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Population structure and patterns of genetic variation in a pearl oyster (Pinctada radiata) native to the Arabian Gulf

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Abstract

The pearl oyster (Pinctada radiata), a marine species native to the Arabian Gulf in the Indian Ocean is common in Qatari waters where it comprises 95% of pearl oyster stocks found naturally there. P. radiata therefore, has been used extensively as a natural bio-indicator for monitoring pollution in the Arabian Gulf. Sustainability of P. radiata wild stocks are now significantly threatened in the Arabian Gulf region as a result of both natural (e.g. extreme salinity, high temperatures, high evaporation rates and low flushing rates) and anthropogenic factors (e.g. rapid development of coastline areas, overfishing and heavy exploitation). Long term sustainability and conservation of this local resource in the Arabian Gulf has therefore become a serious issue, but their survival can significantly contribute to maintenance of healthy marine ecosystems across the region. The current study assessed natural levels and patterns of genetic variation in Arabian Gulf populations of the native pearl oyster, P. radiata to define wild population structure. Potential intrinsic (e.g. pelagic larval phase and life history traits) and extrinsic factors (e.g. water current, wave action, water temperature and prevailing wind direction) that could influence wild population structure were investigated. MtDNA (COI) sequences were used here to define levels and patterns of genetic diversity within and among three sample sites in Qatar territorial waters. Results of the statistical analysis that partitioned variation (AMOVA) were significant

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(P=0.0234) and showed that all variation was present within P. radiata sampled sites (94.61%) and differentiation among sites was relatively low (5.39%).

Keywords: Pearl oyster, P. radiata, gene flow, population structure, Arabian Gulf, Qatar, GSSs, SSRs.

1. Introduction

Biodiversity is expressed as the variability between organisms on Earth as a whole or within a specific ecosystem (Harrington et al. 2010). Environments around the world change dramatically, and as a result, biodiversity is being depleted very quickly as a result of the impacts of human factors. The composition of biological communities has been altered directly through human activities for example the overexploitation of natural resources and population expansion, and indirectly through high levels of pollution, habitat destruction, the impacts of invasive species and climate change (Hooper et al. 2005; Frankham 2003). The United Nations has recognized that human actions have accelerated rates of extinction by up to 1,000 times higher than the natural background rate (UN 2010a). An increasing number of species face extinction in the near future, while numerous others exhibit a decrease in a limited population making them more vulnerable to extinction in the future. Hence, maintaining adequate levels of genetic diversity is a critical issue in order to save the species / groups. Endangered / threatened by extinction or depletion and to maintain long-term resilience against future environmental change (Frankham 2003; Allendorf and Luikart 2007).

Genetic diversity in any species is a resource that manifests itself at two different levels of hierarchy. The first level is clear and includes genetic differences between individuals within a population, for example eye color in humans, flower colors in

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plants, etc. The second level of genetic diversity is found between different populations (Allendorf 1983; Frankham, Ballou and Briscoe 2004). The International Union for Conservation of Nature (IUCN) has emphasized three primary priorities that include preserving genetic diversity, species diversity, and ecosystem diversity (Frankham 1995). Genetic diversity is a fundamental requirement for any society that provides an evolutionary potential for adapting to contemporary environmental change. Hence, maintaining genetic diversity is imperative in order to save endangered species / groups from the threat of extinction and to maintain their long-term potential against future environmental change. This can be addressed through the appropriate application of genetic theory and molecular DNA techniques to identify and document levels and patterns of genetic variation within and between populations of conservation interest (Frankham, Ballou and Briscoe 2004; Frankham 2003; Feral 2002)

2. Literature review

Over recent decades, molecular approaches have been used extensively to address many environmental questions (Freeland 2005). It has gained wide acceptance due to its efficiency, usefulness, relative strength and flexibility (Selkoe and Toonen 2006). Thus, the genetic markers were useful in solving a number of environmental questions. In addition, the set of available genetic markers has rapidly advanced to address individual limitations of some types of markers (Feral 2002). Molecular genetic techniques have the potential to provide invaluable insights at various levels, including, for assessing levels of genetic diversity (within and between populations), assessing levels of gene flow, determining population structure, inferring patterns of evolutionary biogeography, estimating phylogenetic association and mapping lineage (Feral 2002). They can also address environmental questions including an assessment of parameters of importance such as population bottlenecks, effective population size,

