

**INDONESIAN GENE FLOW AND IMPLICATIONS FOR MARINE PROTECTED
AREAS**

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ABSTRACT

Indonesia is the fourth most populous country in the world, comprised of more than 17,500 islands covering approximately 5000 km in an east to west orientation. It has a complex geological history resulting in a coastline of approximately 95,000 km, the fourth largest coastline in the world and the largest of any tropical nation. The Indonesian Archipelago encompasses the majority of the coral triangle, an area known to harbor the planet's greatest shallow-water biodiversity.

The coral reef ecosystems of Indonesia are in serious decline and a lack of knowledge pertaining to genetic connectivity and gene flow has been identified by several international non-governmental organizations (NGOs) as one of the many steps that require addressing in the design of successful marine protected areas (MPAs) and conservation strategies.

I employ a genetic approach, using highly variable microsatellite loci to investigate the population structure, genetic connectivity and gene flow of several marine invertebrates and two marine angiosperms collected throughout the Indonesian Archipelago.

Several study species demonstrated evidence of population structuring throughout the archipelago and I propose that the radically different habitats found on the shallow Sunda Shelf and the deep waters of the central Indonesia, coupled with the drying of the Sunda Shelf during times of glacial maxima, and the subsequent creation of new habitat by flooding the Sunda Shelf, has played a significant role in structuring populations and accounts for the observed genetic divergence in several of the surveyed species.

This work identifies regions of unique biodiversity throughout Indonesia and allows the best available scientific knowledge pertaining to genetic connectivity in the region to be integrated into future conservation strategy and policy.

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CHAPTER 1: INTRODUCTION

Genetic connectivity and gene flow are important in maintaining viable populations of organisms. Populations that are connected with other populations and exchange genes are able to maintain or increase genetic diversity thus reducing the risk of extinction (Spielman et al. 2004).

Coral reefs occupy approximately 0.1% of the world's ocean surface, yet they contain roughly 25% of all known marine species. They are the most diverse of all marine ecosystems and are frequently described as the rainforests of the sea. Despite this recognition, coral reefs face significant conservation challenges and understanding where coral reef associated recruits are coming from and going to is becoming an increasingly pressing concern for management agencies. For example, if a patch of reef is destroyed through natural (e.g., hurricanes) or anthropogenically-induced disturbances (e.g., sedimentation or dredging) the recovery of the destroyed reef relies upon a supply of new recruits from surrounding areas. Without these new recruits destroyed reefs will not recover, and it has been suggested that an understanding of where these recruits are coming from must be addressed before successful conservation initiatives and policy can be implemented (Roberts 1997; Birrell et al. 2008).

In terrestrial environments it is relatively easy to track individuals throughout their entire lifetime, direct tagging methods such as radio collars or via unique identification tags are routinely employed to do so. In the marine environment the direct tracking of individuals is nearly impossible. Many marine organisms have life histories that include a microscopic pelagic larval dispersal phase, which can last from days to months. This larva is far too small to successfully track via direct methods and this difficulty is compounded by the length of the pelagic larval duration (PLD). PLD, generally, is species specific and can range from days to months. It is logistically infeasible to directly track planktonic larvae from the point of origin

to the point of settlement and, because of this, genetic methods have been developed in efforts to understand where larvae are going, coming from and the magnitude of larval exchange.

To address this lack of understanding of gene flow throughout Indonesia, a carefully selected suite of marine invertebrates and marine angiosperms was chosen. Criteria for inclusion was based upon the ease of identification, known abundance, ease of capture and dispersal potential. Sampling sites were chosen to maximize coverage throughout the archipelago whilst taking into account known potential barriers to gene flow (e.g., the Indonesian throughflow current and the Halmahera eddy).

This study employs highly polymorphic microsatellite loci to uncover patterns, direction and magnitude of gene flow in several common marine invertebrates and angiosperms. Areas that are connected by larval exchange should show similar allele frequencies due to the homogenizing effects of gene flow. Areas that are not connected are likely to have different allele frequencies as a result of genetic drift acting upon isolated populations. Thus, by assessing and comparing allele frequencies in two, or more, geographic locations, we can make inferences on which areas are or are not connected by larval exchange.

This study benefitted greatly from advances in DNA sequencing. Prior to these advances, microsatellite loci were commonly regarded as expensive, troublesome, time consuming and technically difficult to isolate (Selkoe & Toonen 2006). The advent of massively parallel next generation DNA sequencing allowed microsatellite libraries to be developed in this study within two weeks for approximately \$400 per library. This is in stark contrast to the often prohibitory cost and time investment required before the advent of next generation technologies, where development of microsatellite libraries and testing could take several months to years and cost several thousands of dollars.

Traditional methods of microsatellite development would often result in only small numbers of microsatellite loci leaving the researcher with few choices regarding which loci to use. The libraries developed for the work described in this study frequently contained thousands of potential microsatellite loci allowing the selection of only the best and most well behaved loci; a luxury not afforded before the advent of next generation DNA sequencing.

The purpose of this work was to identify the direction and magnitude of gene flow within the Indonesian Archipelago and identify regions of unique biodiversity with a view to incorporating this into future marine conservation initiatives.

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CHAPTER 2: Population genetic subdivision of seagrasses, *Syringodium isoetifolium* and *Thalassia hemprichii*, in the Indo-Malay archipelago

Abstract

The population genetics of two seagrasses, *Syringodium isoetifolium* and *Thalassia hemprichii* were assessed throughout the Indonesian Archipelago. I genotyped 165 *S. isoetifolium* blades at 15 microsatellite loci from 14 sampling sites and 389 *T. hemprichii* blades at 17 microsatellite loci from 16 sampling sites. Bayesian clustering methods revealed two genetically distinguishable clusters in each species, although the geographic boundaries of these clusters differed. *Syringodium isoetifolium* has a cluster that is located exclusively on the shallow Sunda Shelf and appears to follow the demarcation defined by Wallace's line. *Thalassia hemprichii*, however, has a genetically distinguishable cluster located within the Banda Sea. The Banda Sea has unique physical oceanographic conditions, which I speculate may be driving the observed differentiation in *T. hemprichii*. These patterns of differentiation illuminate the processes responsible for creating and maintaining the elevated levels of biodiversity in the region. Historical sea level changes on the Sunda Shelf, and oceanographic patterns in the Banda Sea, both contribute to population differentiation, the starting point for speciation.

Introduction

Seagrasses are important components of marine ecosystems, providing habitat, nursery grounds and food for a wide variety of organisms (Lubbers et al. 1990; Heck et al. 1995; de la Torre-Castro & Ronnback 2004). At the same time, seagrasses act as buffers at the interface between terrestrial and oceanic environments. They stabilize sediments with their dense networks of roots and rhizomes, helping to prevent the erosion and destruction of coastal environments (Barbier et al. 2011). As a buffer between the land and ocean, seagrass beds play an important role in nutrient cycling and the removal of land based nutrients and sediment inputs. The removal of these inputs is achieved through direct and indirect mechanisms, where seagrass blades decrease water flow facilitating the deposition of sediments and active filtering of suspended particulate matter by seagrass-associated suspension feeders (Gacia et al. 1999). The roots and rhizomes aid in the retention of nutrient and sediment preventing resuspension (Gacia & Duarte 2001). The removal and stabilization of these inputs results in higher clarity and nutrient poor waters that are conducive to the survival and growth of coral reefs. Despite the recognized importance of seagrass beds, they are subject to the anthropogenic pressures associated with growing coastal populations such as increased sedimentation, degraded water quality and mechanical damage to seagrass habitats (Curran et al. 2002; Short et al. 2011).

The Indonesian Archipelago contains the largest portion of the Coral Triangle, an area spanning six countries and containing the planets greatest abundance of shallow water marine biodiversity (Briggs 2000; Allen & Werner 2002; Hoeksema 2007; Allen 2008). Despite only covering just over 1.5 % of the world's total ocean area, the Coral Triangle contains more than 75 % of all known coral species, approximately 30% of the planet's coral reefs, nearly 40 % of all known fish species, and is considered to be the center of seagrass biodiversity, making the Coral Triangle a global priority for conservation (Hughes et al. 2002; Roberts et

al. 2002; Spalding et al. 2003; Briggs 2005; Short et al. 2007; Allen 2008; Veron et al. 2009). Wallacea, an area within the Indonesian portion of the Coral Triangle, has been identified as a global biodiversity hotspot (Myers et al. 2012). Wallacea is demarked in the west by the biogeographical line that was described by Wallace (1860) and dubbed 'Wallace's Line' by Huxley (1868). The eastern edge is bounded by Lydekker's line (Lydekker 1896). Wallace's line follows the eastern boundary of the Sunda Shelf while Lydekker's line follows the western boundary of the Sahul Shelf (Figure 1). Wallacea includes deep water channels, and this region formed the only connection between the tropical Pacific and Indian Ocean waters during periods of glacial maxima (Voris 2000). It is likely that Wallacea acted as a refuge permitting the persistence of species when the Sunda and Sahul Shelf were exposed during periods of low sea level.

Genetic diversity and population connectivity are rarely considered in the design of marine conservation strategies even though they are regarded as important components of conservation strategy and marine protected area (MPA) design (Sala et al. 2002; Bejer et al. 2014; Evans et al. 2014). Globally, seagrass beds, along with other marine and coastal ecosystems, are suffering from the negative influences created by the three billion humans living within 160 km of the coast, a number expected to increase over the coming decades (Economist 2014; Kaiser et al. 2005). Consequently it is becoming increasingly important to understand the genetic diversity and gene flow that is shaping seagrass beds and populations. As seagrass habitat is increasingly fragmented by human activities, maintaining viable populations will become increasingly important and difficult. If these populations are lost, vital ecosystem services also disappear, and a domino effect will be felt throughout coral reefs and other marine ecosystems.

This study aims to assess the genetic diversity and gene flow of two seagrass species that can be found throughout Indonesia; *Syringodium isoetifolium* and *Thalassia hemprichii*.

Both species have a widespread distribution from east Africa to the western Pacific (IUCN 2010a, b) and both species are able to reproduce both sexually and asexually. Sexual reproduction takes place via hydrophily (water dispersed pollen) and relies on the ability of pollen to find stigmas of recipient female plants. Pollination success declines rapidly with increasing distance between male and females. Both species produce seeds when sexually fertilized and these seeds have been observed to float for between two and 14 days (Lacap et al. 2002; Olesen et al. 2004). Asexual reproduction takes place through extension of the rhizome or through fragmentation. The products of fragmentation can be transported long distances on oceanic currents to establish new populations.

A common method used in the United States to restore and re-establish seagrass beds is through transplants from a donor bed to an impacted area (Fonseca et al. 1998). Considering the genetic diversity of the donor beds along with that of the recipient beds is important to the long-term persistence of the transplanted beds, and transplants from genetically polymorphic populations have a higher probability of survival (Procaccini & Piazzini 2001; van Katwijk et al. 2009; Evans et al. 2014). By revealing genetic diversity in both species, this study can guide future seagrass conservation and restoration initiatives.

Successful marine conservation programs require a holistic approach that encompasses terrestrial ecosystems (e.g., agricultural practices, habitat destruction for housing, etc.) along with nursery habitats such as seagrass beds, mangroves, and coral reefs (Wilkinson & Salvat 2012, 2014; Kroon et al. 2014; Sheppard 2014; Sale et al. 2014). By examining the genetic diversity and population structure of two widely distributed seagrasses throughout Indonesia, I hope to aid in the on-going efforts to protect and maintain the processes that have created the incredible biological diversity observed in Indonesia and throughout the Coral Triangle. This study adds to other work on an increasingly diverse range of taxa to understand gene flow and population connectivity in the region (Lourie & Vincent

2004; Rohfritsch & Borsa 2005; Kochzius & Nuryanto 2008; Timm & Kochzius 2008; Barber et al. 2011; Ackiss et al. 2013; Arlyza et al. 2013; DeBour et al. 2014).

Materials and Methods

A total of 257 *S. isoetifolium* and 406 *T. hemprichii* blades were collected throughout the Indonesian Archipelago between May and August of 2010 and 2011 (Figure 1). Both species reproduce sexually and asexually, and fragmentation can be important in establishing new populations. Because of these factors, all samples (i.e., blade fragments) were collected approximately 20 m apart from each other. All blades were immediately desiccated with silica gel and stored in silica gel until DNA extraction could be performed. DNA was extracted from the blades using the NucleoSpin[®] Plant II kit (Machery Nagel GmbH and Co., Bethlehem, PA, USA) with the manufacturer's CTAB protocol. Multiplex polymerase chain reactions (PCR) were performed using primers developed specifically for *S. isoetifolium* and *T. hemprichii* (Wainwright et al. 2013a, b) with additional primers for *S. isoetifolium* developed by Matsuki et al. (2013). Reactions for *S. isoetifolium* and *T. hemprichii* contained 2.5 μL of MyTaq[™] HS Mix (Bioline, Taunton, MA, USA), 0.5 μL of deionized water and 1 μL of primer mix and 1 μL of DNA diluted 1:250 for both *S. isoetifolium* and *T. hemprichii*. Primers for each locus in the multiplex reaction consisted of three primers utilizing a modified M13-tailed primer method described by Boutin-Ganache et al. (2001) to produce fluorescently-labeled amplicons. Primer mixes contained 0.35 pmol of tailed forward primer, 1.5 pmol of the reverse and dye-labeled primers for each locus (see Wainwright et al. (2013a, b) for details). All multiplex mixes were designed with Multplex Manager (Holleley & Geerts 2009) and details of each multiplex mix can be found in Appendix A, Tables S1 and S2.

PCR conditions followed a two-step protocol beginning with 2 minutes at 95° C, followed by 30 cycles of 95° C for 30 seconds and 55° C for 2.5 minutes as per

manufacturer's instructions. PCR products were resolved with an ABI 3730 Genetic Analyzer and sized with GENEIOUS 6.1.6 (Biomatters, San Francisco, CA, USA; <http://www.geneious.com/>). MICRO-CHECKER 2.3.3 (Van Oosterhout et al. 2004) was used to test for possible scoring errors and the presence of null alleles. Error rates (Tables S3 and S4) were calculated for each locus in both species by re-genotyping a random subset of 10% of the total number of samples as in Selkoe and Toonen (2006). Potential deviations from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium, and locus-by-locus summary statistics were calculated with ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010; Appendix A Tables S5 and S6)

The genetic structure of *S. isoetifolium* and *T. hemprichii* populations was investigated using STRUCTURE 2.3.4 (Pritchard et al. 2000). For both species, I used an admixture model with location information as a prior and a burn in period of 100,000 steps followed by another 100,000 steps. K values were estimated by initially setting the value of K from 1 to the maximum number of sample locations for each species. Each value of K was run for a total of 10 iterations and the optimum K was determined using the method of Evanno et al. (2005) applied in STRUCTURE HARVESTER 0.6.94 (Earl 2012). The value of K was determined using all loci in each species and then confirmed using only loci that showed a 0% error rate in *S. isoetifolium* and a $\leq 5\%$ error rate in *T. hemprichii* (Appendix A, Figures S1 to S4). Convergence was assumed if the alpha and likelihood values were stable and all runs returned similar results. STRUCTURE results were visualized using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and DISTRUCT 1.1 (Rosenberg 2004) implemented in CLUMPAK (Kopelman et al. 2015). AMOVA analysis was performed using the default settings in ARLEQUIN and groups were assigned based on the results of STRUCTURE. Pairwise F'_{ST} and F_{ST} values were calculated with GENODIVE 2.0b23 (Meirmans & Van Tienderen 2004). A standardized measure of genetic differentiation was employed (F'_{ST}) because traditional F_{ST}

values can become constrained and artificially low when using highly polymorphic loci (e.g., the microsatellites employed in this study; see Bird et al. (2011) for a complete discussion on the various merits and failings of the different metrics used to measure fixation and genetic differentiation). Patterns of isolation by distance (IBD) were tested using F'_{ST} and the default settings in the web-based program Isolation by Distance Web Service 3.23 (Jensen et al. 2005; <http://ibdws.sdsu.edu/~ibdws/>). Geographical distances between samples were calculated in Google Earth as the shortest possible distance between populations via sea.

Both data sets were checked for the presence of clones with GENODIVE 2.0b23 (Meirmans & Van Tienderen 2004). I define a clone as one or more specimens that are genetically indistinguishable from each other at the surveyed loci, each individual was compared to all other collected individuals. A total of 92 (35.8%) *S. isoetifolium* and 17 (4.2%) *T. hemprichii* clones were found and removed. *S. isoetifolium* clones were found in all sampled locations and *T. hemprichii* clones were found only in the Luwuk and Manado sampling locations. No clones were included in the analyses.

The inbreeding coefficient (F_{IS}) was calculated using FSTAT v2.9.3.2 (Goudet 1995).

Results

Syringodium isoetifolium

A total of 257 *S. isoetifolium* blades were collected from 14 sites and genotyped initially at 26 loci, but 11 of these loci were subsequently removed because of difficulties in achieving successful amplification or difficulties encountered when scoring as a result of stutter and or null alleles (see Appendix A, Table S5 for details of loci included in analysis). Once clones had been removed, 165 blades were included in all analyses.

Several loci were monomorphic in one sampling location but were polymorphic in another. For example, locus SI55 had four alleles in the 22 individuals collected from Alor while this locus was monomorphic in the 10 specimens collected from Karimunjawa

(Appendix A, Table S5). The maximum number of alleles observed was 10 at locus SII15 in specimens from Halmahera (Appendix A table S5). Expected and observed heterozygosities ranged from 0.063 to 0.856 and 0.00 to 1.00, respectively. Several samples have loci that deviate significantly from Hardy-Weinberg equilibrium following Bonferroni adjustments ($P = 0.003$; Appendix A, Table S5). Tests for linkage disequilibrium showed less than 1% of the loci surveyed showed evidence of significant linkage disequilibrium after Bonferroni adjustment ($P = 0.0002$). No single locus pair, however, was responsible for the disequilibrium and no discernible patterns were evident in the pairs of loci showing significant disequilibrium. All calculated values of F'_{ST} and F_{ST} were statistically significant at $P \leq 0.03$ (Table 1)

Following the methods implemented by Evanno et al. (2005), it was determined that the most likely value of $K = 2$ (see Appendix A, Figure S1 and S2). STRUCTURE plots indicate two distinct groups: a western group on the Sunda Shelf and an east-central group. Error rates for each locus were calculated and STRUCTURE runs were performed in three different ways: using (1) only loci showing no error, (2) all loci except SII17 (locus SII17 had the highest error rate) and (3) all loci included (see Appendix A, Table S3 for details of error rates). All three methods showed the same assignments of samples to the same clusters (Figure 2).

An AMOVA was performed using data from all loci on the two clusters resulting from STRUCTURE analysis and 67.6% of the variation is contained within the populations (Table 2). There was some evidence of a pattern of IBD over all populations although it was not significant ($r = 0.179$, $P = 0.085$) and no evidence of IBD was observed within any of the clusters defined by STRUCTURE. Analysis of the inbreeding coefficient (F_{IS}) indicated that 11 out of the 14 sampled sites had negative values, indicating outbreeding while the remaining three sites all had low positive values (Appendix A, Table S7)

Thalassia hemprichii

A total of 406 *T. hemprichii* blades were collected from 16 sites throughout Indonesia and genotyped at 20 loci. Three loci were subsequently discarded because of difficulties in achieving successful amplification or difficulties encountered when scoring as a result of stutter or null alleles (see Appendix A, Table S6 for details of loci included in analysis). A total of 389 blades were used in analyses once clones had been removed. Expected and observed heterozygosities ranged from 0.092 to 0.910 and 0.059 to 1.00, respectively. The number of alleles observed across all loci ranged from 2 to 11 and several samples have loci that deviate significantly from Hardy-Weinberg equilibrium following Bonferroni adjustments ($P = 0.003$; Appendix A, Table S6). Tests for linkage disequilibrium revealed that less than 1% of the surveyed loci showed evidence of significant linkage disequilibrium after Bonferroni corrections ($P = 0.0001$). All values of F'_{ST} and F_{ST} were significant at $P \leq 0.001$ (Table 3).

Employing the methods described by Evanno et al. (2005) the most likely value of $K = 2$ (see Appendix A, Figures S3 and S4). STRUCTURE runs were performed in four different ways: using (1) only loci showing a less than 5% error rate, (2) only loci with a less than 7% error rate, (3) only loci with a less than 10% error rate and (4) all loci included (Appendix A, Table S4). All four methods showed the same assignments of samples to the same clusters (Figure 3). Several AMOVA analyses were performed using data from all loci and the strongest support was for groups composed of sample sites Pari, Belitung, Natuna, Karimunjawa, Sanur, Derawan, Bira, Komodo, Alor, Wakatobi, and Manado and a group with the remaining sample sites (Table 4). A significant pattern of IBD was observed over all populations ($r = 0.366$, $p = 0.004$), however, no evidence of IBD was observed within the clusters defined by STRUCTURE. Analysis of the inbreeding coefficient (F_{IS}) indicated that all

sampled sites were possibly undergoing outbreeding as indicated by negative values (Appendix A, Table S8)

Discussion

Both *S. isoetifolium* and *T. hemprichii* show genetic structuring throughout the Indonesian Archipelago as indicated by the significant values observed in all pairwise comparisons of F_{ST} and F'_{ST} . The STRUCTURE analyses reveal that both species can be divided into two genetically distinct units (Figures 2 and 3), however, the locations of these units are different for each species. *S. isoetifolium* has one group found exclusively on the Sunda Shelf while the other is found across central and eastern parts of the archipelago. It is likely that the exposure of the Sunda Shelf during periods of glacial maxima (Tomascik et al. 1997) accounts for the genetic structure observed in *S. isoetifolium*. Work on *Rhizophora apiculata*, a widespread Indonesian mangrove tree, also shows a genetically distinct population on the Sunda Shelf supporting the idea that exposure of the Sunda Shelf during periods of glacial maxima could be driving the observed genetic structure (Yahya et al. 2014). Differentiation on the Sunda Shelf is likely the result of habitat loss during glacial maxima when sea levels dropped by as much as 120 m below present day levels (Voris 2000) and the consequent repopulation of the Sunda Shelf as glaciers retreat and sea level rises creating new habitat that could be exploited. The continental margin of the Sunda Shelf is known as the Wallace line (Huxley 1868). Several studies have shown that genetic differentiation can be observed on either side of the Wallace line, such as in the genus *Cerithidea* (marine snails), *Echinolittorina* snails, *Hippocampus trimaculatus* (three-spot seahorse) and several species of marine fish (Lourie & Vincent 2004; Collette 2005; Reid et al. 2006, 2013).

