incidence of disease (Hoegh-Guldberg and Bruno 2010, Hughes et al. 2010), and with a smaller effective population likely have less standing genetic variation to draw on for adaptation. Further, the demographic consequences of a higher susceptibility to disturbances, whether lethal or sublethal, are likely to be reduced reproductive output and recruitment (Oliver and Babcock 1992,

Hughes et al. 2000, Levitan et al. 2004) and therefore, a slower rate of recovery from disturbance. In the worst instances, recovery from severe disturbances would be severely compromised if reproductive isolation was further compounded by Allee effects (Knowlton 2001). Further integrated taxonomic study that includes micro-morphological examination of the *Acropora aspera* lineages in tandem with investigations of reproductive biology is required to resolve species boundaries within the Kimberley *A. aspera* complex.

## 6.4 Conclusions

This study utilised thousands of genome-wide SNPS to reveal that populations of the brooding coral I. brueggemanni and the broadcast spawning coral A. aspera are characterised by strong geographic structure over multiple scales in NWA. For the brooder, collections within the inshore Kimberley comprised one species, with no evidence of cryptic diversity. Thus, the brooding mode of reproduction in this species appears to maintain abundant populations by local recruitment over scales of a few hundred metres, with occasional longer distance dispersal over scales of few tens of kilometres that prevents inter-specific genetic divergence. In contrast, the morphospecies A. aspera comprises several discrete lineages in NWA that not only occur in sympatry but also exhibit genetic affinities across geographically distant sites. The implication is that reproductive barriers exist between lineages in the broadcast spawner, most likely through a combination of allopatric speciation followed by reconnection during sea level change, in conjunction with ecological divergence through localised adaptation to heterogeneous environments and reproductive isolation through difference in the timing of spawning. Consistent with a greater propensity for widespread dispersal in spawning corals, the level of genetic subdivision within the Acropora asp-c lineage ( $F_{ST} = 0.101$ ) was half that of the brooder I. brueggemanni ( $F_{ST}$  = 0.230). However, the general patterns of ecological connectivity were remarkably similar between the two corals; migration is rare among reefs that are separated by more than a few tens of kilometres, the Sunday Strait appears to be a semi-permeable barrier in which Tide Rip and Mermaid Islands provide stepping stone connections between the Dampier Peninsula and Buccaneer Archipelago, and the offshore populations of Ashmore Reef are separate evolutionary significant units from those of the inshore reefs of the Kimberley. These common findings among two species with different reproductive modes suggest our conclusions maybe applicable to many hard corals in the region, and have important implications for spatial management strategies aimed at maximising the resilience of these ecosystems to climate change and other human induced disturbances.

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# 9 Data Availability

Data associated with this research is available on the AIMS Data Access Portal at <u>http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-</u> 22b4240435a7

# **10** Appendices

#### Appendix 1 General Diversity Array Technologies SNP development protocol.

Genome-wide single nucleotide polymorphism (SNP) data were generated at Diversity Arrays Technology (DArT) with DArTseq methodology using the next generation sequencing platform. DArTseq represents a new implementation of sequencing of complexity reduced representations (Altshuler et al. 2000) and recent applications of this concept using the next generation sequencing platforms (Elshire et al. 2011). Detailed protocols are provided in Kilian et al. (2012), and examples of recent applications are Cruz et al. (2013) and Raman et al. (2014). The method is conceptually similar to RAD-seq methods (Baird et al. 2008), but in comparison, because generation of restriction fragments with appropriate adapters is more straightforward during the complexity reduction stage, there is a high degree of qualitative and quantitative reproducibility in sampling genomic fragments. A subsample of six to eight individuals from 12 sites (n = 94) spread across the entire sample area (to avoid ascertainment bias) was used to optimise the DArTseq methodology. Four methods of complexity reduction were tested in corals (data not presented) to select the most appropriate method based on both the size of the representation and the fraction of a genome selected for assays, and the PstI-Hpall method was selected. DNA samples were processed in digestion/ligation reactions principally as per Kilian et al (2012) but replacing a single Pstl-compatible adaptor with two different adaptors corresponding to two different Restriction Enzyme (RE) overhangs. The PstI-compatible adapter was designed to include Illumina flowcell attachment sequence, sequencing primer sequence and "staggered", varying length barcode region, similar to that reported by Elshire et al. (2011). Reverse adapter contained flowcell attachment region and Hpall-compatible overhang sequence.