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and individual migration rates (Selkoe and Toonen 2006; Palumbi 1996). Beneficial application and widespread use has resulted in a rich diversity of molecular markers which include: allozymes, mtDNA, RFLPs, RAPDs, SNPs, ESTs, and SSRs (micro-satellites) to name but a few (Feral 2002; Selkoe and Toonen 2006) for example, mitochondrial DNA (mtDNA) has become a very common marker for characterizing phylogenetic relationships and used in geography studies (Avise 2000; Bermingham and Moritz 1998). Although there are limitations to inference from mtDNA data, ease of application means that, for many applications, mtDNA has become the marker of choice for addressing many evolutionary and environmental questions (Selkoe and Toonen 2006).

2.1 Assessment of the natural population composition

Molecular phylogenetics has many beneficial applications. It has played an increasingly important role in conservation biology. For example, it provides a powerful tool for identifying clusters that have been vulnerable to rapid diversification or recent extinction. It also has the ability to assess an individual's contribution to overall population genetic diversity (Moritz 1995). Phylogeographic analysis of scoring has been used for many applications including revealing the extent of correlation between coded species through the identification of genetic boundaries ((Palumbi 1997; Arbogast and Kenagy 2001; Bermingham and Moritz 1998; Palumbi 1996). This approach has also been useful in determining the origin of alien species (such as invasive or introduced species) and as a means of tracing pathways to colonization and has also proven important in distinguishing between indigenous and introduced populations (by human activities) (Freeland 2005; Beebee and Rowe 2008; Palumbi 1996).

2.2 Pearl oyster and Pinctada radiata

Pearl oyster is a traditional term that has been applied widely to bivalves that belong to two widely recognized genera; Pinctada (Roding, 1798) and Pteria (Scopoli, 1777),

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both sets of species belonging to the Family Pteriidae (Gray, 1874). Genera in the Family Pteriidae are characterized by possession of an oblique shell with a straight hinge. There are several criteria that distinguish members of the two genera including the arrangement of hinge teeth and, morphological and anatomical character variation (Hayes 1972; Lamprell, Whitehead and Healy 1998; Mikkelsen et al. 2004). Variation in shell colour and shape are influenced considerably by both genetic and environmental factors (Wada 1984).

Pinctada radiata is abundant in the Arabian Gulf region of the north-western Indian Ocean and the Red Sea (Al- Khayat and Al-Ansi 2008). Although current evidence suggests that P. radiata has not established populations in the Mediterranean Sea, many records report that P. radiata is an invasive species that may have been introduced unintentionally to the latter region. There are very few studies that have examined genetic diversity at any scale in Pinctada radiata, so little is known about the levels and patterns of variation in this species. There are no gene sequencing studies yet, however it has been explicitly attempted to examine population variance in P. radiata. Therefore, there is a large gap in terms of assessing the normal levels and patterns of genetic diversity in P. radiata and determining the demographics of this classification via its normal distribution. Therefore, this paper aims to assess the levels and patterns of genetic variation in the Arabian pearl oyster, Pinctada radiata, a species native to the Persian Gulf in the Indian Ocean, and it aims to develop SSR markers for P. radiata target species using the NGST (Ion Torrent) approach.

3. Population Structure Inferred from Mitochondrial (COI) Sequence Data

3.1 Introduction

Genetic diversity and population structure of aquatic species can be influenced by many factors, some intrinsic to the organism (e.g. life-history traits) while others are

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extrinsic to them (e.g. physical barriers to dispersal). Gene flow is an important force that can affect population structure. Essentially gene flow is the amount of effective dispersal that occurs among subpopulations (demes) that in turn, will influence population structure and the amount of genetic variation that is maintained within and between natural populations or even demes, over time. It is apparent that differences in the amount of genetic diversity present within and among populations is a function potentially, of many forces generated from the combined effects of processes including gene flow, genetic drift, mutation and various forms of natural selection. Genetic drift, mutation and natural selection generally enhance divergence among sub-populations while gene flow has the opposing effect by homogenizing diversity across populations and inhibiting subpopulation development or population structure (Bohonak 1999; Balloux and Lugon-Moulin 2002).