T. hemprichii also consists of two genetically distinct groups in the Indonesian Archipelago. One group is found within the Banda Sea while the other encompasses the

Sunda Shelf and extends east into central Indonesia. This structure cannot be explained by the exposure of the Sunda Shelf at glacial maxima or by barrier effects resulting from the Indonesian throughflow current. The Banda Sea is a somewhat enclosed body of water bounded by the Banda Arc, a horseshoe-shaped archipelago created by complex tectonic and volcanic activity. Additionally this region contains some of the deepest water on the planet with depths in the Weber Basin surpassing 7000 m (van Aken et al. 2009). Considering the extreme water depths in this region, the absence of a direct passage of water from the Indonesian throughflow (which runs north to south through the Makassar Strait) and the potential isolating effect of the Banda Arc, could result in the divergence seen in populations of *T. hemprichii* as it becomes locally adapted. Wang et al. (2015) show that the Banda Sea has distinct oceanographic conditions relative to the surrounding marine environment. The Banda Sea, on the whole, has a lower sea surface temperature and a lower chlorophyll a concentration when compared to water outside of this region. Whether or not the Banda Arc or the deep water in this region is responsible for these oceanographic differences is unknown and whether or not these environmental conditions are the ultimate cause of the differentiation we see in *T. hemprichii* is also unknown. It does, however, provide the opportunity for selection to act upon populations in this region. Lydekker (1896) proposed a line that defines the eastern geographical limit of oriental fauna, and Lydekker's line nearly perfectly follows the Banda Arc and passes slightly to the east of Halmahera. The pattern of differentiation observed in *T. hemprichii* from the east of the archipelago follows the easterly limit defined by Lydekker's line with populations from the eastern islands of Tual, Ambon and Halmahera all belonging to the same cluster found in the Banda Sea. The lack of samples from around New Guinea and east of Lydekker's line only allows me to speculate whether or not this line is acting as a barrier preventing dispersal of *T. hemprichii*. The unique oceanographic conditions in the Banda Sea, and the placement of Lydekker's line along the

margins of the shallow Sahul Shelf, however, gives credence to the idea that a different habitat on the shallow Sahul Shelf plays a role in the genetic structuring of *T. hemprichii* populations. Other studies have detected population differentiation of the Sahul Shelf relative to the rest of the Archipelago (Kochzius & Nuryanto 2008; Ackiss et al. 2013; DeBoer et al. 2014; Yahya et al. 2014) giving support to the idea that periods of lowered sea level, coupled with local extirpations and the subsequent recolonization of newly available habitat, may be driving genetic differentiation in the region. I also speculate that it is likely the deep waters of central Indonesia are acting as a species refuge during periods of glacial maxima and as a source of recruits when sea levels rose to their present day levels.

Given the permeable nature and the seasonal shifts in water currents throughout Indonesia, it is unlikely that these currents are solely responsible for creating the barriers responsible for restricting gene flow. The Indonesian throughflow constantly moves in a north to south direction through the Makassar Strait at up to $10.5 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ (Pandey & Pandey 2006) creating what should be a formidable barrier to dispersal. Despite this obstacle, recent studies by Debour et al. (2014) and Ackiss et al. (2013) have shown that populations of the clam, *Tridacna crocea*, and fish, *Caesio cuning*, do not show any genetic differentiation across this barrier, consistent with our finding that *T. hemprichii* are not genetically distinguishable on either side of the Indonesian throughflow current. Other water currents throughout the archipelago vary in direction, dependent upon the prevailing wind patterns of the northeast monsoon (December and January), and the southeast monsoons (June to July) (Tomascik et al. 1997). The shifting nature of these currents and the observed ability of many marine taxa to spawn over a period of several months or multiple times over the course of a year should facilitate archipelago wide dispersal (Babcock 1992; Stobart et al. 1993; Penland et al. 2004; Mangubhai & Harrison 2008; Rosser 2008; Baird et al. 2009; Padilla-Gamiño & Gates 2012). Consequently there should be very few, if any places, in

Indonesia that are completely isolated by water currents. Despite the potential homogenizing effects of these multidirectional, seasonal water currents, my work does identify a genetically distinct cluster of *T. hemprichii* within the Banda Sea.

Several theories have been proposed to explain the high levels of biodiversity in the Coral Triangle; (1) Center of speciation model which states that new species are created in the Coral Triangle, (2) Center of accumulation model which suggests that new species are formed in periphery regions and eventually make their way into the Coral Triangle and (3) Center of overlap model that attributes the high biodiversity to the fact that Indian and Pacific ocean species overlap in the area known as the Coral Triangle. The idea that *T. hemprichii* could be adapting to local environmental conditions lends support to the center of origin theory to explain for the abundant biodiversity observed in this area. (See Bowen et al. (2013) for a summary of the proposed theories that are likely working in unison to create the elevated levels of biodiversity observed in the region).

S. isoetifolium and *T. hemprichii* frequently co-occur within the same seagrass meadows, both reproduce asexually or sexually and both have similar dispersal mechanisms, the similarity in these life history characteristics make it extremely likely that both species occupy a comparable, but different ecological niche. Despite these similarities, *S. isoetifolium* and *T. hemprichii* show contrasting patterns of genetic structure. Similar contrasting patterns have been observed in three closely related, endemic Hawaiian limpets (*Cellana* sp) (Bird et al. 2007). It is not surprising that two species, despite sharing similar ecology, reproductive, and dispersal strategies show contrasting genetic structure, and several studies conclude that dispersal potential is not a good predictor of genetic structure in the marine environment (Bradbury & Bentzen 2007; Shanks 2009; Riginos et al. 2011 Selkoe & Toonen 2011).

When comparing the values of F_{IS} for both species (Appendix A, Tables S7 and S8), both appear to be using outcrossing as a reproductive strategy, a common strategy in plants (Wright et al. 2013). The F_{IS} values of *S. isoetifolium* (Appendix A, Table S7) collected from the genetic cluster found on the Sunda Shelf are, on the whole, lower than those measured through the rest of Indonesia indicating a greater degree of outcrossing. A higher prevalence of outcrossing could facilitate adaptation to conditions on the Sunda Shelf. In contrast, the F_{IS} values for *T. hemprichii* are similar throughout Indonesia (Appendix A, Table S8).

Numerous seagrass restoration projects throughout the world have attempted restoration through the transplantation of mature plants, seedlings or seeds (Thorhaug 1987; West et al. 1990; Campbell & Paling 2003; Fishman et al. 2004). Previous work has shown that genetic variability of the transplants is an important consideration when developing transplantation plans and genetically polymorphic transplants have a greater chance of survival (Procaccini & Piazz 2001; Williams 2001; Hughes & Stachowicz 2004; Hughes & Stachowicz 2009). If the ultimate goal of seagrass restoration projects is the maintenance of genetic diversity, consideration should be given to the origin and destination of transplants. *S. isoetifolium* and *T. hemprichii* both show evidence of two genetically distinct clusters within Indonesia. If transplants from one genetic cluster are transplanted into another genetic cluster, at best, this unique biodiversity will be lost and at worst the transplantation may fail due to maladaptation. By examining the variation observed at microsatellite loci in *S. isoetifolium* and *T. hemprichii*, I shed light on the genetic variation in both species, to aid informed decisions on any future transplantation initiatives for these species.

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Figures

Figure 1. Map showing the location of sampling sites throughout Indonesia. TH indicates *Thalassia hemprichii* and SI indicates *Syringodium isoetifolium*. Numbers in parenthesis show sample size from the respective sampling location. Also shown are the approximate locations of Wallace's line along with Huxley's modification to the line and Lydekker's line.

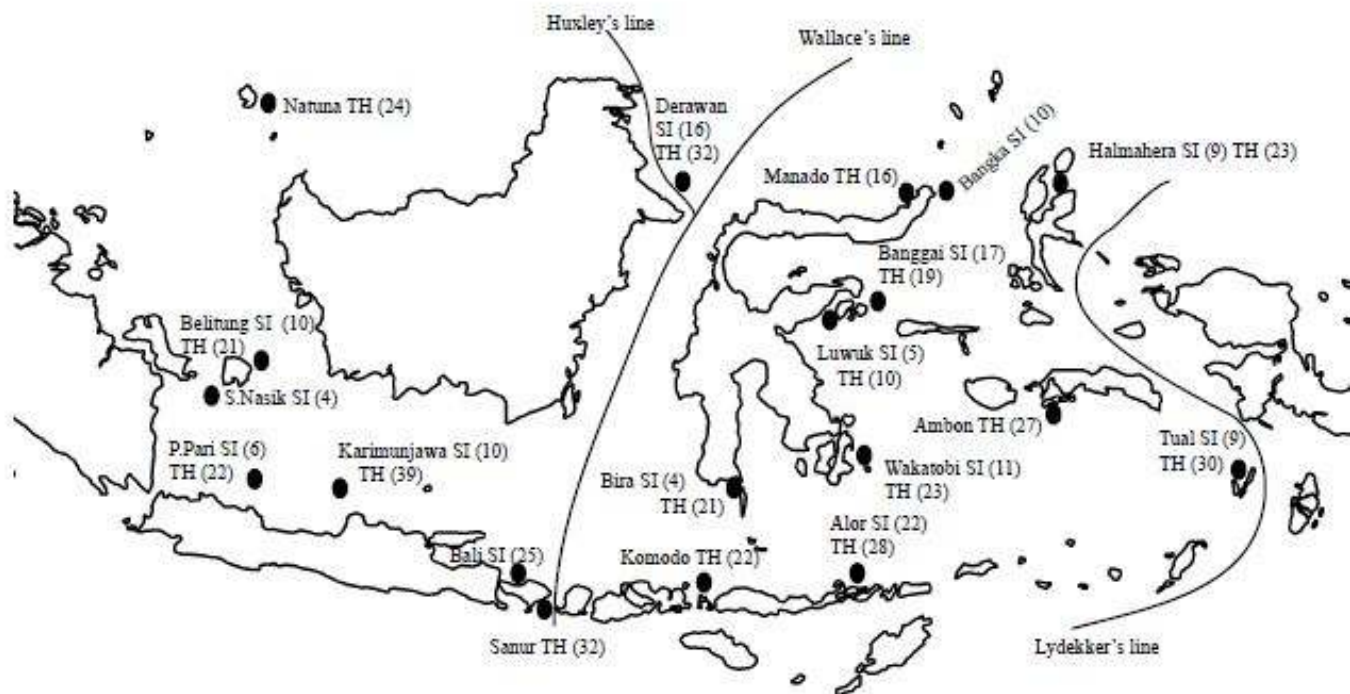
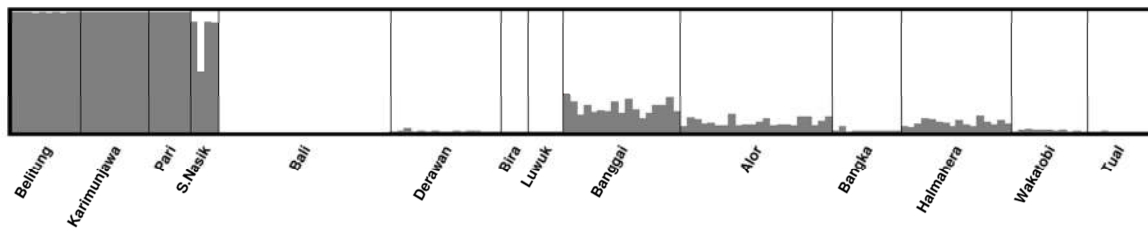
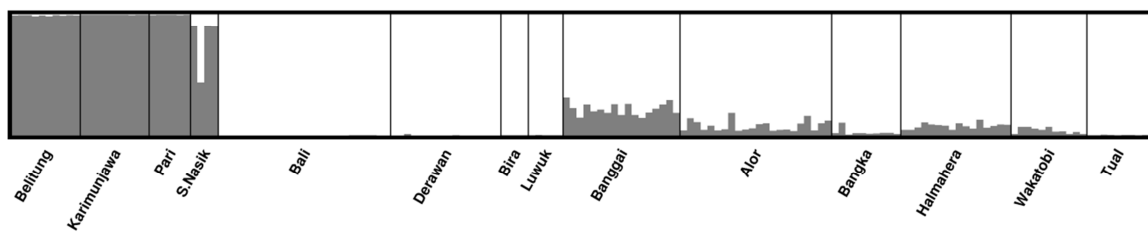


Figure 2. *Syngodium isoetifolium* Bayesian clustering analysis using STRUCTURE 2.3.4. For all runs, $K = 2$ and the likelihood was the average of 10 runs. A) Only loci with a 0% error rate included, B) Locus SI117 removed (locus SI117 had the highest error rate), C) All loci included. See Table 1 for details of locus specific error rates.

A



B



C

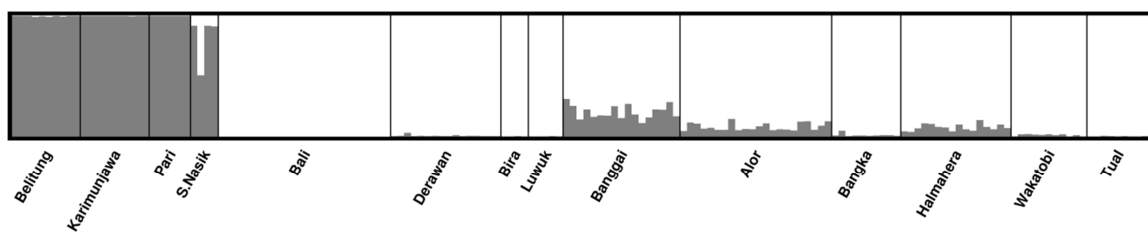
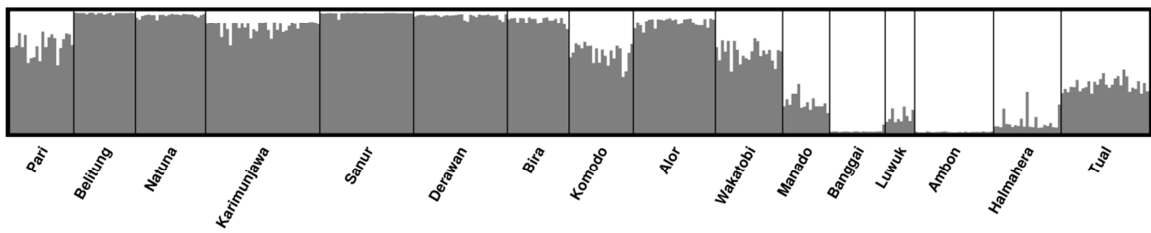
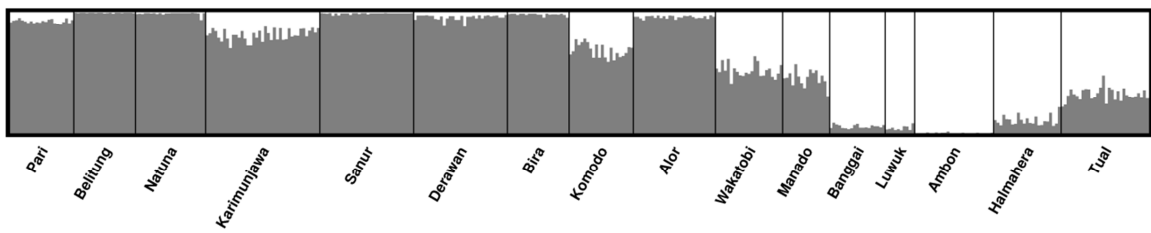


Figure 3. *Thalassia hemprichii* Bayesian clustering analysis using STRUCTURE 2.3.4. For all runs, K=2 and the likelihood was the average of 10 runs. A) Only loci with <5% error rate included, B) Only loci with <7% error rate included, C) All loci.

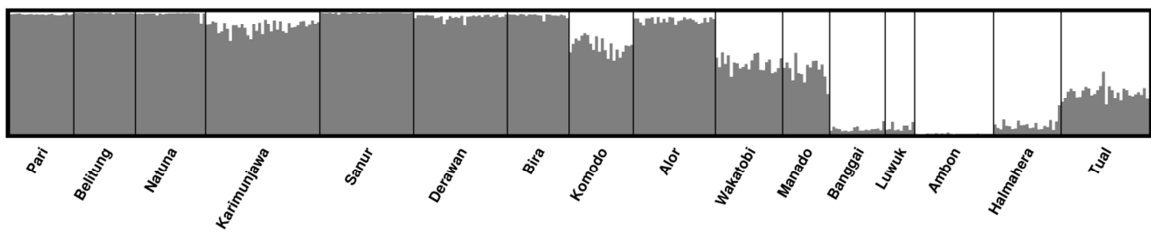
A



B



C



D

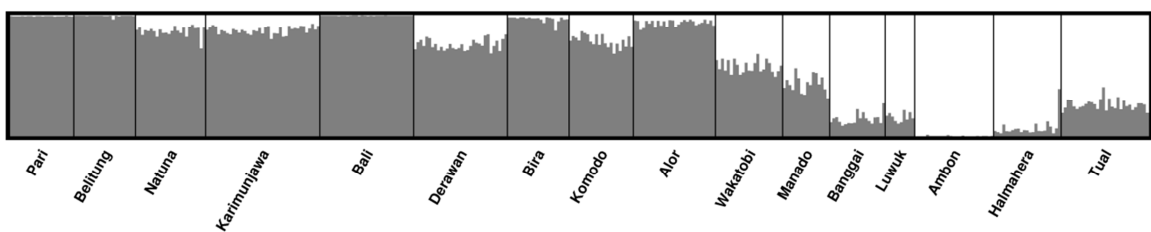


Table 1. *Syringodium isoetifolium* locus specific error rates calculated by re-genotyping a randomly chosen subset of 10% of the total number of samples (see Selkoe & Toonen 2006). Loci Si021 is from Matsuki et al. (2013).

Locus	% Error
SI008	0.0
SI023	0.0
SI027	0.0
SI055	0.0
SI059	0.0
SI060	0.0
SI089	0.0
SI099	0.0
SI103	0.0
SI104	0.0
SI108	2.1
SI109	0.0
SI115	4.2
SI117	18.8
Si021	4.2

Table 2. *Thalassia hemprichii* locus specific error rates calculated by re-genotyping a randomly chosen subset of 10% of the total number of samples (see Selkoe & Toonen 2006).

Locus	% Error
TH07	6.3
TH14	2.1
TH17	6.3
TH32	6.3
TH37	4.2
TH39	8.3
TH43	2.0
TH44	14.6
TH45	14.6
TH48	6.3
TH49	14.6
TH50	6.3
TH53	8.3
TH72	10.4
TH73	4.2
TH75	6.3

Table 3. *Syringodium isoetifolium*. Pair wise F'_{ST} values above and F_{ST} values below the diagonal.

All values are significant $p < 0.03$.

	Alor	Bali	Banggai	Bangka	Belitung	Derawan	Halmahera	Karimunjawa	Luwuk	P.Bira	P.Pari	S.Nasik	Tual	Wakatobi
Alor	--	0.25	0.13	0.25	0.49	0.22	0.21	0.46	0.24	0.17	0.47	0.33	0.63	0.18
Bali	0.15	--	0.36	0.31	0.61	0.43	0.35	0.64	0.34	0.30	0.61	0.53	0.67	0.24
Banggai	0.07	0.21	--	0.34	0.42	0.18	0.16	0.36	0.27	0.24	0.41	0.29	0.60	0.25
Bangka	0.15	0.20	0.19	--	0.52	0.30	0.22	0.56	0.24	0.37	0.60	0.40	0.68	0.35
Belitung	0.29	0.40	0.24	0.33	--	0.51	0.51	0.25	0.62	0.66	0.31	0.20	0.71	0.54
Derawan	0.13	0.27	0.10	0.18	0.31	--	0.16	0.49	0.27	0.29	0.55	0.36	0.67	0.39
Halmahera	0.12	0.21	0.08	0.12	0.30	0.09	--	0.46	0.19	0.26	0.52	0.32	0.62	0.31
Karimunjawa	0.28	0.43	0.21	0.37	0.17	0.31	0.27	--	0.63	0.65	0.30	0.23	0.70	0.50
Luwuk	0.14	0.22	0.15	0.15	0.40	0.16	0.10	0.42	--	0.27	0.66	0.51	0.70	0.38
P.Bira	0.10	0.19	0.13	0.23	0.43	0.17	0.14	0.43	0.17	--	0.66	0.54	0.72	0.28
P.Pari	0.29	0.40	0.24	0.39	0.21	0.34	0.30	0.21	0.45	0.44	--	0.31	0.75	0.47
S.Nasik	0.19	0.34	0.16	0.25	0.13	0.22	0.18	0.15	0.32	0.33	0.21	--	0.68	0.43
Tual	0.37	0.43	0.33	0.42	0.46	0.40	0.35	0.45	0.44	0.45	0.49	0.42	--	0.64
Wakatobi	0.10	0.15	0.13	0.21	0.34	0.22	0.17	0.31	0.23	0.17	0.30	0.25	0.38	--

Table 4. *Syringodium isoetifolium* AMOVA results based upon *a priori* population groupings as determined by STRUCTURE 2.3.4 (Pritchard et al. 2000).

	Degrees of Freedom	Variance Component	% Variation	Fixation Indices	<i>P</i> Value
Among groups (F_{CT})	1	0.73	16.33	0.16	< 0.001
Among populations within groups (F_{SC})	12	0.72	16.11	0.19	< 0.001
Within populations (F_{ST})	316	3.03	67.57	0.32	< 0.001

Table 5. *Thalassia hemprichii* pair wise F'_{ST} values above and F_{ST} values below the diagonal. All values are significant $p < 0.001$.