Only "mixed fragments" (PstI-HpaII) are effectively amplified in 30 rounds of PCR using the following reaction conditions: PCR conditions consisted of an initial denaturation at 94 °C for 1 min followed by 30 cycles of 94 °C for 20 sec, 58 °C for 30 sec and 72 °C for 45 sec, with a final extension step at 72 °C for 7 min. After PCR, equimolar amounts of amplification products from each sample of the 96-well microtiter plate were bulked and applied to c-Bot (Illumina) bridge PCR followed by sequencing on Illumina Hiseq2500. The sequencing (single read) was run for 77 cycles.

Once optimised, the above method was used to generate sequences from the entire collection, and then processed using proprietary DArT analytical pipelines. In the primary pipeline the FASTQ files are first processed to filter away poor quality sequences, applying more stringent selection criteria to the barcode region compared to the rest of the sequence. In that way the assignments of the sequences to specific samples carried in the "barcode split" step are very reliable. Approximately 2,500,000 (+/- 7%) sequences per barcode/sample were used in marker calling. Finally, identical sequences were collapsed into "fastqcall files", which were groomed using DArT's proprietary algorithm that corrects low quality bases from singleton tags using collapsed tags with multiple members as a template. These files are used in the secondary pipeline for DArT PL's proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14). All tags from all libraries were clustered using DArT PL's C++ algorithm at the threshold distance of 3, followed by parsing of the clusters into separate SNP loci using a range of technical parameters, especially the balance of read counts for the allelic pairs. Additional selection criteria were added to the algorithm based on analysis of approximately 1,000 controlled cross populations. Testing for Mendelian distribution of alleles in these populations facilitated selection of technical parameters discriminating true allelic variants from paralogous sequences.





APPENDIX 2 Fig. A1 Histograms of descriptive statistics of 23, 165 SNPS for the entire *Isopora brueggemanni* data set showing reproducibility (A), call rate (B), coverage (C), SNP allele frequency (D) and heterozygosity (E), and summary of numbers of loci remaining after each filter was applied.



APPENDIX 2 Fig. A2 Plot of  $\Delta K$  for increasing K from STRUCTURE analyses of the entire *Isopora brueggemanni* collection without any prior information and run with correlated allele frequency model.



Appendix 2 Fig. A3 Barplots from STRUCTURE analysis using the LOCPRIOR model showing membership coefficients for K = 2 to 10 of the entire *Isopora brueggemanni* collection. Major modes calculated in CLUMPAK are presented.

Appendix 2 Table A1 Pairwise F <sub>ST</sub> estimates between sites for <i>I. brueggemanni</i> in the Kimberley below diagonal, and P-	
values significance based on 999 permutations are shown above diagonal.	

	Ashmore	West_Montalivet	Hedley_Is	Irvine_Is	Bathhurst_W	Longitude_Is	Frazer_ls	Barret_Rock	Asshlyn_Is	Pope_ls	Tide_Rip_Is	Mermaid_Is	Janinko	Ngoorroodool	Jalan	Noyon	Ardinoogoon	Kooljaman
Ashmore	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
West_Montalivet	0.227	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Hedley_Is	0.459	0.285	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Irvine_ls	0.448	0.284	0.135	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Bathhurst_W	0.472	0.319	0.148	0.046	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Longitude_Is	0.451	0.287	0.131	0.058	0.064	0.000	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Frazer_Is	0.451	0.287	0.115	0.049	0.054	0.025	0.000	0.049	0.008	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Barret_Rock	0.449	0.288	0.114	0.054	0.062	0.035	0.009	0.000	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Asshlyn_Is	0.460	0.298	0.126	0.064	0.068	0.043	0.020	0.022	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Pope_Is	0.436	0.277	0.152	0.075	0.103	0.061	0.058	0.054	0.074	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Tide_Rip_Is	0.472	0.314	0.140	0.077	0.072	0.054	0.035	0.037	0.034	0.096	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Mermaid_Is	0.457	0.307	0.148	0.083	0.076	0.048	0.050	0.051	0.057	0.090	0.046	0.000	0.001	0.001	0.001	0.001	0.001	0.001
Janinko	0.459	0.299	0.155	0.081	0.082	0.066	0.057	0.060	0.069	0.099	0.062	0.042	0.000	0.025	0.003	0.001	0.001	0.001
Ngoorroodool	0.470	0.310	0.183	0.105	0.100	0.084	0.083	0.086	0.092	0.124	0.080	0.049	0.017	0.000	0.002	0.001	0.001	0.001
Jalan	0.448	0.292	0.154	0.087	0.101	0.072	0.064	0.063	0.075	0.087	0.076	0.059	0.028	0.041	0.000	0.001	0.001	0.001
Noyon	0.478	0.325	0.193	0.124	0.126	0.105	0.103	0.102	0.107	0.141	0.106	0.078	0.051	0.046	0.061	0.000	0.001	0.001
Ardinoogoon	0.475	0.317	0.180	0.104	0.115	0.101	0.085	0.089	0.098	0.117	0.099	0.089	0.066	0.082	0.080	0.103	0.000	0.001
Kooljaman	0.524	0.387	0.304	0.249	0.254	0.236	0.223	0.226	0.231	0.234	0.242	0.228	0.220	0.245	0.212	0.260	0.242	0.000