	Alor	Ambon	Sanur	Banggai	Belitung	Bira	Derawan	Halmahera	Karimunjawa	Komodo	Luwuk	Natuna	Manado	P.Pari
Alor	--	0.52	0.31	0.42	0.38	0.26	0.30	0.51	0.20	0.20	0.58	0.40	0.38	0.26
Ambon	0.23	--	0.64	0.35	0.63	0.61	0.51	0.38	0.49	0.48	0.58	0.58	0.46	0.63
Sanur	0.14	0.30	--	0.53	0.43	0.42	0.45	0.53	0.25	0.23	0.62	0.58	0.52	0.21
Banggai	0.17	0.14	0.23	--	0.54	0.46	0.43	0.20	0.37	0.36	0.24	0.55	0.30	0.47
Belitung	0.16	0.27	0.20	0.21	--	0.33	0.40	0.58	0.24	0.34	0.65	0.38	0.44	0.34
Bira	0.11	0.26	0.19	0.18	0.14	--	0.28	0.52	0.25	0.26	0.66	0.31	0.44	0.34
Derawan	0.12	0.21	0.19	0.16	0.16	0.11	--	0.42	0.34	0.33	0.64	0.28	0.30	0.42
Halmahera	0.22	0.16	0.24	0.08	0.24	0.22	0.16	--	0.49	0.46	0.36	0.61	0.39	0.53
Karimunjawa	0.08	0.20	0.11	0.14	0.10	0.10	0.13	0.19	--	0.12	0.47	0.40	0.27	0.14
Komodo	0.08	0.20	0.10	0.14	0.14	0.11	0.13	0.19	0.05	--	0.49	0.49	0.32	0.24
Luwuk	0.25	0.25	0.29	0.09	0.27	0.27	0.25	0.15	0.18	0.20	--	0.75	0.44	0.53
Natuna	0.17	0.25	0.27	0.22	0.16	0.13	0.11	0.26	0.16	0.20	0.31	--	0.45	0.48
Manado	0.15	0.18	0.22	0.10	0.17	0.17	0.11	0.15	0.10	0.12	0.16	0.17	--	0.40
P.Pari	0.11	0.27	0.10	0.19	0.15	0.15	0.17	0.22	0.06	0.10	0.22	0.20	0.16	--

Table 6. *Thalassia hemprichii* AMOVA results based upon *a priori* population groupings as determined by STRUCTURE 2.3.4 (Pritchard et al. 2000).

	Degrees of Freedom	Variance Component	% Variation	Fixation Indices	<i>P</i> Value
Among groups (F_{CT})	1	0.34	5.93	0.06	< 0.001
Among populations within groups (F_{SC})	14	0.72	12.46	0.13	< 0.001
Within populations (F_{ST})	762	4.75	81.61	0.18	< 0.001

CHAPTER 3: Population genetics of the collector urchin, *Tripneustes gratilla*, in the Indo-Malay archipelago

Abstract

The population genetics of 353 collector urchins, *Tripneustes gratilla*, from 16 sampling sites throughout Indonesia was investigated using 12 microsatellite loci. Bayesian clustering methods revealed two genetically distinct clusters, one located predominantly on the shallow Sunda Shelf and the second in the deep waters associated with the biogeographic region known as Wallacea. I propose that extirpation due to loss of marine habitat caused by lowering of sea levels during periods of glacial maxima, followed by recolonization as glaciers retreat could be responsible for the genetic differentiation observed. The identification of cryptic diversity should aid in the delineation of hotspots of biodiversity allowing countries to meet global conservation priorities such as those defined by the Convention on Biological Diversity and The International Union for Conservation of Nature. These data indicate the Sunda Shelf is a semi-isolated region within the Coral Triangle biodiversity hotspot, and possibly a source of evolutionary novelty.

Introduction

Tripneustes gratilla is a widespread Indo-Pacific sea urchin that is commercially exploited as a food source throughout South East Asia and the Coral Triangle (Kasim 2009). Global sea urchin production peaked in 1995 with an estimated 120,306 tons harvested followed by a collapse of many urchin fisheries (Juinio-Meñez 2008). While the number of *T. gratilla* harvested from Indonesian waters is unknown, *T. gratilla* has been identified as the most important urchin species in the adjacent Philippine fishery (Juinio-Meñez 2008). The harvesting of *T. gratilla* for commercial purposes created a nearly half million US\$ industry in the Philippines before unregulated harvesting caused a collapse in 1992 (Juinio-Meñez 2008). Similar unregulated harvesting is occurring in Indonesia and if Indonesian fisheries have not already collapsed they are likely to soon. The high commercial value of *T. gratilla* has spurred research into aquaculture programs designed to support commercial exports and the recovery of wild stocks of *T. gratilla* (Juinio-Meñez 1998; 2008; Dworjanyn et al. 2007).

T. gratilla has a pelagic larval duration of between 42 and 52 days (Junio-Meñez et al. 1998) and gametes of males and females have been shown to be negatively buoyant, once these gametes come into contact with the substratum they resist dislodgment in what is thought to be a mechanism to increase settlement success (Thomas 1994).

The fishery collapses are particularly worrisome because *T. gratilla* is considered a keystone species capable of controlling invasive algal species. In Hawai'i, experiments using *T. gratilla* as a biocontrol agent have demonstrated that it can reduce the biomass of invasive *Kappaphycus* spp. and other alien or invasive macroalgae (Conklin & Smith 2005; Stimson et al. 2007; Westbrook et al. 2015). Despite this apparent benefit of controlling invasive algae, other studies have shown that population outbreaks of *T. gratilla* have a detrimental effect on seagrass and algal populations through overgrazing (Eklöf et al. 2008; Valentine & Edgar 2010).

Previous work by Lessios et al. (2003) has shown that populations of *T. gratilla* throughout the Pacific and Indian Oceans are genetically indistinguishable across both oceans when comparing mitochondrial cytochrome oxidase I (COI) sequence data. The same study, however, also uncovered large and significant F_{ST} values at a regional level indicating local genetic differentiation. The population genetics of *T. gratilla* have been examined at a finer scale in the Philippines by Malay et al. (2002) and Casilagan et al. (2013). Malay et al. (2002) used six allozyme loci and Casilagan et al. (2013) employed both mtDNA COI and microsatellites in efforts to elucidate population structure throughout the Philippine archipelago. Both of these studies found no significant population structuring possibly indicating high levels of gene flow in *T. gratilla* populations from the Philippines or a lack of resolution in the genetic markers employed (Malay et al. 2002; Horne 2014).

The Coral Triangle contains the greatest shallow water biodiversity on the planet and Indonesia contains the largest portion of the Coral Triangle (Briggs 2000; Allen & Werner 2002; Hoeksema 2007; Allen 2008). Despite this recognition, a report by the World Resources Institute (WRI) in 2012 classified more than 85% of the reefs of the Coral Triangle as currently threatened by local stressors and nearly half of all the reefs in the Coral Triangle were classified as highly or very highly threatened.

As these threats become more prevalent, there is an urgent need to design effective marine conservation strategies and policies. Successful marine conservation strategies require a holistic approach, incorporating conservation issues from terrestrial ecosystems (e.g., habitat destruction resulting from poor land management practices, agricultural runoff, increasing sedimentation inputs, etc.) coupled with the preservation of marine nursery grounds, seagrass beds, mangroves and coral reefs (Kroon et al. 2014; Wilkinson & Salvat 2012; Sheppard 2014; Sale et al. 2014). Genetic diversity and population connectivity are rarely considered in the creation of new Marine Protected Areas (MPA) even though they are

considered important components of successful conservation initiatives (Sala et al 2002; Evans et al. 2014). By examining the population genetics and population structure in *T. gratilla*, I add to the growing body of work that has already been performed in the region in the hope that genetic information, especially the genetic connectivity of multiple species, can be incorporated into future marine conservation strategies. (Lourie & Vincent 2004; Rohlfritsch & Borsa 2005; Kochzius 2008; Timm & Kochzius 2008; Barber et al. 2011; Ackiss et al. 2013; Arlyza et al. 2013; DeBoer et al. 2014)

Several theories have been proposed to explain the high levels of biodiversity in the Coral Triangle; (1) Center of speciation model which states that new species are created in the Coral Triangle, (2) Center of accumulation model which suggests that new species are formed in periphery regions and eventually make their way into the Coral Triangle and (3) Center of overlap model that attributes the high biodiversity to the fact that Indian and Pacific ocean species overlap in the area known as the Coral Triangle (see Bowen et al. (2013) for a more comprehensive review). It is likely that all of these processes have a role in promoting the high levels of biodiversity (Briggs 2003; Jokiel & Martinelli 1992; Eble et al. 2011; Gaither & Rocha 2013).

The Indonesian Archipelago can be divided into three very broad regions, two of which are dominated by the shallow Sunda shelf in western Indonesia and Sahul shelf in eastern Indonesia (Figure 1). The third, called Wallacea, is characterized by deep water and volcanic islands. During periods of glacial maxima, sea levels dropped by as much as 120m (Voris 2000), exposing both the Sunda and Sahul Shelves extirpating marine species in these areas. The region known as Wallacea is bounded by the Wallace line in the west (Huxley 1868) and Lydecker's line (Lydekker 1896) in the east (Figure 1). The passageway created by the deep waters of Wallacea formed the primary marine connection between the tropical Pacific and Indian Oceans during periods of glaciation. On account of the remarkably high

levels of biodiversity observed within this region of the Coral Triangle, Wallacea has been identified as a global biodiversity hotspot (Myers et al. 2000) and it is possible that this region acted as a refuge allowing species persistence during times of low sea level.

By examining the population genetics of *T. gratilla*, I aim to shed light on the processes that are creating and maintaining marine biodiversity in Indonesia and the Coral Triangle region with the ultimate goal of assisting on-going conservation efforts in the region.

Materials and Methods

Several tube feet were removed from each sampled individual and preserved in a 20% salt saturated DMSO solution. In total, 353 individuals were collected from 16 sampling sites throughout the Indonesian Archipelago between May and August of 2010 and 2011 (Figure 2). DNA extraction followed a modified DNeasy Blood and Tissue Kit protocol (Qiagen, Valencia, California, USA). Proteinase K was purchased from Creative BioMart (Shirley, New York, USA). EconoSpin All-In-One Mini Spin Columns were substituted for Qiagen spin columns (Epoch Life Science, Missouri City, Texas, USA). All extraction parameters (volumes, times and temperatures) followed the DNeasy Blood and Tissue Kit instructions for animal tissues.

Multiplex polymerase chain reactions (PCR) were performed using primers specifically developed for *T. gratilla* (Wainwright et al. 2012) in 5 μ L reactions. Each reaction contained 2.5 μ L of MyTaq™ HS Mix (Bioline, Taunton, MA, USA), 1 μ L of DNA diluted 1:250, 0.5 μ L of deionized water and 1 μ L of primer mix. Each multiplex reaction contained three primers utilizing a modified M13-tailed primer method described by Boutin-Ganache et al. (2001) to produce fluorescently labeled amplicons. Primer mix contained 0.35 pmol of a tailed forward primer, 1.5 pmol of the reverse and dye-labeled primers for each locus (see Wainwright et al. 2012 for details). Multiplex mixes were designed with Multiplex Manager (Holleley & Geerts 2009) and details of each multiplex mix can be found in Appendix B,

Table S1. PCR conditions followed a two-step protocol beginning with 2 minutes at 95° C, followed by 30 cycles of 95° C for 30 seconds and 55° C for 2.5 minutes, per manufacturer's instruction.

PCR products were resolved with an ABI 3730 Genetic Analyzer and sized with GENEIOUS 6.1.6 (Biomatters, San Francisco, CA, USA; <http://www.geneious.com/>). MICRO-CHECKER 2.3.3 (Van Oosterhout et al. 2004) was used to test for possible scoring errors and the presence of null alleles. Error rates (Table 1) were calculated for each locus by re-genotyping a random 10% of specimens (Selkoe & Toonen 2006). Deviations from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium, and locus-by-locus summary statistics were calculated with ARLEQUIN 3.5.1.2 (Excoffier & Lischer 2010; Appendix B, Table S2)

The genetic structure between populations was investigated using the program STRUCTURE 2.3.4 (Pritchard et al. 2000). Error rates were calculated for each locus (Table 1) and STRUCTURE runs were performed in four ways; (1) all loci, (2) only loci with $\leq 2.1\%$ error rate (3) only loci that showed $\leq 10\%$ null alleles over all sampled locations and (4) only loci that had ≤ 2 populations out of HWE over all sampled locations (see Appendix B, Table S2 for details of null alleles). I used an admixture model with location information as a prior and a burn in period of 100,000 steps followed by another 100,000 steps. K values were estimated by initially setting the value of K from 1 to the maximum number of samples (16). Each value of K was run for 10 iterations and the optimum K was determined using the method of Evanno et al. (2005) applied in STRUCTURE HARVESTER 0.6.94 (Earl 2012). The value of K was initially determined using all loci and then confirmed using only loci that showed $\leq 2.1\%$ error rate (Appendix B, Figures S1 and S2). Convergence was assumed if the alpha and likelihood values were stable and all runs returned similar results. STRUCTURE results were visualized using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and DISTRUCT 1.1 (Rosenberg 2004) implemented in CLUMPAK (Kopelman et al. 2015). AMOVA analysis was

performed using the default settings in ARLEQUIN and groups were assigned based on the results of STRUCTURE. Pairwise F'_{ST} and F_{ST} values were calculated with GENODIVE 2.0b23 (Meirmans & Van Tienderen 2004). See Bird et al. (2011) for a complete discussion on the various merits and failings of the different metrics used to measure genetic differentiation. Patterns of isolation by distance were tested using F'_{ST} and the default settings in the web-based program Isolation by Distance Web Service 3.23 (Jensen et al. 2005; <http://ibdws.sdsu.edu/~ibdws/>). Geographical distances between populations were estimated in Google Earth as the shortest oceanic distance between samples.

Results

A total of 353 individuals were initially genotyped at 19 loci, but seven loci were subsequently removed because of difficulties in achieving successful amplification or as a result of stutter and null alleles (see Appendix B, Table S1 for details of loci included in analysis).

The number of alleles per locus ranged from three to 30. Observed and expected heterozygosities ranged from 0.154 to 1.000 and 0.280 to 0.967 respectively (Appendix B, Table S2). Several samples have loci that deviate significantly from Hardy-Weinberg equilibrium (HWE) following Bonferroni adjustments ($P = 0.004$). One of the major HWE expectations is the assumption of random mating. The gametes of both of male and female *T. gratillia* are negatively buoyant and able to resist dislodgement once they come into contact with substrate. These factors make it extremely likely that the assumption of random mating is violated by natural processes. Deviations from the expectations of HWE are commonly attributed to issues associated with the incorrect scoring and amplification (null alleles) of the microsatellite loci employed. This, however, may not always be the case in that the life history characteristics of many invertebrate species could be responsible for the observed deviations from HWE. After Bonferroni adjustment, no pair of loci showed any evidence of

significant linkage disequilibrium ($p = 0.0003$). Several pairs of sample sites showed significant F'_{ST} and F_{ST} values $p = < 0.05$ (Table 2).

Following Evanno et al. (2005), the most likely value of $K = 2$ (See Appendix B, Figures S1 and S2 for details of Evanno's delta K statistic). STRUCTURE plots indicate two genetically distinct groups: one group is predominantly on the Sunda Shelf and the other group extends in an easterly direction from the Sunda Shelf (Figure 3). When comparing the four different data sets with differing numbers of loci depending upon error rates, the presence of null alleles or deviations from HWE, all four data sets showed the same assignments of samples to the same clusters (Figure 3).

Discussion

Populations of the sea urchin *T. gratilla* show evidence of genetic structuring throughout the Indonesian Archipelago, as indicated by the significant F'_{ST} and F_{ST} (Table 2). STRUCTURE analysis indicates two genetically distinguishable units in Indonesia. One cluster is found in the west of the archipelago predominantly in the shallow waters associated with the Sunda Shelf and the other cluster is found in the deep waters that dominate central and eastern Indonesia. (Figures 1 and 3).

The processes that contribute to population differentiation and ultimately speciation in the ocean are poorly understood (Moura et al. 2014). Mayr (1954) concluded that speciation in the oceans follows the same rules as speciation in terrestrial environments (i.e., barriers prevent gene flow and the resulting isolation allows speciation in allopatry). The absence of obvious barriers to gene flow and the highly dispersive nature of marine organisms have caused many to question whether speciation in the ocean is entirely reliant upon isolation (Miglietta et al. 2011). There is growing body of literature suggesting that selection may be the driving force behind population differentiation and ultimately speciation in the oceans (Ruzzante et al. 1996; Naciri et al. 1999; Bierne et al. 2003; Beheregaray & Sunnucks 2001;

Jorgensen et al. 2005; Nosil et al. 2009; Schluter 2009; White et al. 2010; Bird et al. 2012; Bowen et al. 2013; Shafer et al. 2013).

The observed pattern of differentiation in *T. gratilla* from Indonesia could be explained by barrier effects resulting from the Indonesian Throughflow (ITF) current. This current moves up to $10.5 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ in a north to south direction through the Makassar Strait (Fig. 2; Pandey & Pandey 2006). The ITF could be acting as a barrier preventing gene flow between populations found in the east and west of the archipelago. The resulting isolation could be responsible for the two genetically distinct populations in Indonesia, however, water currents throughout the archipelago reverse and flow in the opposite direction dependent upon the season and the direction of the prevailing winds. The multidirectional nature of these water currents and the ability of many marine taxa to spawn multiple times over the course of the year should promote archipelago wide dispersal and a lack of observable population structure (Babcock 1992; Stobart et al. 1993; Penland et al. 2004; Mangubhai & Harrison 2008; Rosser 2008; Baird et al. 2009; Padilla-Gamiño & Gates 2012). For comparisons, Lessios and Robertson (2006) found that 18 out of 20 species of reef fish surveyed on either side of the Eastern Pacific Barrier (EPB) showed little significant differentiation. The EPB is an approximate 5000 km stretch of deep water between central Pacific islands and continental coastlines of the Americas, dubbed an “impassable barrier” by Darwin (1880). If larvae can cross the EPB as demonstrated by Lessios & Robertson (2006) then larvae should also cross the much smaller barrier created by the ITF. With two genetically distinct units of *T. gratilla* in Indonesia, I postulate that habitat differences and ecological release could be driving the observed patterns of differentiation. The idea that differing habitats throughout Indonesia could be driving differentiation is further supported by the work of Tremblay & Halpin (2012) who modeled a virtual cloud of larvae, tracking hypothetical settlement as the virtual larvae moved through seascapes controlled by ocean

currents and larval characteristics. They showed that connectivity between the Sunda Shelf and Wallacea was extremely limited, and this was likely caused by habitat differences between the regions.

One of the genetically distinct clusters of *T. gratilla* is found predominantly on the Sunda Shelf, an area of shallow water with an average depth of approximately 70 m (Hanebuth et al. 2002). The other cluster is found in Wallacea, a region dominated by deep water. This region contains some of the deepest water on the planet with water depths exceeding 7000 m (van Aken et al. 2009). The Sunda shelf does have a detectably lower salinity when compared to other bodies of water in Indonesia especially those of central/eastern Indonesia (Levitus & Boyer 1998), a result of fresh water input from the large landmasses that enclose the Sunda Shelf. In addition to this physical difference between the water bodies, the Sunda shelf is dry during glacial maxima when water levels drop as much as 120 m (Voris 2000). The drying of the Sunda Shelf causes habitat loss and the extirpation of marine creatures. In contrast, the deep waters of central and eastern Indonesia could be acting as a refuge allowing species to persist during periods of lowered sea level. This deep water could also be acting as a source of recruits that can colonize newly available when the Sunda Shelf is flooded.

The drying of the Sunda and Sahul shelves along with the consequent flooding when glaciers subside creates an opportunity for species that persisted in the deep waters of Wallacea to expand their ranges into new habitat. Species involved in these expansions could benefit from ecological release facilitating the genetic differentiation seen in populations of *T. gratilla* on the Sunda Shelf and Wallacea (Yoder et al. 2010; Crandall et al. 2011; Waters et al. 2012). Genetic surfing, a process of accelerated genetic drift that can alter allele frequencies as an expansion front passes over new habitat, can cause differentiation of populations (Excoffier & Ray 2008; Excoffier et al. 2009; Slatkin & Excoffier 2012; Horne

2014). Habitat inundation as glacier's retreat creates an excellent opportunity for genetic surfing to cause the observed differentiation.

Waters et al. (2014) described a 'founder takes all' process where rapid expansion of founder alleles occurs and, as the founders reach high densities, competition prevents newly arriving recruits from becoming established. Like genetic surfing, this is a bottleneck effect where strong genetic drift causes allele frequencies to change in the new habitat when compared to the original source of the alleles. *T. gratilla* has a pelagic larval duration of between 42 and 52 days (Junio-Meñez et al. 1998), which should facilitate long distance dispersal events into areas of newly available habitat. Hence the founder takes all process could account for the differentiation observed between the new habitats of the Sunda Shelf and much older habitats of Wallacea. Post-glacial colonization of the Sunda Shelf would provide an unparalleled opportunity for founder populations to reach high densities, possibly in the absence of predators, while more recent immigrants from the source population could be outcompeted or swamped by local reproduction, producing the observed differentiation.

The preservation of biodiversity is essential for the maintenance of ecosystems services that provide food security for millions of humans (Duffy 2003). Biodiversity hotspots are rightfully regarded as a global conservation priority as defined by the Convention on Biological Diversity and the International Union for Conservation of Nature (Myers et al. 2000; Hughes et al. 2002; Roberts et al. 2002; Millennium Ecosystem Assessment World Resources Institute 2005; Convention on Biological Diversity 2010; Brooks 2006; Selig et al. 2014). The identification of cryptic diversity in the widespread tropical sea urchin *T. gratilla* from the Indonesian Archipelago could warrant separate management plans specifically designed for each population. The differentiation between populations on the shallow Sunda Shelf and deep Wallacea invokes the possibility that *T. gratilla* in these regions may be on separate evolutionary trajectories. Given enough time,

reproductive isolation between these two regions, coupled with ecological differences, could lead to parapatric speciation. This gives support to the center of origin hypothesis as a mechanism that is able to create the elevated levels of biodiversity seen in the Coral Triangle and throughout the Indonesian Archipelago.

This work adds to the growing body of research that has taken place or is taking place in Indonesia and the Coral Triangle to advance conservation initiatives in the region. I hope that by shedding light on processes that are structuring populations genetically along with the processes that are creating and maintaining biodiversity in the region, holistic and all-encompassing, comprehensive management plans can protect this unique environment for millennia to come.

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Figures

Figure 1. Map showing the locations of the Sunda Shelf, Wallacea and the Sahul Shelf. The light grey area indicates the maximum exposure of land during periods of glacial maxima (sea levels 120 m lower than present day). Also indicated are the approximate positions of Wallace's and Lydekker's lines. Figure adapted from Vorris (2000) and downloaded from the The Field Museum of Natural History (<http://www.fieldmuseum.org/pleistocene-sea-level-maps>).

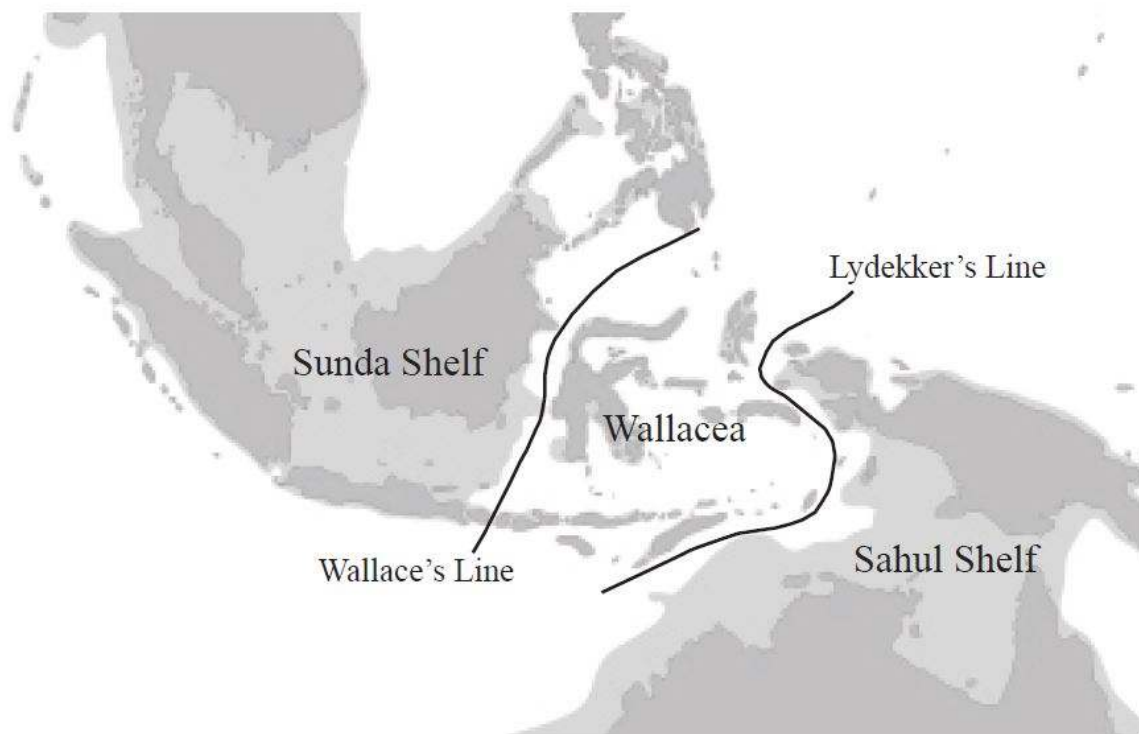


Figure 2. Map the showing location of sample sites throughout Indonesia. Numbers in parenthesis indicate sample size from respective sampling location. Indonesian Through Flow and Makassar Strait are indicated.

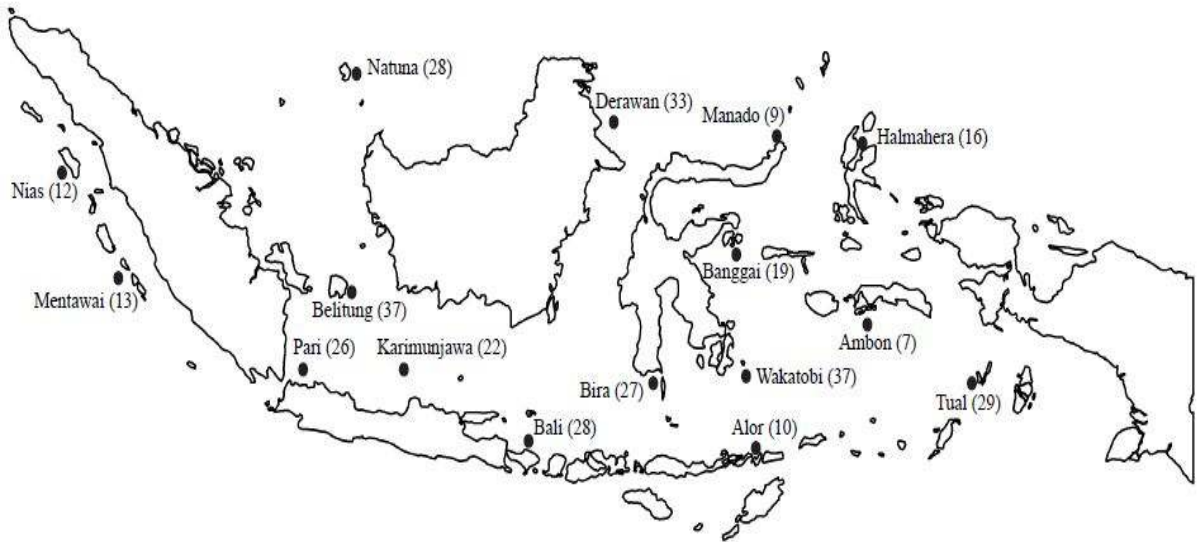
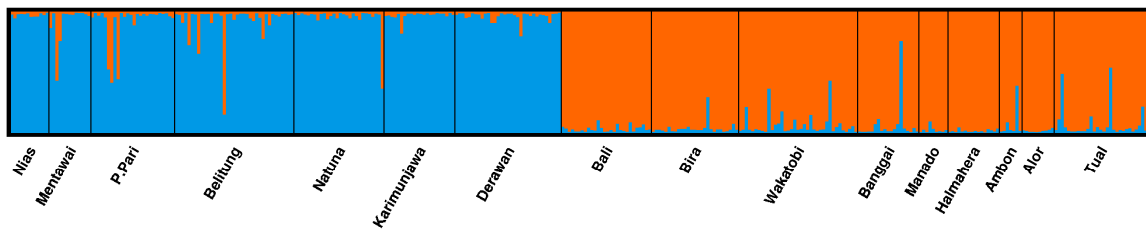
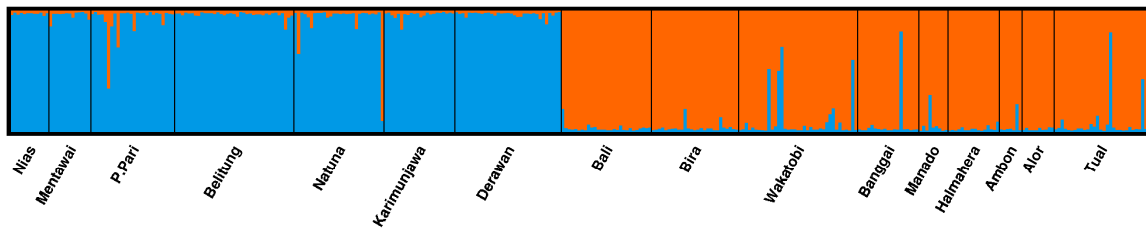


Figure 3. *Tripneustes gratilla* Bayesian clustering analysis using STRUCTURE 2.3.4. For all runs, $K=2$ and the likelihood was the average of 10 runs. A), All loci B), Only loci with $\leq 2,1\%$ error rate included C) Only loci showing a frequency of $\leq 10\%$ null alleles over all sampled populations, D) Only loci that had ≤ 2 populations out of HWE over all sampled locations.

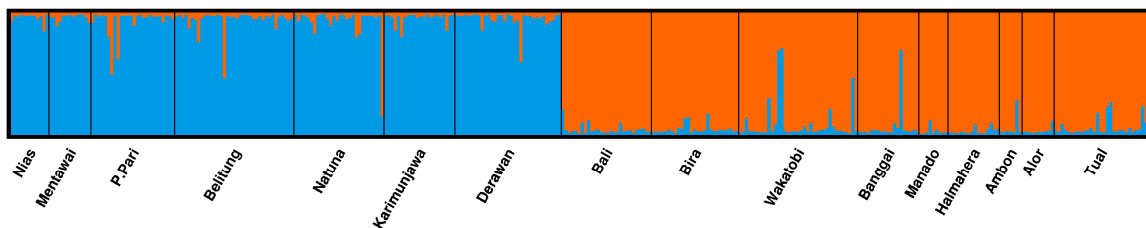
A)



B)



C)



D)

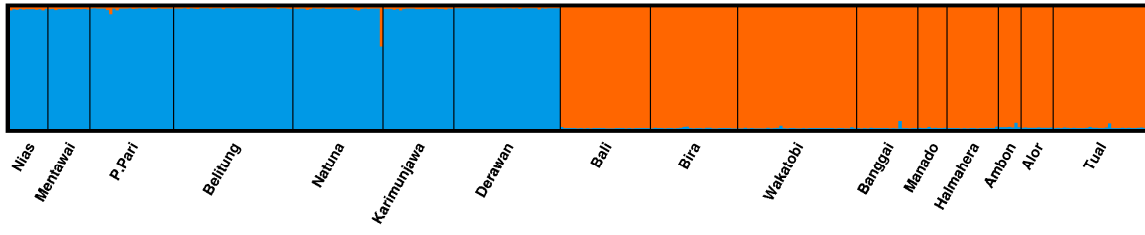


Table 1. *Tripneustes gratilla* locus specific error rates calculated by re-genotyping a randomly chosen subset of 10% of the total number of samples (see Selkoe & Toonen 2006).

Locus	% Error
TG20	4.2
TG60	0.0
TG01	2.1
TG04	2.1
TG07	2.1
TG28	0.0
TG26	6.3
TG39	0.0
TG27	0.0
TG11	8.3
TG02	4.2
TG51	6.3

Table 2, *Tripneustes gratilla* pair wise F'_{ST} values above and F_{ST} values below the diagonal. Bolded values are significant $P \leq 0.05$.

	Alor	Ambon	Bali	Banggai	Belitung	Bira	Derawan	Halmahera	Karimunjawa	Manado	Mentawai	Natuna	Nias	Pari	Tual	Wakatobi
Alor	--	0.20	0.18	0.13	0.25	0.20	0.30	0.32	0.25	0.26	0.32	0.26	0.44	0.26	0.13	0.18
Ambon	0.06	--	0.03	-0.16	0.04	-0.04	0.11	0.18	0.11	0.03	0.10	0.10	0.19	0.09	0.00	-0.03
Bali	0.04	0.01	--	-0.04	0.10	0.00	0.12	0.06	0.12	0.07	0.14	0.08	0.21	0.10	0.03	-0.01
Banggai	0.04	-0.01	0.00	--	0.07	-0.06	0.06	0.09	0.08	0.02	0.08	0.05	0.14	0.08	-0.02	-0.05
Belitung	0.05	0.01	0.02	0.02	--	0.07	0.04	0.19	0.04	0.20	0.06	-0.01	0.13	0.02	0.09	0.07
Bira	0.05	0.00	0.00	-0.01	0.02	--	0.10	0.08	0.11	0.09	0.13	0.09	0.20	0.09	0.00	-0.03
Derawan	0.07	0.03	0.03	0.02	0.01	0.02	--	0.22	0.01	0.25	0.00	0.03	0.06	0.00	0.12	0.10
Halmahera	0.07	0.04	0.01	0.03	0.04	0.02	0.05	--	0.26	0.20	0.22	0.17	0.34	0.18	0.13	0.08
Karimunjawa	0.06	0.03	0.03	0.02	0.01	0.02	0.00	0.05	--	0.21	0.01	0.05	0.09	0.00	0.12	0.09
Manado	0.06	0.02	0.02	0.02	0.04	0.02	0.06	0.04	0.05	--	0.23	0.17	0.28	0.19	0.09	0.07
Mentawai	0.08	0.04	0.03	0.03	0.02	0.03	0.00	0.05	0.01	0.06	--	0.05	0.06	0.03	0.14	0.12
Natuna	0.05	0.03	0.02	0.02	0.00	0.02	0.01	0.03	0.01	0.03	0.01	--	0.13	0.00	0.08	0.05
Nias	0.11	0.06	0.05	0.05	0.03	0.05	0.02	0.08	0.03	0.07	0.02	0.03	--	0.07	0.22	0.18
Pari	0.06	0.03	0.02	0.02	0.01	0.02	0.00	0.04	0.00	0.04	0.01	0.00	0.02	--	0.10	0.08
Tual	0.03	0.01	0.01	0.00	0.02	0.00	0.03	0.03	0.03	0.02	0.03	0.02	0.06	0.02	--	0.01
Wakatobi	0.04	0.01	0.00	0.00	0.02	0.00	0.02	0.02	0.02	0.02	0.03	0.01	0.05	0.02	0.00	--

Table 3. *Tripneustes gratilla* AMOVA results based upon population groupings determined by STRUCTURE 2.3.4 (Pritchard et al. 2000).

	Degrees of Freedom	Variance Component	Percentage of Variation	Fixation Indices	<i>P</i>
Among groups (F_{CT})	1	0.07	1.74	0.02	<0.001
Among populations within groups (F_{SC})	14	0.06	1.26	0.01	<0.001
Within populations (F_{ST})	690	4.81	97.00	0.03	<0.001

CHAPTER 4: Population genetics of the banded coral shrimp, *Stenopus hispidus*, in the Indo-Malay archipelago

Abstract

The population genetics of 387 individual *Stenopus hispidus* collected from 11 sampling locations throughout Indonesia was examined with 15 microsatellite loci. Bayesian clustering methods revealed evidence for three genetically distinct clusters . While genetic structure in this marine invertebrate is low, it is unexpected on this geographic scale, in a species with a global distribution and a pelagic larval duration of up to 210 days. Factors other than larval dispersal must be promoting population divisions. In this case, genetic partitions correspond to differing habitat types, possibly allowing selection to augment oceanographic and geographic barriers to gene flow. The identification of cryptic diversity in *S. hispidus*, and concordance with population separations observed in other reef biota, will aid in the identification of global hotspots of biodiversity allowing countries to meet conservation priorities such as those defined by the Convention on Biological Diversity and The International Union for Conservation of Nature.

Introduction

Stenopus hispidus, the banded coral shrimp has a pan-tropical distribution extending into some temperate areas. Members of the genus *Stenopus* are among the 10 most traded species of marine ornamentals worldwide, between 1998 and 2003 exporters recorded 42,802 individuals collected and exported globally (Wabnitz et al. 2003). The real numbers are likely to be much higher as a result of illegal, unregulated and unreported fishing. Indonesia is a major exporter in the marine ornamental trade, accounting for 44% of the global trade (Wabnitz et al. 2003). The Solomon Islands, like Indonesia, is one of the six countries that contain portions of the Coral Triangle, and it is likely that similar, or more intensive collecting of *S. hispidus* is occurring throughout the more populous Indonesian Archipelago.

S. hispidus is one of a number of stenopodidean shrimps that have symbiotic relationships with fish removing external parasites and injured flesh (Limbaugh et al. 1961). Cleaning shrimps (e.g., *S. hispidus*), are part of a community of cleaners and intact communities of cleaning organisms can play an important role in maintaining reef fish health and the structuring of fish communities (Bshary et al. 2007). Limbaugh (1961) demonstrated that the removal of cleaning organisms can cause fish to move to areas that did have them, and fish that remained in areas without cleaning species became infected by fungal growth. Bshary (2003) and Grutter (2003) also demonstrated that the presence of cleaning organisms promotes local fish diversity. Many reef fish and invertebrates play an active grazing role, which can aid in the control and prevention of algal overgrowth. If grazing is reduced via the removal of cleaning species for the aquarium trade as suggested by Bshary (2003) and Grutter (2003), coral reefs can become smothered by faster growing algae resulting in habitat loss and ecosystem instability (Adam et al. 2015). Coral reefs on the whole are notoriously overfished (Jackson et al. 2001) and if organisms such as cleaner wrasse and cleaner shrimps (i.e. *S. hispidus*) are removed to supply the increasing global demand for the aquarium trade,

it is likely that fish biomass and diversity will decrease further (Limbaugh 1961).

Consequently, it is becoming increasingly important to understand the genetics that are shaping populations of *S. hispidus* to manage exploitation of this important coral reef associated invertebrate.

Once the female *S. hispidus* has mated, a mass of eggs are deposited under the abdomen. These eggs hatch approximately 16 days later and then pass through nine larval stages before settlement. The larval phase can be extended for up to 210 days in the absence of suitable habitat (Fletcher et al. 1995). This long larval duration gives *S. hispidus* the potential to disperse great distances, and I hypothesize that this long dispersal potential coupled with the seasonal directionality of water currents throughout the Indonesian Archipelago (Tomascik et al. 1997) should facilitate archipelago wide genetic homogeneity and an absence of population structure.

The Indonesian Archipelago contains the largest portion of the Coral Triangle, a region spanning six countries. The Coral Triangle contains the greatest shallow water biodiversity on the planet (Briggs 2000; Allen & Werner 2002; Hoeksema 2007; Allen 2008), but the processes that have created this remarkable biodiversity are poorly understood. Mayr (1954) concluded that speciation in the oceans follows the same rules as speciation in terrestrial environments where barriers prevent gene flow and the resulting isolation promotes speciation in allopatry. This model of speciation can be difficult to apply in a three-dimensional marine environment with few apparent barriers to dispersal. This is especially true throughout the current-swept Indonesian Archipelago where currents should facilitate larval dispersal

Several theories explaining the high levels of biodiversity in the region have been developed and it is likely that at some point in time each theory has contributed to create the elevated levels of biodiversity observed. The three main theories are; (1) Center of speciation

model, which states that new species are created in the Coral Triangle, (2) Center of accumulation model, which suggests that new species are formed in periphery regions and eventually make their way into the Coral Triangle and (3) Center of overlap model that attributes the high biodiversity to the fact that Indian and Pacific ocean flora and fauna overlap in the area known as the Coral Triangle. See Bowen et al. (2013) for a more comprehensive discussion of these ideas and support for each hypothesis has been uncovered (Jokiel 1992; Briggs 2003; Eble et al. 2011; Cowman & Bellwood 2013; Gaither & Rocha 2013).

An understanding of genetic connectivity pertaining to marine organisms has been identified as a missing, yet important component of Marine Protected Area (MPA) design (Sala et al 2002; Evans et al. 2014). By examining the population structure in *S. hispidus*, I add to the growing body of work that has already been completed in the region (Lourie & Vincent 2004; Rohfritsch & Borsa 2005; Kochzius 2008; Timm & Kochzius 2008; Barber et al. 2011; Ackiss et al. 2013; Arlyza et al. 2013; DeBour et al. 2014) with the intention that future MPA design and conservation policy within Indonesia will incorporate genetic information into new, all-encompassing marine conservation strategies.

Materials and Methods

A single chela (claw) was collected from 387 individuals at 11 sampling sites throughout the Indonesian Archipelago between 2010 and 2011 (Figure 1). All chelae were stored in a 20% salt saturated DMSO solution and DNA extraction followed a modified DNeasy Blood and Tissue Kit protocol (Qiagen, Valencia, California, USA). Proteinase K was purchased from Creative BioMart (Shirley, New York, USA). EconoSpin All-In-One Mini Spin Columns were substituted for Qiagen spin columns (Epoch Life Science, Missouri

City, Texas, USA). All extraction parameters (volumes, times and temperatures) followed instructions for the DNeasy Blood and Tissue Kit for animal tissues.

Multiplex polymerase chain reactions (PCR) were performed using primers specifically developed for *S. hispidus* (Wainwright et al. 2012) in 5 μ L reactions. Each reaction contained; 2.5 μ L of MyTaq™ HS Mix (Bioline, Taunton, MA, USA), 1 μ L of DNA diluted 1:50, 0.5 μ L of deionized water and 1 μ L of primer mix. Multiplex reaction consisted of three primers utilizing a modified M13-tailed primer method described by Boutin-Ganache et al. (2001) to produce fluorescently labelled amplicons. Primer mix contained 0.35 pmol of tailed forward primer, 1.5 pmol of the reverse and dye-labeled primers for each locus (see Wainwright et al. 2012 for details). All multiplex mixes were designed with MULTIPLEX MANAGER (Holleley and Geerts 2009) and details of each multiplex mix can be found in Appendix C, Table S1. PCR conditions followed a two-step protocol beginning with 2 minutes at 95° C, followed by 30 cycles of 95° C for 30 seconds and 50° C for 2.5 minutes, as per manufacturer's instruction.

PCR products were resolved with an ABI 3730 Genetic Analyzer and sized with GENEIOUS 6.1.6 (Biomatters, San Francisco, CA, USA; <http://www.geneious.com/>). MICRO-CHECKER 2.3.3 (Van Oosterhout et al. 2004) was used to test for possible scoring errors and the presence of null alleles. Error rates (Table 1) were calculated for each locus by re-genotyping an arbitrary chosen subset of 48 samples, as recommended by Selkoe & Toonen (2006). Potential deviations from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium, and locus-by-locus summary statistics were calculated with ARLEQUIN 3.5.1.2 (Excoffier & Lischer 2010; Appendix C, Table S2)

The genetic structure between populations was investigated using the program STRUCTURE 2.3.4 (Pritchard et al. 2000). Error rates were calculated for each locus and STRUCTURE runs were performed in two different ways; (1) only loci showing a $\leq 2.1\%$ error

rate and (2) all loci included (see Table 1 for details of locus specific error rates). I used an admixture model with location information as a prior and a burn in period of 100,000 steps followed by another 100,000 steps. K values were estimated by initially setting the value of K from 1 to 10 (the number of sample locations). Each value of K was run for 10 iterations and the optimum K was determined using the method of Evanno et al. (2005) applied in STRUCTURE HARVESTER 0.6.94 (Earl 2012). The value of K was initially determined using all loci and then confirmed using only loci that showed a $\leq 2.1\%$ error rate (Appendix C, figures S1 and S2). Convergence was assumed if the alpha and likelihood values were stable and all runs returned similar results. STRUCTURE results were visualized using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and DISTRUCT 1.1 (Rosenberg 2004) implemented in CLUMPAK (Kopelman et al. 2015). AMOVA analysis was performed using the default settings in ARLEQUIN and groups were assigned based on the results of STRUCTURE. Pairwise F'_{ST} and F_{ST} values were calculated with GENODIVE 2.0b23 (Meirmans & Van Tienderen 2004). See Bird et al. (2011) for a complete discussion on the various merits and failings of the different metrics used to measure genetic differentiation. Patterns of isolation by distance were tested using F'_{ST} and the default settings in the web-based program Isolation by Distance Web Service 3.23 (Jenson et al. 2005; <http://ibdws.sdsu.edu/~ibdws/>). Geographical distances between populations were calculated in Google Earth as the shortest possible distance between populations via sea.

Results

Initially 387 individuals were genotyped at 18 loci, but three loci were subsequently removed because of difficulties in achieving successful amplification or scoring as a result of stutter and or non-specific amplification (see Appendix C, Table S2 for details of loci included in analysis).