APPENDIX 3 Fig. A1 Histograms of descriptive statistics of 34, 304 SNPS for the entire *Acropora aspera* data set showing reproducibility (A), call rate (B), coverage (C), SNP allele frequency (D) and heterozygosity (E), and summary of numbers of loci remaining after each filter was applied.



APPENDIX 3 Fig. A2 Histograms of descriptive statistics of 27, 304 SNPS for the Acropora asp-c data set showing reproducibility (A), call rate (B), coverage (C), SNP allele frequency (D) and heterozygosity (E), and summary of numbers of loci remaining after each filter was applied.



APPENDIX 3 Fig. A3 Plot of  $\Delta K$  for increasing K from STRUCTURE analyses of the entire *Acropora aspera* collection without any prior information and run with correlated allele frequency model.



Appendix 3 Fig. A4 Barplots from STRUCTURE analysis showing membership coefficients for K = 2 to 8 of the entire Acropora aspera collection. Major modes calculated in CLUMPAK are presented.



APPENDIX 3 Fig. A5 Plot of  $\Delta K$  for increasing K from STRUCTURE analyses of the Acropora asp-c lineage with prior information on sampling location and run with correlated allele frequency model.



APPENDIX 3 Fig. A6 Barplots from LOCPRIOR runs in STRUCTURE showing membership coefficients for K = 2 to 8 of colonies in the Acropora asp-c lineage. Major modes calculated in CLUMPAK are presented.

Appendix 3 Table A1 Pairwise  $F_{ST}$  estimates between sites for *Acropora* asp-c in the Kimberley below diagonal, and P-values based on 999 permutations are shown above diagonal. Sites with sample size <4 were excluded.

	Ashmore_Reef	Condilac_Is	White_Is	Bathurst_E_Sat	Bowles_Reef	Barret_Rock	Asshlyn_Is	Pope_Rock	Tide_Rip	Mermaid_Is	Janinko	Aloon
Ashmore_Reef	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Condilac_Is	0.419	0.000	0.009	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
White_Is	0.425	0.030	0.000	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.001	0.001
Bathurst_E_Sat	0.369	0.074	0.068	0.000	0.275	0.119	0.212	0.360	0.084	0.018	0.001	0.002
Bowles_Reef	0.376	0.072	0.066	0.003	0.000	0.369	0.365	0.393	0.186	0.036	0.001	0.002
Barret_Rock	0.341	0.068	0.064	0.006	0.000	0.000	0.334	0.369	0.066	0.028	0.001	0.001
Asshlyn_Is	0.321	0.067	0.065	0.003	0.000	0.000	0.000	0.342	0.048	0.013	0.001	0.001
Pope_Rock	0.377	0.068	0.060	0.001	0.000	0.000	0.000	0.000	0.209	0.047	0.002	0.001
Tide_Rip	0.349	0.064	0.065	0.009	0.004	0.007	0.007	0.004	0.000	0.230	0.002	0.003
Mermaid_Is	0.367	0.068	0.066	0.020	0.015	0.015	0.015	0.014	0.002	0.000	0.002	0.006
Janinko	0.397	0.091	0.097	0.060	0.055	0.057	0.052	0.056	0.035	0.036	0.000	0.189
Aloon	0.446	0.098	0.103	0.066	0.056	0.061	0.055	0.058	0.039	0.039	0.005	0.000





Appendix 4 Fig. A1 Particle tracks from oceanographic model run over 8 days in different austral seasons. Orange circles represent release sites, with particles released from each site designated by a unique colour. Data courtesy of Ming Feng (CSIRO; WAMSI Kimberley Project 2.2.7), and plots courtesy Dirk Slawinski (CSIRO).



Appendix 4 Fig. A2 Particle tracks from oceanographic model run over 40 days in different austral seasons. Orange circles represent release sites, with particles released from each site designated by a unique colour. Data courtesy of Ming Feng (CSIRO; WAMSI Kimberley Project 2.2.7), and plots courtesy Dirk Slawinski (CSIRO).