The minimum number of alleles observed was two at locus Sten32 in the sample from Bira and the maximum number of alleles (22) was observed in the sample from Manado at locus Sten23. Observed and expected heterozygosities ranged from 0.172 to 0.974 and 0.200 to 0.933 respectively (Appendix C, Table S2). Several samples have loci that deviate significantly from Hardy-Weinberg equilibrium following Bonferroni adjustments ($P = 0.003$) and several loci show evidence of null alleles (Appendix C, Table S2). After Bonferroni adjustment, no pairs of loci showed significant linkage disequilibrium ($P = 0.0002$). Several pairs of sample sites showed significant F'_{ST} and F_{ST} values $P = < 0.05$ (Table 2)

Following Evanno et al. (2005), the most likely value of $K = 3$ (See Appendix C, Figures S1 and S2). One group was comprised of sampling locations Bali, Derwan, Bira, Manado and Komodo. A second group consisted of Banggai, Ambon, Halmahera, Alor and Wakatobi with a third group consisting of the sample from Tual (Figure 2). When comparing both data sets with differing numbers of loci depending upon error rates, both data sets showed the same assignments of populations to the same clusters (Figure 2).

An AMOVA was performed using data from all loci on the three clusters resulting from STRUCTURE analysis and 98.2% of the variation is contained within the populations (Table 3). There was no significant pattern of IBD over all populations ($r = 0.1312$, $p = 0.7641$).

Discussion

The population structure of *S. hispidus* in Indonesia is characterized by three genetically distinguishable regions corresponding to the western, central and eastern portions of Wallacea (Figure 1). The central region of Wallacea contains the Banda Sea, with depths in the Weber basin exceeding 7000 m (van Aken et al. 2009). Wang et al. (2015) used a set of biophysical parameters, including sea surface temperatures, chlorophyll a concentration, currents and salinity patterns to delineate marine regions throughout central Indonesia. Using

these parameters, Wang et al. (2015) divided central Indonesia and Wallacea into distinctive biophysical regions, each of the defined regions is expected to show similar habitat type and ecosystem functioning. The clusters defined by STRUCTURE follow these delineations very closely. It is possible that *S. hispidus* is becoming adapted to differing local environmental regimes, those individuals not suited to a particular habitat are selected against and this could be contributing to reproductive isolation. Oceanographic barriers to gene flow could be augmented by ecological differences, the starting point for parapatric speciation (Rocha & Bowen 2008). Once this isolation becomes effective, neutral markers (e.g., the microsatellites used in this study) would begin to diverge as the homogenizing effects of gene flow are curtailed.

Deviations from Hardy–Weinberg equilibrium (HWE) are frequently attributed to incorrect scoring, locus specific amplification issues or other data errors. The Hardy-Weinberg equilibrium is based on a number of key assumptions, which, if broken may lead to deviations. The assumption of random mating may be frequently violated in marine organisms, especially those with a sessile adult phase. Whilst *S. hispidus* cannot be defined as truly sessile, adults show a very high degree of site fidelity, some degree of territoriality, and home ranges of less than 1 m² (Limbaugh et al. 1961; Chockley & St. Mary 2003). Truly random mating is unlikely in these circumstances and consequently the HWE assumption of random union of gametes might be violated. It is probable that real biological processes are causing genuine departures from HWE and these departures are not the artifacts of incorrect scoring or locus specific issues. The deviations from HWE that we observed in *S. hispidus* may be widespread and common in marine taxa (DeBoer et al. 2014)

The majority of samples in this study were collected from a region of Indonesia known as Wallacea, bounded by Wallace’s line to the west (Huxley 1868) and Lydecker’s line

(Lydekker 1896) to the east (Appendix C, Figure S3). Wallacea has exceptionally high biodiversity and this area has been identified as a global biodiversity hotspot (Myers et al 2012). Despite having a distance of approximately 2000 km at its widest point and a discernible lack of any obvious barriers to dispersal, microsatellite data reveal three genetically distinguishable regions. The discovery of these partitions is surprising given the high dispersal potential of *S. hispidus* larvae. When the seasonal directionality of water currents are considered along with the high dispersal potential of *S. hispidus*, it would be logical to assume that genetic homogeneity should result. Work by Lessios et al. (2003) on the widely distributed sea urchin genus *Tripneustes* demonstrated that genetic structure could be absent on a global scale, but present on a regional scale. Although I am unable to address global genetic structure in *S. hispidus*, I did detect weak genetic structure at the regional scale throughout Indonesia (Figure 2 and Table 2). This observation is consistent with the findings of Weersing & Toonen (2009) who found that pelagic larval duration (PLD) is a poor predictor of gene flow and population structure. These observations help to explain and corroborate the existence of the weak genetic structure observed in *S. hispidus*. This structure was observed across relatively short distances in a species that has a very large range and very long PLD (Fletcher et al. 1995).

The identification and preservation of biodiversity has been recognized as essential for maintaining ecosystem services, providing food security throughout the planet as well as maintaining stable ecosystems and promoting resilience in changing climates (Duffy 2003, Millennium Ecosystem Assessment World Resources Institute 2005). Despite the 200+ day PLD of *S. hispidus*, the three genetically distinct clusters demonstrate that gene flow is restricted over the relatively short distances that make up the sampling region. Applying similar methods to other marine species throughout Indonesia and the Coral Triangle should allow us to identify other regions that contain cryptic biodiversity (Roberts et al. 2002). The

identification of this cryptic biodiversity can be used to designate high priority areas for conservation. For example, if several studies using different taxa all uncover previously unknown cryptic diversity in the same region, then this region would warrant a higher investment in conservation programs and enforcement to maintain and promote biodiversity. The identification of these areas allows global conservation priorities such as those defined by the Convention on Biological Diversity and the International Union for Conservation of Nature to be achieved (Myers et al. 2000; Hughes et al. 2002; Roberts et al. 2002; Millennium Ecosystem Assessment World Resources Institute 2005; Convention on Biological Diversity 2010; Brooks 2006; Selig et al. 2014). The cryptic diversity identified by this work is either the result of genetic drift acting upon isolated populations or selective pressures that have become apparent in a geographically localized fashion (Gorospe & Karl 2015). These selective pressures could be contributing to the formation of barriers to gene flow. If this is occurring, isolation may be the result accounting for the observed divergence seen in the neutral loci employed in this study. A growing body of literature is beginning to suggest that selective pressures may play an important role in population differentiation and may be one of the main drivers of speciation in the oceans, adding weight to our idea that populations of *S. hispidus* could be adapting to local environmental conditions (Ruzzante et al. 1996; Naciri et al. 1999; Bierne et al. 2003; Beheregaray & Sunnucks 2001; Jorgensen et al. 2005; White et al. 2010; Bird et al. 2012; Bowen et al. 2013; Shafer et al. 2013).

This research complements other work on a variety of Indonesian marine taxa in efforts to uncover patterns and magnitude of gene flow (Barber et al. 2000; 2006; 2011; Lourie & Vincent 2004; DeBoer et al. 2008, 2014; Kochzius & Nuryanto 2008; Timm & Kochzius 2008; Vagelli et al 2009; Carpenter et al. 2011; Starger et al. 2010; Timm et al. 2012; Ackiss et al. 2013; Deboer et al. 2014; Jackson et al. 2014).

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By adding to the body of work that has already been completed, I hope to resolve processes that have created the elevated biodiversity in the region. These studies in turn allow resource managers and policy makers to incorporate the best available scientific knowledge pertaining to genetic connectivity into conservation policy.

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Figures

Figure 1, Map showing the location of sample sites throughout Indonesia. Numbers in parenthesis indicate sample size from respective sampling location.

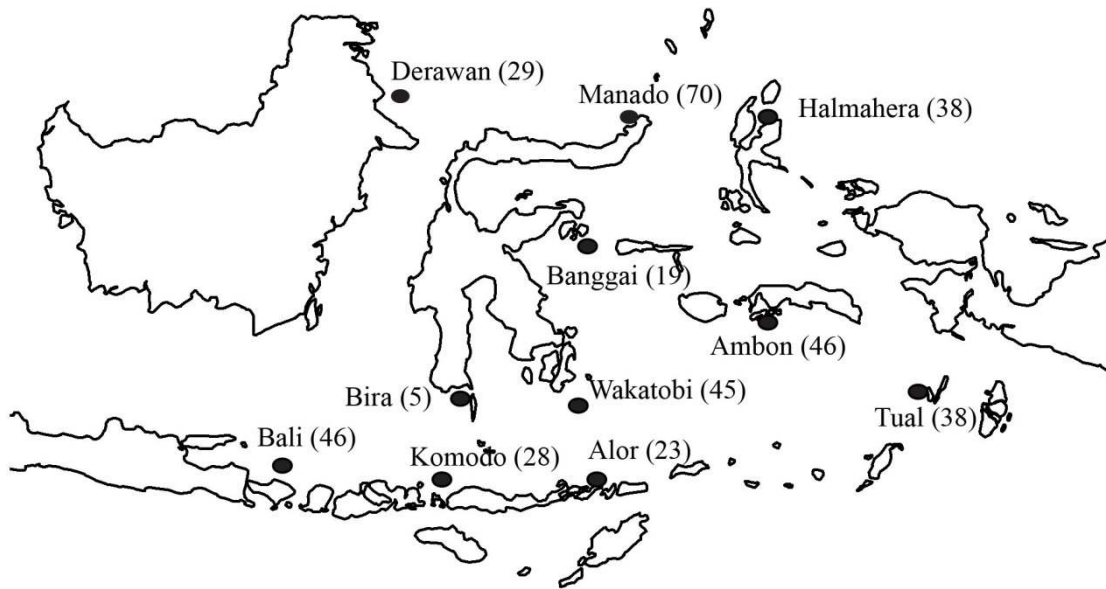


Table 1, Locus specific error rates calculated by re-genotyping an arbitrary chosen subset of 48 samples (see Selkoe & Toonen 2006).

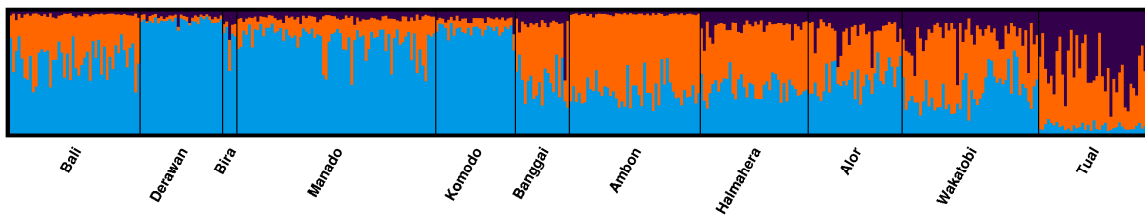
Locus	% Error
Sten01	2.1
Sten05	2.1
Sten10	0.0
Sten21	2.1
Sten23	2.1
Sten24	2.1
Sten27	2.1
Sten32	8.3
Sten36	2.1
Sten43	2.1
Sten72	0.0
Sten73	0.0
Sten76	8.3
Sten92	2.1
Sten94	4.2

Table 2, *Stenopus hispidus* pairwise F'_{ST} above and F_{ST} values below the diagonal. Bolded values are **not** significant at $p \leq 0.05$.

	Bali	Derawan	Bira	Manado	Komodo	Banggai	Ambon	Halmahera	Alor	Wakatobi	Tual
Bali	--	0.09	0.14	0.02	0.06	0.03	0.05	0.05	0.02	0.05	0.06
Derawan	0.03	--	0.02	0.04	-0.01	0.05	0.14	0.11	0.07	0.11	0.13
Bira	0.05	0.02	--	0.09	0.05	0.09	0.17	0.14	0.11	0.10	0.12
Manado	0.01	0.01	0.03	--	0.02	0.03	0.08	0.05	0.03	0.07	0.08
Komodo	0.02	0.00	0.03	0.01	--	0.01	0.10	0.07	0.06	0.06	0.11
Banggai	0.01	0.02	0.04	0.01	0.01	--	0.01	0.01	0.01	0.01	0.03
Ambon	0.02	0.05	0.06	0.02	0.03	0.01	--	0.01	0.02	0.00	0.04
Halmahera	0.01	0.03	0.04	0.01	0.02	0.01	0.01	--	0.03	0.02	0.04
Alor	0.01	0.03	0.03	0.01	0.02	0.01	0.01	0.01	--	0.02	0.00
Wakatobi	0.02	0.04	0.05	0.02	0.03	0.01	0.01	0.01	0.01	--	0.01
Tual	0.02	0.04	0.04	0.02	0.03	0.01	0.01	0.01	0.00	0.01	--

Figure 2, *Stenopus hispidus* Bayesian clustering analysis using STRUCTURE 2.3.4. For both runs, K=3 and the likelihood was the average of 10 runs. Sample sites arranged in an approximate west to east orientation. A) Only loci with $\leq 2,1\%$ error rate included, B) All loci.

A)



B)

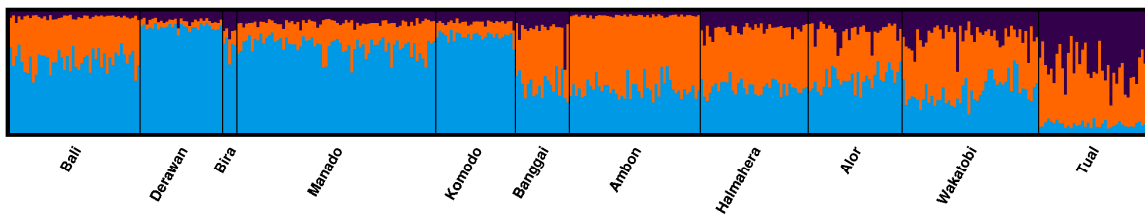


Table 3. *Stenopus hispidus* AMOVA results based upon population groupings as determined by STRUCTURE 2.3.4 (Pritchard et al. 2000).

	Degrees of Freedom	Variance Component	% Variation	Fixation Indices	<i>P</i>
Among groups (F_{CT})	2	0.03	0.65	0.006	0.010
Among populations within groups (F_{SC})	8	0.06	1.18	0.012	< 0.001
Within populations (F_{ST})	763	4.78	98.17	0.183	< 0.001

Chapter 5: Summary and conclusions.

The work detailed in previous chapters demonstrates that distinct genetic clusters can be identified throughout the Indonesian Archipelago in several species. This differentiation has implications for the preservation of biodiversity and the future evolutionary potential of marine species found throughout the Coral Triangle and Southeast Asia.

It is not unreasonable to suggest that the differing habitat conditions encountered throughout the archipelago (i.e., shallow shelves and deep waters of central Indonesia) may be responsible for the observed differentiation.

As a result of the Halmahera Eddy (HE) and the Indonesian Throughflow (ITF) the Banda Sea could be somewhat isolated from the Indian and Pacific Oceans. This isolation in conjunction with the observable differences in salinity, sea surface temperatures and chlorophyll a concentrations of the Banda Sea could be facilitating adaption to the unique environmental conditions in a geographically localized fashion (Gorospe & Karl 2015).

Chapters 2 & 3 provide support for the idea that the different habitat types seen across the relatively shallow waters of the Sunda Shelf (average water depths of 70 m) (Hanebuth et al. 2002) and the deep waters of central Indonesia, which can surpass 7000 m in the Weber Basin (van Aken et al. 2009) could be facilitating the observed genetic differentiation seen in several species. Results from chapter 2 show a general pattern of east-west differentiation in two species of seagrass; the same pattern is observable in *Tripneustes gratilla* (chapter 3). In addition to habitat differences this east-west differentiation could also be explained by colonization of the Sunda Shelf after periods of glacial maxima. During periods of glacial maxima sea levels dropped by 120 m completely exposing the Sunda Shelf to air causing the loss of all marine species found here. Sea levels returned to present day levels approximately 6000 years ago (Voris 2000) creating an unparalleled opportunity to exploit new habitat in

the likely absence of predators and competitors. Further work taking a coalescence based approach using DNA sequence data could provide support for this idea.

The results for the seagrass (Chapter 2), *Thalassia hemprichii* do not show a perfect pattern of east-west; differentiation as observed in *T. gratilla* and *Syringodium isoetifolium*. The genetic cluster observed across the Sunda Shelf in *T. hemprichii* extends out through the Lesser Sunda Islands. This extension is a likely consequence of the Indonesian Throughflow aiding in the dispersal of fragments or propagules from the Sunda Shelf as the ITF current makes its way through the Lesser Sunda Islands before exiting into the Indian Ocean.

Chapter 4 shows that genetic structure can exist over relatively short distances in a species that has a long pelagic larval duration (PLD). *Stenopus hispidus* larvae can remain in the water column for 210 days and given the nature of water currents, that can reverse and flow in opposite directions, I hypothesized that complete genetic homogeneity should be the result. Contrarily, I found evidence for three distinct genetic clusters. Green & Mous (2008) delineated the Coral Triangle into a set of ecoregions. An ecoregion is described as; a large unit of land or water containing a geographically distinct assemblage of species, natural communities, and environmental conditions. These ecoregions nearly perfectly explain the pattern of differentiation seen in *S. hispidus*. It is possible that *S. hispidus* is becoming locally adapted to the same conditions that allowed ecoregions to be identified and defined.

A growing body of literature is beginning to highlight the importance of local recruitment in the maintenance and recovery of populations following a disturbance (Jones et al. 1999; 2005; Almany et al. 2007; Planes et al. 2009; Saenz-Agudelo et al. 2011; Hogan et al. 2012; Salles et al. 2015; Markey et al. 2016). The observation of three distinct genetic clusters over a relatively short distance (1000 km) in a species with such a long PLD suggests that a degree of local recruitment in *S. hispidus* may be occurring, adding weight to the idea

that marine systems are not as open once thought, and local recruitment could be very important in the maintenance or recovery of marine populations following a disturbance.

The computer program Marxan (<http://www.uq.edu.au/marxan/>) has been specifically developed as a decision support tool aiding in conservation planning by identifying conservation priorities allowing them to be protected in the most cost efficient manner. Beger et al. (2014) specifically incorporated genetic data in Marxan planning and showed that conservation priorities changed when genetic data are included. If the differing results caused by the inclusion of genetic data are correct, or more accurate, then a compelling case can be made for the importance of genetics in conservation planning.

Genetic techniques and molecular methods can be used to identify regions of high genetic diversity. It is possible that these regions could contain the genetic diversity or adaptive potential allowing adaptation to climate change induced threats such as ocean acidification and coral bleaching (Selkoe et al. 2016). These areas of high genetic diversity should be designated as priority areas for conservation. Without the use of genetic tools, this diversity could be missed by even the most experienced field biologist.

The rapid decline in the cost of DNA sequencing associated with high throughput next generation technologies, make it much more feasible to attempt mass scale parentage analysis studies. Parentage analysis has the potential to unlock the “black box of larval dispersal” and actually uncover source and sink populations. If this does become possible, areas of reef that can be identified as exporters of larvae should receive a higher investment in the resources required for conservation.

The application of genetics to answer questions pertaining to biodiversity has the ability to greatly increase our understanding of the processes that have created it. Distinct genetic clusters can be identified throughout Indonesia. If the goal of conservation is to

conserve evolutionary potential a strong case can be made for conserving equal proportions of each genetic cluster. It is this evolutionary potential that that could allow future generations to adapt and persist in the face of future climate change or new and emerging threats. The preservation of biodiversity at all levels has been identified as a priority and is fundamental to the strategic plan on biodiversity defined by the United Nations and United Nations Environment Programme Convention on Biological Diversity (CBD). Given enough time the observed differentiation could lead to the creation of new species greatly enhancing and aiding in the maintenance of biodiversity; a priority for the International Union for the Conservation of Nature and the CBD.

The use of genetics and molecular tools in a conservation setting does begin to answer fundamental questions on how open or closed are marine populations. Traditionally, as a consequence of the long pelagic larval durations frequently observed in marine organisms, marine systems were viewed as open. That view is now beginning to be challenged in several species that have the potential to disperse great distances. Despite this dispersal potential, genetic structure can be identified over relatively short distances (e.g., *Stenopus hispidus*; Chapter 4) meaning that local recruitment could be a much more important process than originally thought.

There will not be a one size fits all solution to marine conservation. Marine conservation will require the synthesis of data from many disciplines (e.g., biological, oceanographic and social sciences) if it is to be successful. This work, along with others in the region that detail genetics and gene flow, does begin to shed light on the evolutionary processes that have and are shaping the Coral Triangle and the marine environment of S.E. Asia. This allows policy makers and resource managers to use the best available science pertaining to genetics, gene flow and evolution in future conservation plans. Admittedly,

genetics is not a silver bullet that will make conservation successful, but it can be an important consideration for the success of conservation programs. Nonetheless, there is still much work to be performed in the region before we understand the full implications of connectivity and gene flow in relation to conservation.

If work like this is to have a bearing on conservation it needs to be communicated to policy makers and resource managers in a manner that is understandable and applicable. The implications of this type work may not be immediately apparent to someone who has not spent eight years studying it. Frequently, resource managers and policy makers have to make rapid decisions, if the full implications of a body of work (e.g., genetics) are not successfully communicated it can be glossed over and excluded from conservation plans. As demonstrated by *Beger et al. (2014)* the inclusion of genetic data can change conservation priorities. Omission of genetic data resulting from poor communication could be detrimental to future conservation planning.

With a concerted effort from the many fields involved in conservation, along with the integration of the resulting data from each of these disciplines we stand the greatest possible chance of preventing the so called coral reef crisis and ensuring the persistence of coral reef ecosystems into the distant future.

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APPENDIX A

Table S1. *Syringodium isoetifolium*. Details of primers used in each multiplex mix. Bolded primers indicate which primers were not used in the analysis due to null alleles or scoring difficulties. Primers in mixes 6 and 7 are from Matsuki et al. (2013).

Mix	Primers and Labels
1	SI59 FAM, SI60 VIC, SI32 NED
2	SI55 FAM, SI23 VIC, SI99 NED, SI27 PET
3	SI109 FAM, SI08 VIC, SI57 NED
4	SI81 FAM, SI110 VIC , SI 115 NED, SI 104 PET
5	SI117 FAM, SI89 VIC, SI103 NED, SI 108 PET
6	Si021 FAM, Syris004-2 NED, Syris270 VIC, Syris277 PET
7	Si003 NED, Si0014 PET, Si019 FAM, Syris284 VIC

Table S2. *Thalassia hemprichii*. Details of primers used in each multiplex mix. Bolded primers indicate which primers were not used in the analysis due to null alleles or scoring difficulties.

Mix	Primers and Labels
1	TH43 FAM, TH53 VIC, TH72 NED, TH14 PET
2	TH7 FAM, TH44 VIC, TH45 NED, TH75 PET
3	TH2 FAM , TH37 VIC, TH50 NED, TH66 PET
4	TH32 VIC, TH39 NED, TH73 PET, TH17 FAM
5	TH48FAM, TH49 VIC, TH34 NED , TH51 PET

Table S3. *Syringodium isoetifolium* locus specific error rates calculated by re-genotyping a randomly chosen subset of 10% of the total number of samples (see Selkoe & Toonen 2006). Loci Si021 is from Matsuki et al. (2013).

Locus	% Error
SI08	0.0
SI023	0.0
SI027	0.0
SI055	0.0
SI059	0.0
SI060	0.0
SI089	0.0
SI099	0.0
SI103	0.0
SI104	0.0
SI108	2.1
SI109	0.0
SI115	4.2
SI117	18.8
Si021	4.2

Table S4. *Thalassia hemprichii* locus specific error rates calculated by re-genotyping a randomly chosen subset of 10% of the total number of samples (see Selkoe & Toonen 2006).

Locus	% Error
TH07	6.3
TH14	2.1
TH17	6.3
TH32	6.3
TH37	4.2
TH39	8.3
TH43	2.0
TH44	14.6
TH45	14.6
TH48	6.3
TH49	14.6
TH50	6.3
TH53	8.3
TH72	10.4
TH73	4.2
TH75	6.3

Table S5. *Syringodium isoetifolium* locus by locus summary statistics for each sample. Sample site (number of individuals) number of alleles (N_a), observed (H_o) and expected heterozygosity (H_e). After Bonferroni adjustments, p -values were set to 0.003 to test for departures from Hardy-Weinberg equilibrium and significant values are highlighted in bold. The potential presence of null alleles was tested for using MICROCHECKER 2.3.3 (Van Oosterhout et al. 2004). # indicates locus was monomorphic in this population.

Population (n)	Locus															Mean
	SI08	SI23	SI27	SI55	SI59	SI60	SI89	SI99	SI103	SI104	SI108	SI109	SI115	SI117	SI021	
Alor (22)																
N_a	1	4	6	4	2	2	2	2	3	2	4	3	6	5	7	3.533
H_o	#	0.318	0.591	0.409	0.182	0.182	0.136	0.273	0.636	0.227	0.455	0.364	0.773	0.591	0.864	0.429
H_e	#	0.428	0.617	0.360	0.304	0.406	0.130	0.241	0.582	0.206	0.616	0.348	0.810	0.634	0.799	0.463
P	#	0.023	0.111	1.000	0.107	0.018	1.000	1.000	0.479	1.000	0.003	0.371	0.271	0.488	0.686	
Null alleles	#	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No	
Bali (25)																
N_a	1	2	2	4	2	3	1	1	2	2	3	2	2	4	5	2.400
H_o	#	0.360	0.240	0.320	0.120	0.480	#	#	0.400	0.360	0.360	0.280	0.240	0.760	0.600	0.377
H_e	#	0.497	0.216	0.479	0.184	0.562	#	#	0.470	0.350	0.515	0.246	0.372	0.584	0.685	0.430
P	#	0.223	1.000	0.011	0.198	0.532	#	#	0.662	1.000	0.042	1.000	0.102	0.111	0.069	
Null alleles	#	No	No	No	No	No	#	#	No	No	No	No	No	No	No	
Banggai (17)																
N_a	2	5	5	3	2	2	2	3	4	2	3	3	8	5	7	3.733
H_o	0.118	0.882	0.765	0.471	0.294	0.353	0.059	0.353	0.353	0.294	0.824	0.765	0.941	0.765	0.588	0.522
H_e	0.214	0.779	0.709	0.383	0.258	0.299	0.059	0.314	0.549	0.258	0.676	0.554	0.856	0.774	0.831	0.501
P	0.179	0.059	0.050	0.639	1.000	1.000	1.000	1.000	0.004	1.000	0.008	0.027	0.262	0.002	0.021	
Null alleles	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No	Yes	

Bangka (10)

N_a	1	2	2	2	1	2	1	2	2	2	3	2	5	4	3	2.267
H_o	#	0.600	0.800	0.700	#	0.700	#	0.100	0.300	0.700	0.800	0.700	0.800	0.800	0.800	0.650
H_e	#	0.526	0.505	0.479	#	0.479	#	0.100	0.268	0.479	0.563	0.521	0.716	0.647	0.611	0.491
P	#	1.000	0.173	0.221	#	0.219	#	1.000	1.000	0.220	0.107	0.520	0.012	0.008	0.160	
Null alleles	#	No	No	No	#	No	#	No	No	No	No	No	No	No	No	

Belitung (10)

N_a	2	4	1	1	1	2	1	1	1	2	2	2	3	4	3	2.000
H_o	1.000	0.700	#	#	#	0.700	#	#	#	0.700	0.600	0.700	0.600	0.700	0.800	0.722
H_e	0.526	0.658	#	#	#	0.479	#	#	#	0.521	0.526	0.521	0.637	0.721	0.542	0.570
P	0.006	0.344	#	#	#	0.220	#	#	#	0.520	1.000	0.520	0.441	0.010	0.172	
Null alleles	No	No	#	#	#	No	#	#	#	No	No	No	No	No	No	

Bira (4)

N_a	1	2	3	2	2	1	1	1	2	2	3	1	4	3	2	2.000
H_o	#	0.750	1.000	0.750	0.750	#	#	#	0.750	0.500	0.750	#	1.000	1.000	0.750	0.800
H_e	#	0.536	0.679	0.536	0.536	#	#	#	0.536	0.429	0.750	#	0.786	0.750	0.536	0.607
P	#	1.000	0.316	1.000	1.000	#	#	#	1.000	1.000	0.058	#	0.056	1.000	1.000	
Null alleles	#	No	No	No	No	#	#	#	No	No	No	#	No	No	No	

Derawan (16)

N_a	1	3	3	2	2	3	1	3	2	1	2	3	5	4	5	2.667
H_o	#	0.875	0.625	0.125	0.313	0.438	#	0.313	0.813	#	0.813	0.500	0.875	0.750	0.813	0.604
H_e	#	0.669	0.659	0.121	0.272	0.365	#	0.433	0.514	#	0.514	0.548	0.780	0.726	0.734	0.528
P	#	0.001	0.001	1.000	1.000	1.000	#	0.425	0.040	#	0.039	0.004	0.000	0.000	0.019	
Null alleles	#	No	No	No	No	No	#	No	No	#	No	No	No	No	No	

Halmahera (16)

N_a	2	4	6	2	1	2	2	2	4	2	3	4	10	5	8	3.800
H_o	0.063	0.625	0.500	0.375	#	0.500	0.125	0.375	0.500	0.250	0.438	0.625	0.625	0.688	0.813	0.464
H_e	0.063	0.683	0.550	0.508	#	0.484	0.121	0.315	0.619	0.226	0.635	0.639	0.736	0.724	0.837	0.510
P	1.000	0.308	0.238	0.347	#	1.000	1.000	1.000	0.109	1.000	0.049	0.816	0.221	0.068	0.158	
Null alleles	No	No	No	No	#	No	No	No	No	No	No	No	No	No	No	

Karimunjawa (10)

N_a	2	3	1	1	1	2	3	1	2	2	2	2	2	1	4	1.933
H_o	0.700	1.000	#	#	#	0.600	0.700	#	0.800	0.400	0.100	0.800	0.400	#	0.700	0.620
H_e	0.521	0.574	#	#	#	0.442	0.668	#	0.505	0.442	0.100	0.505	0.337	#	0.742	0.484
P	0.521	0.007	#	#	#	0.479	0.057	#	0.173	1.000	1.000	0.172	1.000	#	0.197	
Null alleles	No	No	#	#	#	No	No	#	No	No	No	No	No	#	No	

Luwuk (5)

N_a	1	2	3	3	1	2	1	1	2	2	3	2	4	5	1	2.200
H_o	#	0.400	0.400	0.600	#	0.400	#	#	0.800	0.200	0.600	0.400	0.600	1.000	#	0.540
H_e	#	0.356	0.644	0.622	#	0.356	#	#	0.533	0.200	0.689	0.356	0.778	0.822	#	0.536
P	#	1.000	0.366	1.000	#	1.000	#	#	0.428	1.000	0.303	1.000	0.186	0.898	#	
Null alleles	#	No	No	No	#	No	#	#	No	No	No	No	No	No	#	

P.Pari (6)

N_a	2	2	1	1	1	2	1	1	2	2	2	2	3	2	1	1.667
H_o	0.833	0.667	#	#	#	0.667	#	#	0.667	0.667	0.667	0.667	0.500	0.667	#	0.667
H_e	0.530	0.485	#	#	#	0.545	#	#	0.545	0.545	0.485	0.545	0.439	0.545	#	0.519
P	0.394	1.000	#	#	#	1.000	#	#	1.000	1.000	1.000	1.000	1.000	1.000	#	
Null alleles	No	No	#	#	#	No	#	#	No	No	No	No	No	No	#	

S.Nasik (4)

N_a	2	3	2	1	1	2	1	1	2	2	2	2	3	3	4	2.067
H_o	0.750	0.750	0.250	#	#	0.750	#	#	0.750	0.250	0.000	0.750	1.000	0.750	0.750	0.614
H_e	0.536	0.607	0.250	#	#	0.536	#	#	0.536	0.250	0.429	0.536	0.679	0.750	0.750	0.532
P	1.000	1.000	1.000	#	#	1.000	#	#	1.000	1.000	0.143	1.000	0.314	0.057	0.317	
Null alleles	No	No	No	#	#	No	#	#	No	No	No	No	No	No	No	
Tual (9)																
N_a	1	3	2	1	1	2	3	1	2	3	4	3	3	1	2	2.133
H_o	#	0.778	1.000	#	#	0.444	0.889	#	0.889	0.667	0.444	0.778	0.778	#	0.000	0.667
H_e	#	0.621	0.529	#	#	0.366	0.621	#	0.523	0.699	0.706	0.660	0.647	#	0.366	0.574
P	#	1.000	0.010	#	#	1.000	0.279	#	0.056	0.006	0.020	0.183	0.428	#	0.012	
Null alleles	#	No	No	#	#	No	No	#	No	No	No	No	No	#	Yes	
Wakatobi (11)																
N_a	1	4	2	2	2	2	2	2	2	2	3	3	6	3	4	2.667
H_o	#	0.727	0.727	0.273	0.364	0.455	0.091	0.273	0.545	0.364	0.636	0.545	0.727	0.636	0.455	0.487
H_e	#	0.654	0.485	0.247	0.485	0.368	0.091	0.247	0.416	0.312	0.567	0.437	0.810	0.645	0.576	0.453
P	#	0.073	0.192	1.000	0.538	1.000	1.000	1.000	0.503	1.000	1.000	1.000	0.028	0.038	0.028	
Null alleles	#	No	No	No	No	No	No	No	No	No	No	No	No	No	No	
Mean Alleles	1	3	3	2	1	2	2	2	2	2	3	2	5	4	4	
Mean H_o	0.577	0.674	0.627	0.447	0.337	0.513	0.333	0.281	0.631	0.429	0.535	0.606	0.704	0.759	0.661	
Mean H_e	0.398	0.577	0.531	0.415	0.340	0.437	0.282	0.275	0.507	0.378	0.555	0.494	0.670	0.694	0.667	

Table S6. *Thalassia hemprichii* locus by locus summary statistics for each sample. Sample site (number of individuals) number of alleles (N_a), observed (H_o) and expected heterozygosity (H_e). After Bonferroni adjustments, p -values were set to 0.003 to test for departures from Hardy-Weinberg equilibrium and significant values are highlighted in bold. The potential presence of null alleles was tested for using MICROCHECKER 2.3.3 (Van Oosterhout et al. 2004).

Population (n)	Locus																Mean
	TH7	TH14	TH17	TH32	TH37	TH39	TH43	TH44	TH45	TH48	TH49	TH50	TH53	TH72	TH73	TH75	
Alor (28)																	
N_a	5	2	3	6	9	6	4	4	6	4	3	3	3	6	6	4	4.625
H_o	0.679	0.536	0.107	0.632	0.786	0.721	0.590	0.679	0.821	0.750	0.786	0.214	0.714	0.821	0.750	0.571	0.635
H_e	0.601	0.503	0.105	0.893	0.779	0.910	0.893	0.632	0.700	0.588	0.554	0.198	0.502	0.732	0.666	0.544	0.612
P	0.162	1.000	1.000	0.000	0.450	0.000	0.001	0.503	0.772	0.056	0.008	1.000	0.018	0.120	0.982	0.666	
Null alleles	No	No	No	Yes	No	No	Yes	No	No	No	No	No	No	No	No	No	
Ambon (27)																	
N_a	3	3	5	6	3	5	4	3	5	4	11	5	5	5	3	3	4.563
H_o	0.407	0.407	0.593	0.926	0.630	1.000	0.519	0.333	0.926	0.481	1.000	0.556	0.778	0.667	0.519	0.481	0.639
H_e	0.434	0.481	0.552	0.772	0.557	0.748	0.454	0.338	0.649	0.395	0.802	0.639	0.679	0.588	0.542	0.400	0.564
P	0.654	0.250	0.659	0.000	0.208	0.000	0.861	1.000	0.005	0.776	0.015	0.031	0.288	0.501	0.455	0.802	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	
Banggai (19)																	
N_a	3	2	4	6	3	4	4	4	7	5	8	5	5	5	5	4	4.625
H_o	0.526	0.526	0.632	1.000	0.579	1.000	0.789	0.684	0.895	0.842	1.000	0.737	0.895	0.579	0.737	0.526	0.747
H_e	0.522	0.444	0.573	0.795	0.582	0.609	0.607	0.649	0.654	0.687	0.811	0.703	0.696	0.708	0.553	0.559	0.635

<i>P</i>	1.000	0.607	0.821	0.188	0.451	0.000	0.133	0.969	0.572	0.410	0.280	0.663	0.340	0.342	0.520	0.650
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No

Belitung (21)

N_a	5	3	2	6	5	6	2	5	4	5	3	3	4	3	3	3	3.875
H_o	0.524	0.571	0.190	1.000	0.762	1.000	0.524	0.429	0.905	0.714	1.000	0.429	0.905	0.714	0.381	0.714	0.673
H_e	0.702	0.539	0.177	0.736	0.708	0.777	0.470	0.497	0.654	0.633	0.575	0.521	0.691	0.563	0.396	0.591	0.577
<i>P</i>	0.073	0.688	1.000	0.004	0.169	0.400	0.664	0.563	0.010	0.397	0.000	0.470	0.054	0.256	0.755	0.185	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Bira (21)

N_a	4	3	2	6	7	5	2	5	4	4	4	4	4	8	3	5	4.375
H_o	0.571	0.238	0.048	0.952	0.857	1.000	0.524	0.762	0.952	0.810	0.667	0.571	0.667	0.857	0.714	0.857	0.690
H_e	0.557	0.220	0.048	0.708	0.840	0.736	0.508	0.732	0.672	0.627	0.484	0.489	0.594	0.763	0.617	0.707	0.581
<i>P</i>	0.045	1.000	1.000	0.020	0.312	0.069	1.000	0.481	0.013	0.147	0.226	0.353	0.280	0.538	0.856	0.481	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Derawan (32)

N_a	5	4	3	7	9	8	2	4	5	6	6	5	7	8	5	4	5.500
H_o	0.844	0.719	0.250	1.000	0.625	1.000	0.406	0.594	0.625	0.969	1.000	0.625	0.938	0.938	0.375	0.563	0.717
H_e	0.747	0.597	0.230	0.793	0.688	0.827	0.365	0.613	0.541	0.734	0.768	0.716	0.772	0.756	0.349	0.536	0.627
<i>P</i>	0.160	0.043	1.000	0.080	0.352	0.183	0.652	0.435	0.533	0.015	0.001	0.504	0.001	0.267	0.590	0.531	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Halmahera (23)

N_a	4	2	4	5	5	5	2	3	4	5	9	4	5	7	2	2	4.250
H_o	0.391	0.391	0.522	1.000	0.652	1.000	0.652	0.739	0.826	0.913	1.000	0.391	0.739	0.957	0.261	0.652	0.693
H_e	0.495	0.476	0.529	0.664	0.677	0.704	0.507	0.592	0.590	0.673	0.814	0.534	0.605	0.814	0.232	0.476	0.587
P	0.008	0.412	1.000	0.000	0.395	0.000	0.220	0.348	0.030	0.064	0.194	0.195	0.892	0.804	1.000	0.093	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Karimunjawa (39)

N_a	5	4	4	7	8	6	3	7	5	6	5	3	6	6	4	5	5.250
H_o	0.513	0.487	0.564	0.974	0.821	1.000	0.641	0.667	0.718	0.667	0.846	0.487	0.538	0.821	0.692	0.667	0.694
H_e	0.623	0.557	0.469	0.731	0.844	0.738	0.483	0.770	0.600	0.557	0.607	0.474	0.494	0.648	0.676	0.648	0.620
P	0.042	0.440	0.539	0.007	0.029	0.001	0.056	0.013	0.215	0.420	0.005	0.706	0.903	0.020	0.023	0.884	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Komodo (22)

N_a	5	3	3	8	8	7	3	4	6	4	5	4	4	7	3	3	4.813
H_o	0.500	0.500	0.273	1.000	0.864	1.000	0.727	0.591	0.909	0.318	1.000	0.636	0.727	0.909	0.818	0.500	0.705
H_e	0.518	0.458	0.317	0.810	0.818	0.786	0.521	0.547	0.715	0.289	0.678	0.698	0.589	0.783	0.664	0.458	0.603
P	0.193	0.273	0.537	0.164	0.756	0.510	0.043	0.074	0.005	1.000	0.000	0.067	0.488	0.347	0.317	0.807	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Luwuk (10)

N_a	2	2	4	5	4	5	3	4	4	5	5	5	3	2	5	3	3.813
H_o	0.500	0.000	0.800	1.000	0.900	1.000	1.000	1.000	0.900	0.800	1.000	1.000	1.000	0.200	0.900	0.200	0.763
H_e	0.395	0.189	0.711	0.663	0.700	0.737	0.668	0.689	0.647	0.758	0.711	0.779	0.574	0.442	0.784	0.195	0.603
P	1.000	0.052	0.688	0.031	0.611	0.046	0.083	0.103	0.034	0.759	0.251	0.285	0.007	0.134	0.408	1.000	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Manado (16)

N_a	5	2	2	7	7	6	4	4	5	5	7	3	6	6	4	3	4.750
H_o	0.750	0.375	0.438	1.000	0.875	1.000	0.938	0.625	0.938	0.938	1.000	0.688	0.938	0.813	0.875	0.688	0.805
H_e	0.643	0.508	0.514	0.796	0.738	0.756	0.679	0.657	0.716	0.698	0.817	0.558	0.750	0.679	0.700	0.506	0.670
P	1.000	0.347	0.637	0.472	0.337	0.091	0.119	0.864	0.124	0.045	0.211	0.024	0.191	0.333	0.313	0.096	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Natuna (24)

N_a	6	2	4	6	7	6	3	6	5	5	5	2	6	8	4	4	4.938
H_o	0.833	0.250	0.125	0.958	0.667	1.000	0.375	0.792	0.542	0.583	0.958	0.333	0.833	0.917	0.667	0.500	0.646
H_e	0.676	0.284	0.122	0.793	0.780	0.743	0.318	0.805	0.566	0.640	0.642	0.284	0.636	0.815	0.659	0.422	0.574
P	0.324	0.501	1.000	0.127	0.284	0.000	1.000	0.523	0.526	0.773	0.001	1.000	0.774	0.002	0.068	1.000	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

P.Pari (22)

N_a	4	2	3	4	6	6	2	5	5	3	3	4	6	5	3	3	4.000
H_o	0.864	0.409	0.318	0.864	0.591	1.000	0.545	0.545	0.955	0.727	0.591	0.455	0.591	0.864	0.773	0.818	0.682
H_e	0.696	0.426	0.284	0.671	0.736	0.756	0.512	0.540	0.603	0.555	0.439	0.517	0.545	0.613	0.591	0.678	0.573
P	0.384	1.000	1.000	0.016	0.245	0.055	1.000	0.745	0.001	0.320	0.168	0.685	0.750	0.063	0.329	0.307	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Sanur (32)

N_a	3	3	4	4	8	8	2	5	4	6	2	3	6	8	6	4	4.750
H_o	0.438	0.094	0.094	0.938	0.813	1.000	0.469	0.688	0.969	0.563	0.875	0.406	0.844	0.969	0.719	0.375	0.641
H_e	0.408	0.092	0.092	0.629	0.749	0.778	0.448	0.645	0.550	0.518	0.500	0.538	0.588	0.791	0.553	0.320	0.512
P	0.837	1.000	1.000	0.000	0.003	0.003	1.000	0.962	0.000	0.267	0.000	0.275	0.024	0.015	0.514	0.701	
Null alleles	No	No	No	Yes	No	No	No	No	No	No	Yes	No	No	No	No	No	

Tual (30)

N_a	5	2	4	7	7	9	3	4	6	5	8	4	6	5	4	3	5.125
H_o	0.467	0.467	0.600	0.933	0.833	0.967	0.433	0.767	0.833	0.767	0.967	0.700	0.700	0.900	0.400	0.467	0.700
H_e	0.495	0.452	0.549	0.751	0.799	0.644	0.463	0.649	0.622	0.621	0.783	0.685	0.680	0.612	0.400	0.386	0.599
P	0.761	1.000	0.592	0.005	0.029	0.001	0.459	0.268	0.344	0.149	0.012	0.427	0.925	0.001	0.349	0.496	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Wakatobi (23)

N_a	6	4	3	8	8	6	5	5	6	3	6	6	6	6	4	4	5.375
H_o	0.652	0.696	0.565	1.000	0.739	1.000	0.522	0.696	0.957	0.652	0.913	0.826	0.739	0.609	0.652	0.739	0.747
H_e	0.619	0.567	0.532	0.758	0.760	0.797	0.587	0.666	0.771	0.517	0.721	0.744	0.625	0.578	0.550	0.545	0.646
P	0.984	0.287	1.000	0.078	0.598	0.050	0.765	1.000	0.059	0.590	0.277	0.592	0.663	0.210	0.398	0.047	
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	

Mean Alleles	4	3	3	6	7	6	3	5	5	5	6	4	5	6	4	4	
Mean H_o	0.605	0.407	0.367	0.949	0.745	0.982	0.590	0.669	0.836	0.710	0.915	0.552	0.787	0.791	0.641	0.578	
Mean H_e	0.569	0.420	0.365	0.757	0.739	0.769	0.534	0.626	0.658	0.599	0.681	0.562	0.632	0.685	0.557	0.503	

Table S7. Inbreeding coefficient (F_{IS}) values for *Syringodium isoetifolium*

Location	F_{IS}
Belitung	-0.286
Karimunjawa	-0.302
Pari	-0.324
S.Nasik	-0.182
Bali	0.126
Derawan	-0.15
Bira	-0.391
Luwuk	-0.009
Banggai	-0.043
Alor	0.076
Bangka	-0.347
Halmahera	0.092
Wakatobi	-0.08
Tual	-0.174

Table S8. Inbreeding coefficient (F_{IS}) values for *Thalassia hemprichii*

Location	F_{IS}
Pari	-0.173
Belitung	-0.179
Natuna	-0.062
Karimunjawa	-0.147
Sanur	-0.22
Derawan	-0.087
Bira	-0.148
Komodo	-0.13
Alor	-0.181
Wakatobi	-0.169
Manando	-0.135
Banggai	-0.134
Luwuk	-0.218
Ambon	-0.143
Halmahera	-0.179
Tual	-0.171

Figure S1. *Syringodium isoetifolium* STRUCTURE HARVESTER output showing results of the implemented Evanno's ΔK method of establishing the most supported value of K (see Evanno et al. 2005 for details). All loci Included.

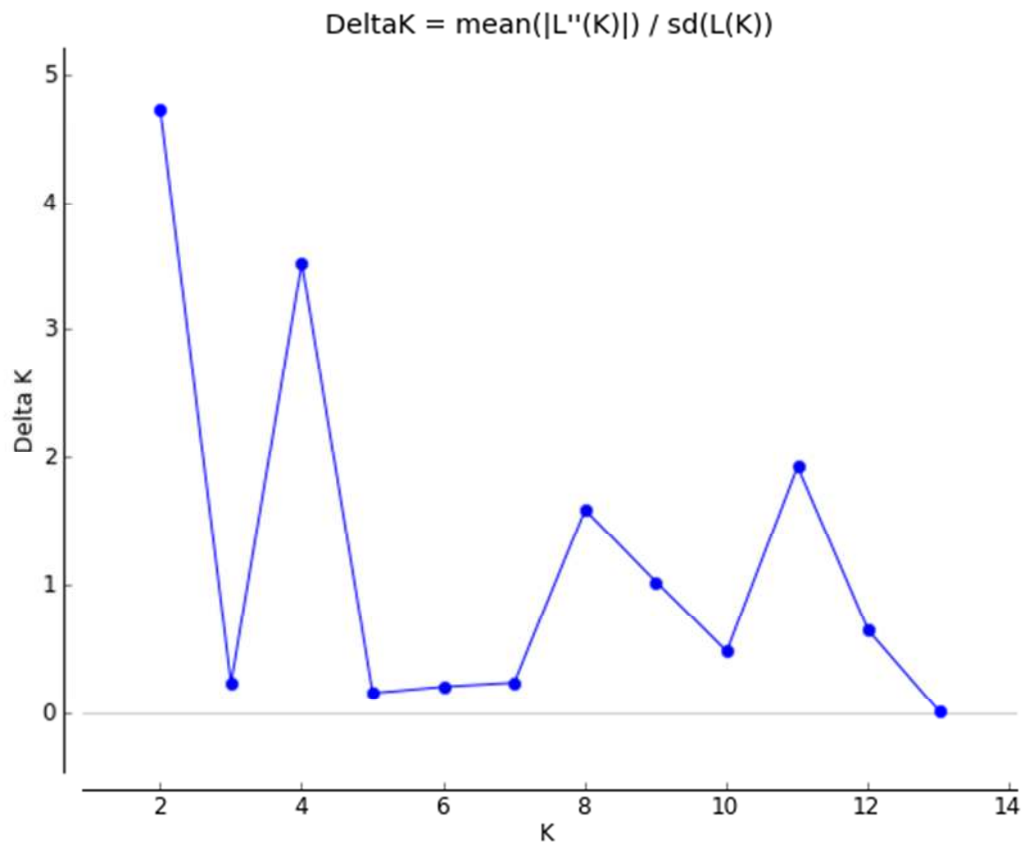


Figure S2. *Syringodium isoetifolium* STRUCTURE HARVESTER output showing results of the implemented Evanno's ΔK method of establishing the most supported value of K (see Evanno et al. 2005 for details). Only loci with a 0% error rate Included.

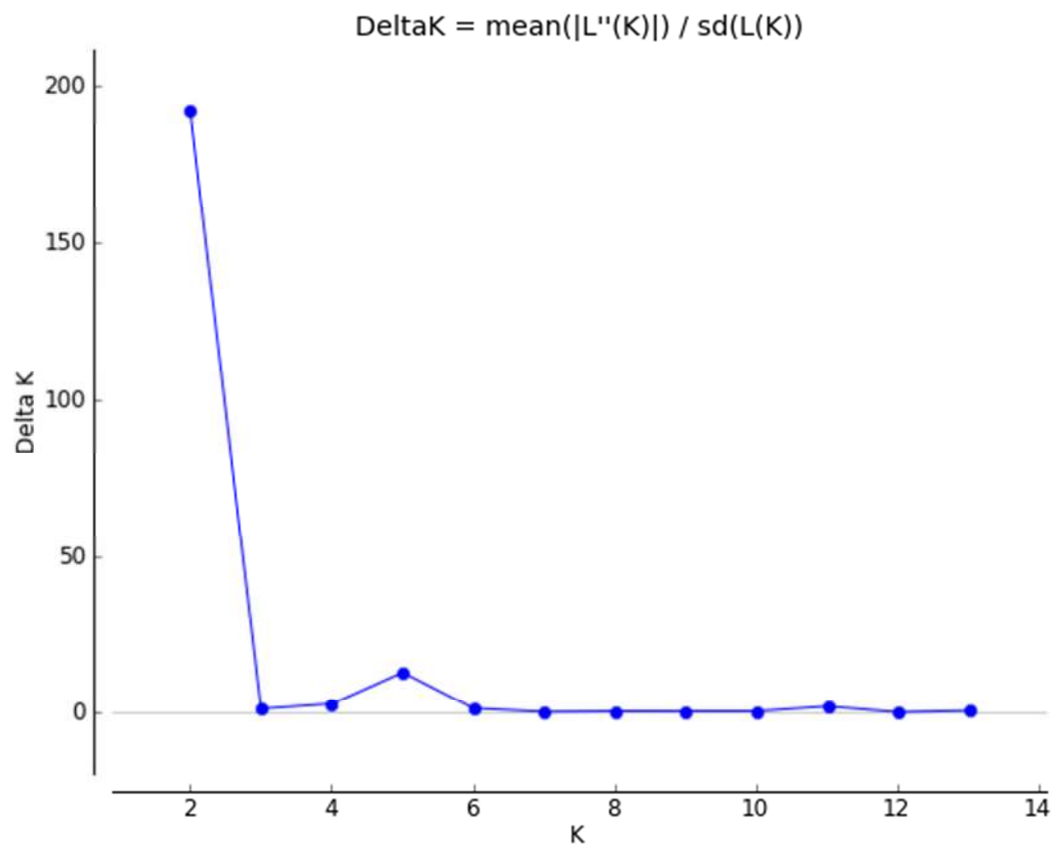


Figure S3. *Thalassia hemprichii* STRUCTURE HARVESTER output showing results of the implemented Evanno's ΔK method of establishing the most supported value of K (see Evanno et al. 2005 for details). All loci included.

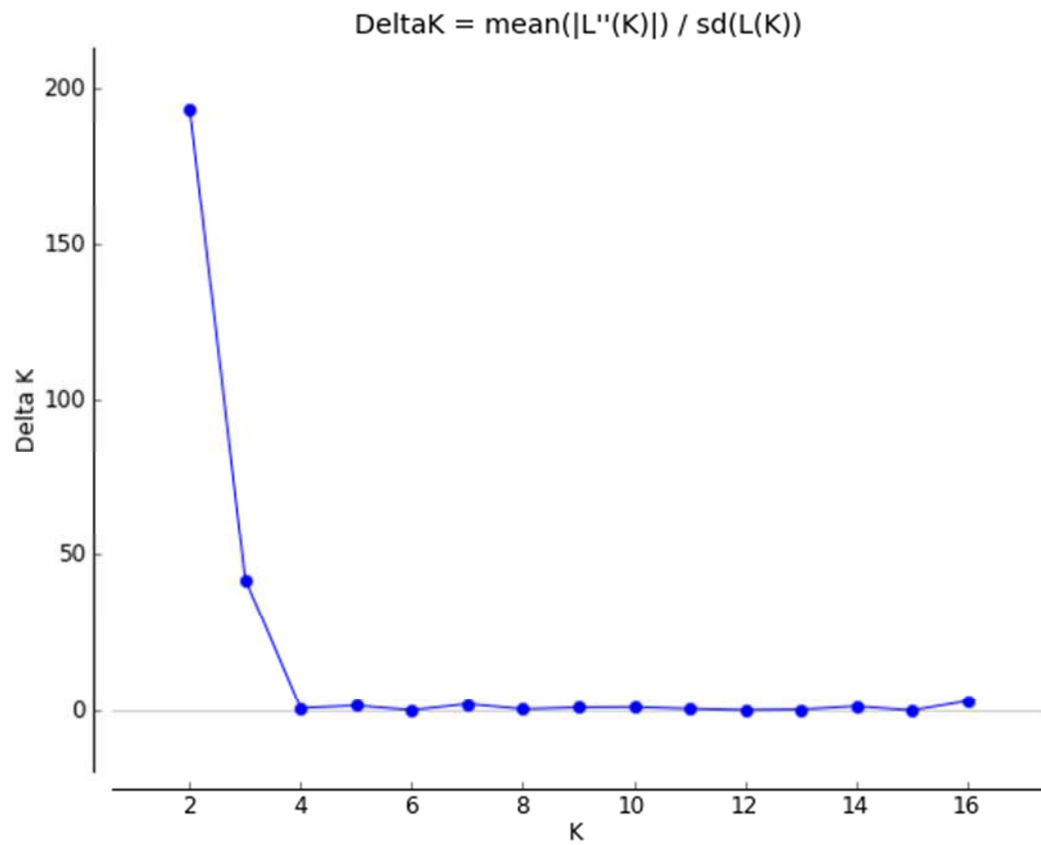
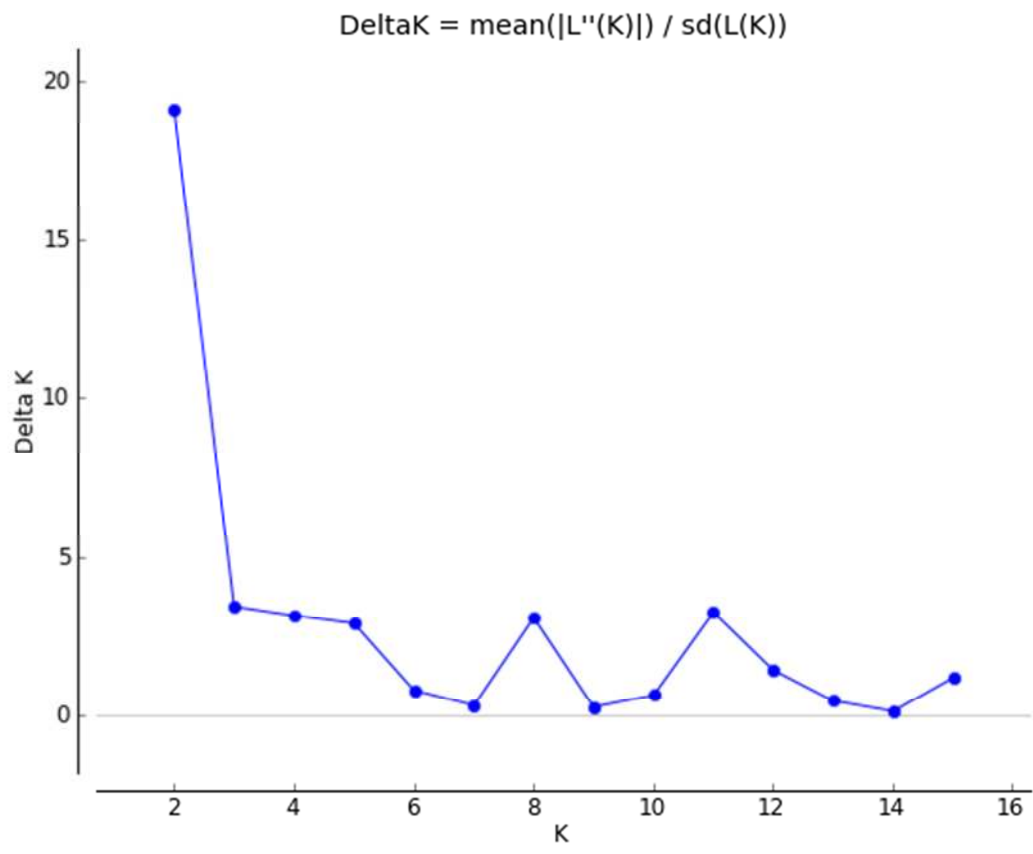


Figure S4. *Thalassia hemprichii* STRUCTURE HARVESTER output showing results of the implemented Evanno's ΔK method of establishing the most supported value of K (see Evanno et al. 2005 for details). Only loci with a 5% or less error rate included.



APPENDIX B

Table S1. Details of primers used in each multiplex mix. Bolded primers indicate primers that were not used in the analysis due to null alleles or scoring difficulties.

Mix	Primers and Labels
1	TG20 FAM, TG61 VIC , TG55 NED , TG60 PET
2	TG01 FAM, TG04 VIC, TG42 NED , TG07 PET
3	TG43 FAM , TG52 VIC , TG26 NED
4	TG39 FAM, TG28 VIC, TG66 NED , TG27 PET
5	TG15 FAM , TG11 VIC, TG02 NED, TG51 PET

Table S2. *Tripneustes gratilla* locus by locus summary statistics for each sample. Sample site (number of individuals), number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity and potential for null alleles. After Bonferroni adjustments, p -values were set to 0.004 to test for departures from Hardy-Weinberg equilibrium and significant values are highlighted in bold. The potential presence of null alleles was tested using MICROCHECKER 2.3.3 (Van Oosterhout et al. 2004).

Population (n)	Locus												Mean
	TG01	TG02	TG04	TG07	TG11	TG20	TG26	TG27	TG28	TG39	TG51	TG60	
Alor (10)													
N_a	8	4	6	5	3	8	9	8	5	7	12	7	7
H_o	0.800	0.600	1.000	1.000	0.800	0.900	0.700	1.000	0.800	0.400	0.900	0.800	0.808
H_e	0.863	0.500	0.784	0.716	0.542	0.889	0.911	0.868	0.668	0.637	0.947	0.879	0.767
P	0.005	1.000	0.372	0.177	0.172	0.001	<0.001	0.082	0.769	0.039	<0.001	0.017	-
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	-
Ambon (7)													
N_a	7	3	6	5	4	10	7	7	6	7	9	9	7
H_o	0.857	0.286	0.714	0.857	0.429	0.571	0.857	0.571	0.714	0.857	0.571	1.000	0.690
H_e	0.846	0.484	0.846	0.758	0.396	0.923	0.857	0.890	0.846	0.813	0.934	0.934	0.794
P	0.546	0.160	0.217	0.509	1.000	0.002	0.912	0.079	0.659	0.977	0.010	1.000	-
Null alleles	No	No	No	No	No	Yes	No	No	No	No	No	No	-
Bali (28)													
N_a	13	7	10	6	5	12	23	13	7	11	25	12	12
H_o	0.964	0.571	0.857	0.536	0.357	0.500	0.929	0.857	0.714	0.821	0.821	0.893	0.735

H_e	0.897	0.690	0.790	0.674	0.473	0.896	0.955	0.900	0.816	0.877	0.964	0.892	0.819
P	0.001	0.014	0.336	0.336	0.009	<0.001	0.013	0.194	0.097	0.433	0.001	0.681	-
Null alleles	No	No	No	No	No	Yes	No	No	No	No	Yes	No	-
Belitung (37)													
N_a	14	8	9	8	9	16	25	13	11	12	30	14	14
H_o	0.892	0.432	0.703	0.919	0.405	0.486	0.892	0.811	0.649	0.811	0.865	0.865	0.727
H_e	0.890	0.622	0.799	0.793	0.361	0.914	0.954	0.892	0.860	0.857	0.961	0.834	0.811
P	0.903	0.006	0.043	<0.001	1.000	<0.001	<0.001	0.236	<0.001	0.946	0.025	0.421	-
Null alleles	No	Yes	No	No	No	Yes	No	No	Yes	No	Yes	No	-
Banggai (19)													
N_a	10	5	8	6	5	11	21	12	8	7	21	14	11
H_o	0.789	0.368	0.632	0.474	0.158	0.211	0.895	0.842	0.579	0.474	0.737	1.000	0.596
H_e	0.872	0.482	0.849	0.640	0.367	0.915	0.964	0.909	0.861	0.825	0.962	0.916	0.797
P	<0.001	0.160	0.053	0.301	0.002	<0.001	0.006	0.018	<0.001	0.001	0.005	0.516	-
Null alleles	No	No	No	No	Yes	Yes	No	No	No	Yes	Yes	No	-
Bira (27)													
N_a	10	7	9	7	5	11	28	14	9	11	24	14	12
H_o	0.889	0.444	0.778	0.556	0.333	0.667	0.926	0.778	0.630	0.778	0.667	0.852	0.691
H_e	0.842	0.496	0.834	0.719	0.412	0.899	0.967	0.911	0.821	0.853	0.962	0.894	0.801
P	0.051	0.391	0.735	0.068	0.076	<0.001	0.071	0.118	0.073	0.664	<0.001	0.081	-
Null alleles	No	No	No	No	No	Yes	No	No	No	No	Yes	No	-
Derawan (33)													
N_a	16	6	9	8	6	16	25	14	13	12	25	9	13
H_o	0.818	0.303	0.818	0.455	0.424	0.485	0.939	0.970	0.515	0.939	0.636	0.788	0.674

H_e	0.901	0.475	0.802	0.571	0.388	0.914	0.959	0.901	0.859	0.874	0.963	0.739	0.779
P	0.015	0.017	0.998	0.007	0.520	<0.001	0.105	0.590	<0.001	0.818	<0.001	<0.001	-
Null alleles	No	No	No	No	No	Yes	No	No	Yes	No	Yes	No	-
Halmahera (16)													
N_a	11	7	8	8	5	10	14	10	5	12	17	11	10
H_o	0.813	0.438	0.750	0.750	0.313	0.688	0.938	0.875	0.688	0.875	0.813	0.938	0.740
H_e	0.861	0.782	0.808	0.790	0.502	0.863	0.915	0.885	0.780	0.917	0.942	0.867	0.826
P	0.063	0.006	0.656	0.830	0.023	0.029	0.043	0.205	0.551	0.096	0.010	0.135	-
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	-
Karimunjawa (22)													
N_a	13	8	8	7	6	13	18	9	10	12	22	7	11
H_o	0.909	0.591	0.727	0.864	0.364	0.500	0.955	0.909	0.455	0.955	0.682	0.864	0.731
H_e	0.913	0.555	0.800	0.743	0.446	0.902	0.949	0.810	0.841	0.879	0.958	0.717	0.793
P	0.209	0.913	0.168	0.130	0.241	0.001	0.695	0.425	0.001	0.794	<0.001	0.597	-
Null alleles	No	No	No	No	No	Yes	No	No	Yes	No	Yes	No	-
Manado (9)													
N_a	8	7	9	7	3	6	12	8	11	6	10	10	8
H_o	0.889	0.889	0.667	0.778	0.556	0.333	0.778	0.778	0.889	0.778	0.444	0.778	0.713
H_e	0.850	0.778	0.882	0.817	0.451	0.784	0.954	0.856	0.882	0.837	0.928	0.928	0.829
P	0.061	0.677	0.056	1.000	1.000	0.002	0.010	0.397	0.897	0.709	0.001	0.271	-
Null alleles	No	No	No	No	No	Yes	No	No	No	No	Yes	No	-
Mentawai (13)													
N_a	8	4	7	7	3	11	14	9	8	11	14	7	10
H_o	0.923	0.308	0.615	0.538	0.154	0.846	0.923	0.769	0.538	0.769	0.615	0.538	1

H_e	0.880	0.458	0.760	0.609	0.280	0.920	0.948	0.880	0.812	0.898	0.951	0.609	0.751
P	0.002	0.075	0.220	0.589	0.230	0.001	0.733	0.177	0.040	0.019	0.002	0.594	-
Null alleles	No	No	No	No	No	No	No	No	Yes	No	Yes	No	-
Natuna (28)													
N_a	14	7	10	8	7	16	23	11	10	12	23	12	13
H_o	0.929	0.607	0.857	0.679	0.393	0.821	0.786	0.893	0.536	0.786	0.750	0.857	0.741
H_e	0.909	0.639	0.849	0.734	0.431	0.931	0.960	0.877	0.838	0.893	0.949	0.885	0.824
P	<0.001	0.410	0.253	0.093	0.162	<0.001	0.016	0.752	0.005	0.112	<0.001	0.222	-
Null alleles	No	No	No	No	No	No	Yes	No	Yes	No	Yes	No	-
Nias (12)													
N_a	6	5	10	5	4	5	14	7	10	9	16	5	8
H_o	0.583	0.583	0.583	0.500	0.333	0.583	1.000	0.833	0.833	0.833	0.917	0.500	0.674
H_e	0.808	0.496	0.895	0.435	0.308	0.786	0.949	0.812	0.917	0.866	0.949	0.435	0.721
P	0.119	1.000	0.013	1.000	1.000	0.335	0.007	0.994	0.255	0.754	0.606	1.000	-
Null alleles	No	No	Yes	No	No	No	No	No	No	No	No	No	-
Pari (26)													
N_a	14	8	8	9	6	14	22	10	12	12	21	9	12
H_o	0.731	0.692	0.692	0.538	0.500	0.846	0.962	0.769	0.769	0.846	0.654	0.769	0.731
H_e	0.902	0.710	0.796	0.716	0.442	0.902	0.955	0.865	0.853	0.891	0.958	0.766	0.813
P	0.063	0.722	0.207	0.005	0.789	0.014	0.034	0.145	0.420	0.156	<0.001	0.320	-
Null alleles	No	No	No	Yes	No	No	No	No	No	No	Yes	No	-
Tual (29)													
N_a	13	7	10	6	6	13	20	13	12	11	23	15	12
H_o	0.931	0.483	0.759	0.862	0.414	0.897	0.931	0.828	0.621	0.690	0.793	0.724	0.744

H_e	0.912	0.469	0.849	0.739	0.409	0.897	0.949	0.891	0.831	0.853	0.952	0.869	0.802
P	0.003	0.419	0.462	0.010	0.492	0.050	0.107	0.025	0.014	0.066	<0.001	0.068	-
Null alleles	No	No	No	No	No	No	No	No	Yes	No	Yes	No	-
Wakatobi (37)													
N_a	15	9	12	8	5	13	27	12	10	13	28	15	14
H_o	0.757	0.622	0.838	0.459	0.405	0.865	0.865	0.784	0.514	0.784	0.703	0.838	0.703
H_e	0.903	0.673	0.869	0.705	0.353	0.908	0.955	0.863	0.805	0.895	0.965	0.902	0.816
P	0.005	0.530	0.034	0.002	1.000	0.021	0.002	0.029	<0.001	0.230	<0.001	<0.001	-
Null alleles	No	No	No	Yes	No	No	No	No	Yes	No	Yes	No	-
Mean Alleles	11	6	9	7	5	12	19	11	9	10	20	11	-
Mean H_o	0.835	0.492	0.753	0.674	0.389	0.657	0.898	0.837	0.637	0.774	0.747	0.812	-
Mean H_e	0.878	0.582	0.826	0.697	0.410	0.890	0.944	0.876	0.831	0.854	0.953	0.817	-

Figure S1. *Tripneustes gratilla* STRUCTURE HARVESTER output showing results of the Evanno's ΔK method of establishing the most supported value of K (see Evanno et al. 2005 for details). All loci Included.

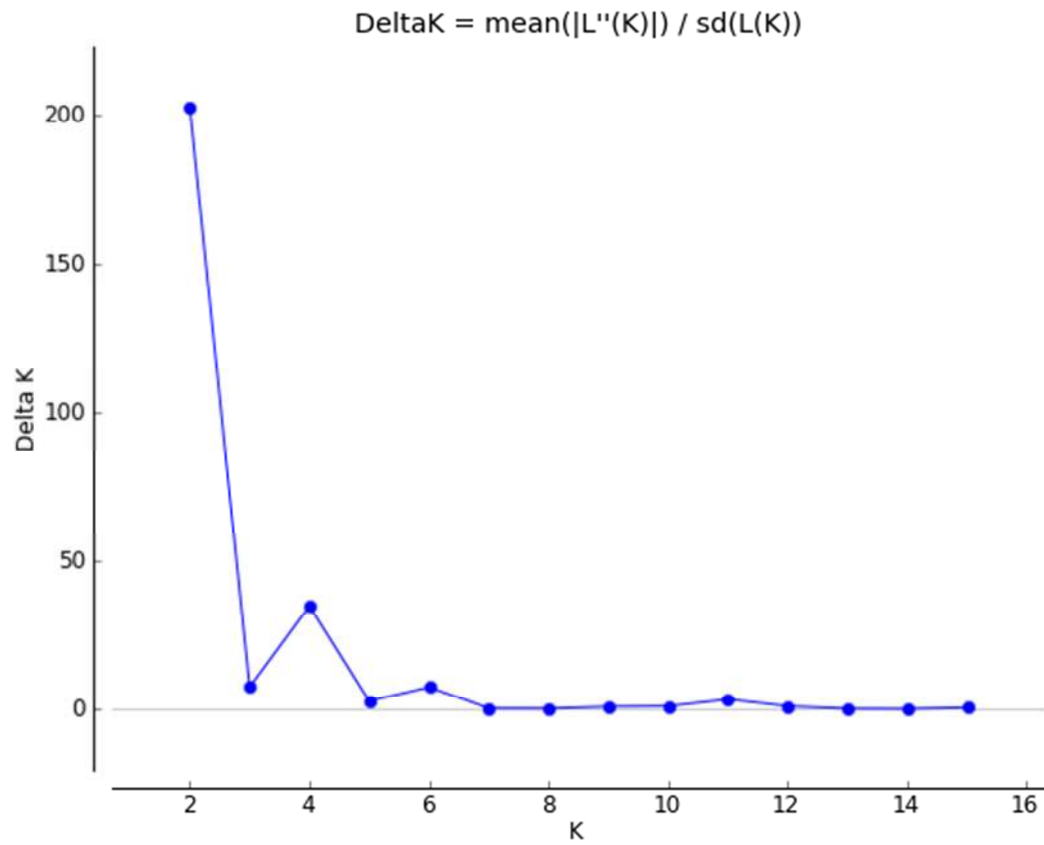
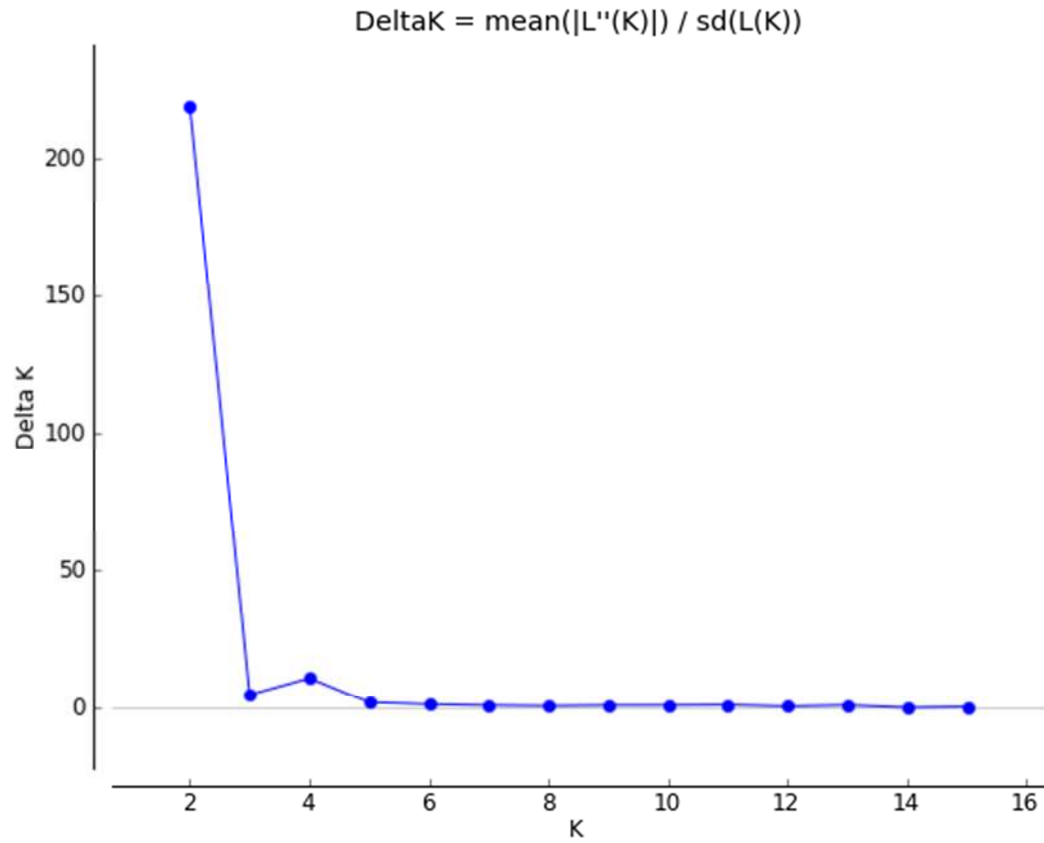


Figure S1. *Tripneustes gratilla* STRUCTURE HARVESTER output showing results of the Evanno's ΔK method of establishing the most supported value of K (see Evanno et al. 2005 for details). Only loci showing a $\leq 2.1\%$ error rate.



APPENDIX C

Table S1. Details of primers used in each multiplex mix. Bolded primers indicate which primers were not used in the analysis due to null alleles or scoring difficulties.

Mix	Primers and Labels
1	Sten76 FAM, Sten08 VIC , Sten27 NED, Sten23 PET
2	Sten94 FAM, Sten05 VIC, Sten72 NED, Sten74 PET
3	Sten10 FAM, Sten43 VIC, Sten24 NED, Sten92 PET
4	Sten01 FAM, Sten 36 VIC, Sten21 NED
5	Sten73 VIC, Sten 91 NED , Sten32 PET

Table S2. *Stenopus hispidus* locus by locus summary statistics for each sample. Sample site (number of individuals), number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity and potential for null alleles. After Bonferroni adjustments, p -values were set to 0.003 to test for departures from Hardy-Weinberg equilibrium and significant values are highlighted in bold. The potential presence of null alleles was tested using MICROCHECKER 2.3.3 (Van Oosterhout et al. 2004).

Population (n)	Locus															Mean
	Sten01	Sten05	Sten10	Sten21	Sten23	Sten24	Sten27	Sten32	Sten36	Sten43	Sten72	Sten73	Sten76	Sten92	Sten94	
Alor (23)																
N_a	9	13	6	5	14	11	9	5	5	7	6	9	4	2	12	7.8
H_o	0.74	0.70	0.74	0.39	0.87	0.87	0.61	0.65	0.61	0.83	0.57	0.78	0.52	0.74	0.83	0.70
H_e	0.64	0.74	0.71	0.41	0.87	0.85	0.73	0.66	0.49	0.77	0.66	0.71	0.43	0.50	0.84	0.67
P	0.92	0.24	0.86	0.31	0.77	0.80	0.00	0.37	0.29	0.30	0.39	0.96	0.83	0.03	0.25	-
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	-
Ambon (46)																
N_a	12	12	6	7	16	14	9	6	3	6	10	10	4	5	7	8.5
H_o	0.85	0.80	0.74	0.39	0.87	0.87	0.74	0.41	0.63	0.55	0.72	0.67	0.63	0.57	0.80	0.68
H_e	0.79	0.78	0.74	0.34	0.88	0.86	0.74	0.59	0.49	0.80	0.71	0.66	0.55	0.83	0.80	0.70
P	0.00	0.03	0.08	1.00	0.12	0.41	0.46	0.01	0.00	0.00	0.89	0.86	0.17	0.00	0.59	-
Null alleles	No	No	No	No	No	No	No	Yes	No	No	No	No	No	Yes	No	-
Bali (46)																
N_a	14	14	8	6	17	14	12	4	7	8	11	2	5	6	10	9.2
H_o	0.87	0.76	0.78	0.30	0.85	0.87	0.89	0.68	0.83	0.89	0.78	0.78	0.67	0.76	0.83	0.77
H_e	0.74	0.74	0.71	0.28	0.87	0.86	0.84	0.68	0.66	0.81	0.73	0.50	0.65	0.55	0.82	0.70
P	0.97	0.43	0.00	1.00	0.50	0.47	0.01	0.00	0.00	0.67	0.96	0.00	0.00	0.02	0.73	-

Null alleles	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	-
Banggai (19)																
N_a	12	10	5	4	15	8	7	5	4	5	9	9	5	4	9	7.4
H_o	0.84	0.84	0.74	0.47	0.89	0.79	0.68	0.47	0.68	0.68	0.67	0.79	0.58	0.68	0.74	0.70
H_e	0.75	0.80	0.66	0.41	0.91	0.78	0.76	0.66	0.68	0.60	0.64	0.72	0.54	0.50	0.78	0.68
P	0.75	0.08	0.96	1.00	0.16	0.75	0.17	0.03	0.41	0.65	0.50	0.39	1.00	0.26	0.97	-
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	-
Bira (5)																
N_a	4	5	5	2	4	7	5	2	4	3	5	4	3	3	4	4.0
H_o	0.60	0.60	0.60	0.40	0.60	0.80	0.60	0.20	0.80	0.80	0.80	0.60	0.60	0.60	0.80	0.63
H_e	0.73	0.87	0.76	0.36	0.78	0.93	0.82	0.20	0.78	0.71	0.87	0.73	0.60	0.60	0.64	0.69
P	0.36	0.06	0.24	1.00	0.19	0.06	0.08	1.00	0.69	0.03	0.29	0.36	0.62	0.62	1.00	-
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	-
Derawan (29)																
N_a	10	12	10	6	14	12	9	6	7	8	8	7	4	6	9	8.5
H_o	0.77	0.90	0.76	0.41	0.90	0.74	0.89	0.43	0.83	0.90	0.71	0.79	0.17	0.46	0.83	0.70
H_e	0.78	0.77	0.76	0.51	0.89	0.87	0.80	0.50	0.78	0.79	0.71	0.72	0.22	0.45	0.84	0.69
P	0.08	0.84	0.35	0.53	0.33	0.003	0.23	0.38	0.69	0.52	0.92	0.55	0.34	0.78	0.03	-
Null alleles	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No	-
Halmahera (38)																
N_a	13	15	7	5	18	12	10	5	7	6	10	9	4	3	9	8.9
H_o	0.66	0.97	0.76	0.39	0.92	0.92	0.87	0.47	0.74	0.76	0.68	0.61	0.58	0.61	0.76	0.71
H_e	0.59	0.91	0.78	0.35	0.91	0.89	0.85	0.69	0.64	0.65	0.72	0.64	0.52	0.44	0.78	0.69
P	0.80	0.98	0.02	1.00	0.40	0.85	0.57	<0.001	<0.001	0.31	0.01	0.74	0.01	0.02	0.35	-

Null alleles	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	-
Komodo (28)																
N_a	15	11	7	5	14	11	11	4	6	10	7	9	4	5	8	8.5
H_o	0.82	0.75	0.71	0.54	0.79	0.72	0.89	0.50	0.79	0.86	0.75	0.67	0.39	0.57	0.86	0.71
H_e	0.86	0.74	0.75	0.60	0.89	0.84	0.81	0.62	0.77	0.83	0.69	0.67	0.34	0.53	0.75	0.71
P	0.38	0.85	0.29	0.19	0.13	0.08	0.10	0.11	0.93	0.09	0.78	0.61	1.00	0.75	0.70	-
Null alleles	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	-
Manado (70)																
N_a	14	14	11	9	22	16	11	7	8	9	12	5	6	6	13	10.9
H_o	0.74	0.83	0.90	0.43	0.87	0.86	0.83	0.56	0.81	0.83	0.83	0.69	0.63	0.50	0.79	0.74
H_e	0.72	0.75	0.81	0.42	0.90	0.87	0.80	0.61	0.71	0.77	0.78	0.53	0.54	0.46	0.76	0.69
P	0.77	0.78	0.64	0.86	0.05	0.05	0.26	<0.001	0.00	0.02	0.46	0.03	0.10	0.04	0.88	-
Null alleles	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	-
Tual (38)																
N_a	11	18	8	5	18	12	4	4	7	7	10	12	4	3	18	9.4
H_o	0.58	0.95	0.89	0.34	0.89	0.79	0.61	0.21	0.74	0.71	0.79	0.68	0.66	0.74	0.89	0.70
H_e	0.51	0.90	0.77	0.39	0.91	0.85	0.47	0.59	0.60	0.68	0.77	0.76	0.63	0.48	0.83	0.67
P	0.94	0.01	0.00	0.07	0.08	0.41	0.29	<0.001	0.19	0.00	0.24	0.44	<0.001	0.00	0.29	-
Null alleles	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	-
Wakatobi (45)																
N_a	17	13	7	6	20	14	6	5	7	9	11	7	5	2	10	9.3
H_o	0.78	0.84	0.82	0.40	0.78	0.84	0.62	0.26	0.71	0.78	0.78	0.56	0.58	0.49	0.76	0.67
H_e	0.70	0.84	0.73	0.38	0.92	0.89	0.58	0.61	0.65	0.74	0.73	0.58	0.59	0.43	0.79	0.68
P	0.76	0.03	0.07	1.00	0.01	0.02	0.12	<0.001	0.00	0.80	0.87	0.31	0.07	0.49	0.71	-
Null alleles	No	No	No	No	Yes	No	No	Yes	No	No	No	No	No	No	No	-

Mean Alleles	12	12	7	5	16	12	8	5	6	7	9	8	4	4	10	8.3
Mean <i>Ho</i>	0.75	0.81	0.77	0.41	0.84	0.82	0.75	0.44	0.74	0.78	0.73	0.69	0.55	0.61	0.81	0.70
Mean <i>He</i>	0.71	0.80	0.74	0.40	0.88	0.86	0.75	0.58	0.66	0.74	0.73	0.66	0.51	0.52	0.78	0.69

Figure S1. *Stenopus hispidus* STRUCTURE HARVESTER output showing results of the implemented Evanno's ΔK method of establishing the most supported value of K (see Evanno et al. 2005 for details). All loci Included.

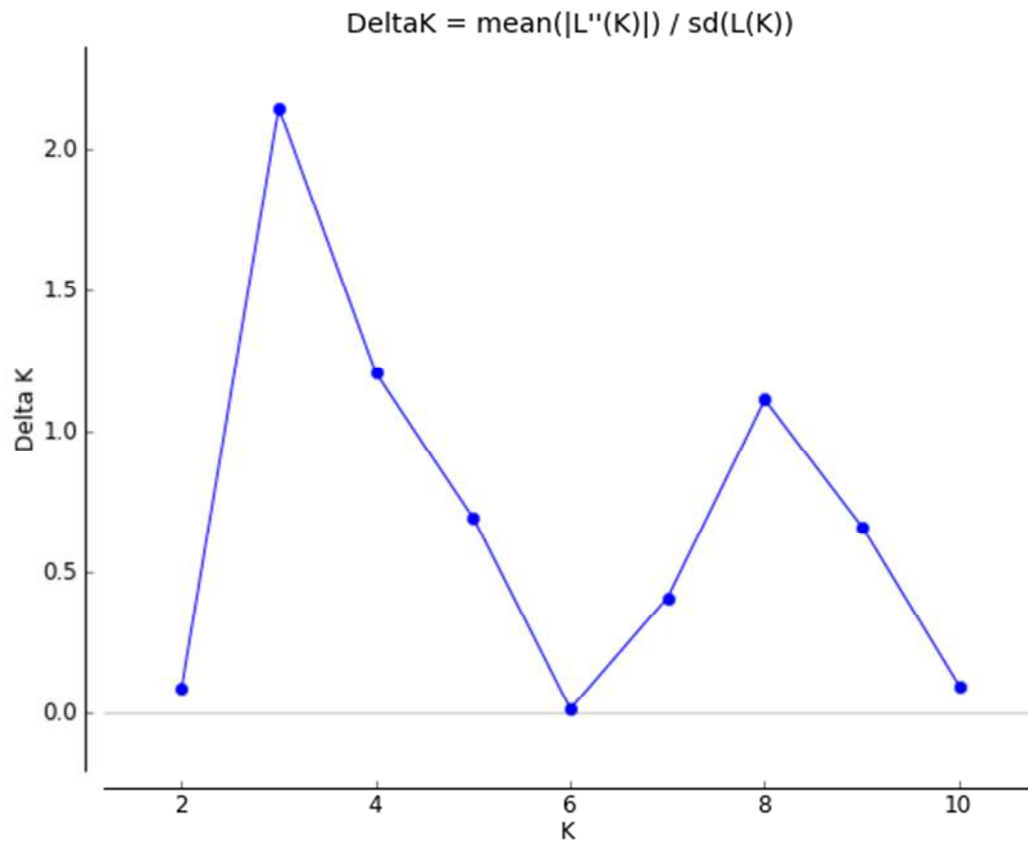
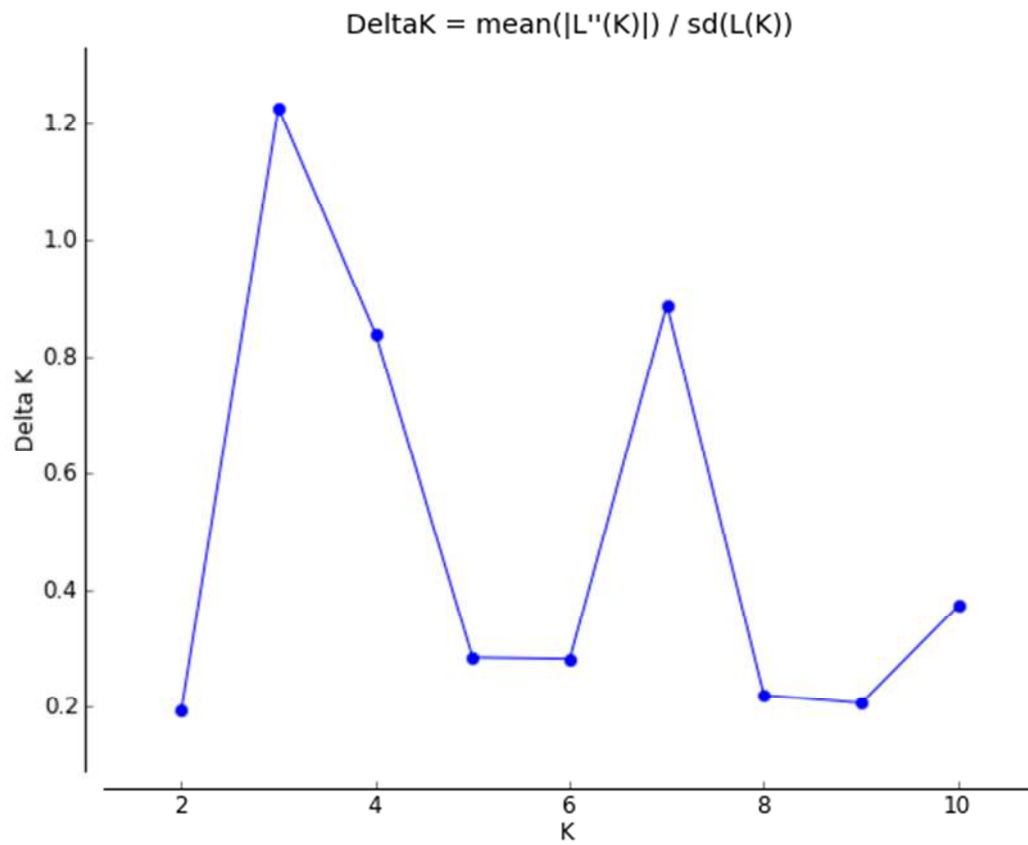


Figure S2. *Stenopus hispidus* STRUCTURE HARVESTER output showing results of the implemented Evanno's ΔK method of establishing the most supported value of K (see Evanno et al. 2005 for details). Only loci showing a $\leq 2.1\%$ error rate.



Appendix C Figure 3, Map showing the locations of the Sunda Shelf, Wallacea and the Sahul Shelf. The light grey area indicates the maximum exposure of land during periods of glacial maxima (sea levels 120 m lower than present day). Also indicated are the approximate positions of Wallace's and Lydekker's lines. Figure adapted from Vorris (2000) and downloaded from the The Field Museum of Natural History (<http://www.fieldmuseum.org/pleistocene-sea-level-maps>).