Some studies have shown however, that trans-oceanic dispersal patterns can often be much more complex and non-intuitive than had previously been anticipated, as a result of the existence of unseen barriers to dispersal and/or behavioural traits that affect gene flow across many habitats and regions of open ocean (Freeland 2005; Bohonak 1999; Doherty, Planes and Mather 1995; Planes, Parroni and Chauvet 1998). A number of molecular studies have investigated levels and patterns of genetic variation and related this to natural distributions in pearl oyster species from diverse regions including Australia, China, Japan and Bahrain. These studies have employed a variety of molecular markers but most commonly; allozymes, mtDNA and microsatellite makers (Southgate and Lucas 2008). In general, the studies have reported low genetic differentiation and high levels of gene flow suggesting regular exchange of migrants between Japanese and Australian populations for P. imbricata (Colgan and Ponder 2002).

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3.2 Methods

Mantle tissues samples from, Pinctada radiata, individuals were collected from the following three locations; Doha Harbour, Ras Bu Fontas and Al-aseeri Bouy in Qatar. Detailed information for each site including sampling dates, site codes and sample sizes are presented in Table 1. Species identification was verified initially in the field via examination of external morphological traits. Fresh mantle tissue samples were first rinsed with 95% ethanol then stored in 99% ethanol in a Biotechnology lab in Qatar before being transported to QUT (Molecular Genetics Research Facility) for genomic DNA (gDNA) extraction. Some samples were extracted using a modified salt extraction method and other samples using a NucleoSpin Tissue extraction kit (MACHEREY-NAGEL).

Table 1: *P. radiata* collection sites and further sampling details.

Collection site	Site code	Latitude and longitude	Sampling dates	Country	Total samples
Doha Harbour	DH	25 18 37 N 51 18 56 E	May 2008	Qatar	42
Ras Abu Fontas	RF	25 18 37 N 51 33 19 E	May 2008	Qatar	40
Al-aseeri Bouy	AB	25 12 992 N 51 37 352 E	Feb 2011	Qatar	25

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Figure 1: Map of Qatar showing sampling sites for P. radiata.

A region of the Cytochrome Oxidase subunit I (COI) gene from the mitochondrial DNA genome (mtDNA) was chosen to assess levels of genetic diversity within and between Qatari P. radiata populations. Mitochondrial DNA has become one of the most popular genetic markers for use in phylogeographic and population genetic studies. It has been applied in more than 80% of phylogeographic studies to date (Beebee and Rowe 2008).

Approximately 450bp of the mtDNA COI gene was amplified using universal primers developed by Folmer et al. (1994). Following unsuccessful mtDNA (COI) amplification in some P. radiata individuals, the degenerate primer (LCO 1490) was replaced with a specific internal primer designed (ACO specific) for the current study using Primer3 (v.0.4.0) software (Rozen and Skaletsky 2000) in order to ensure specificity during PCR amplification. PCRs were run in an EPPENDORF Mastercycler to amplify the target region mtDNA (COI). Agarose gel electrophoresis was used to verify either amplification success or failure. Gels were run for (30-40) mins at 100V and PCR products viewed under UV light.

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Successfully amplified products were purified using a PCR isolation kit from BIOLINE following the manufacture protocols. After purification, amplified products were sequenced in both directions using an EPPENDORF Mastercycler. An Ethanol/EDTA protocol was used to clean up products from sequence reactions. Sequences were generated using an ABI (Applied Biosystems) 3500 Genetic Analyser.

3.3 Data Analysis

Raw sequence data were aligned using ClustalW as implemented in BioEdit v.7.0.9. Chromatographs were used to edit sequence data manually and to verify any unambiguous bases. MEGA v4.0.2 was utilized to estimate evolutionary distance by calculating pairwise nucleotide differences among haplotypes. MEGA software was also used to identify all variable sites including singleton and parsimony-informative sites in mtDNA sequences.

A haplotype phylogenetic tree was constructed following the Neighbour-Joining (NJ) method (Saitou and Nei 1987) based on Kimura 2-parameter model (Kimura 1980). Median joining network (MJ) was another approach used to construct evolutionary relationships among haplotypes using NETWORK v.4.5.1 (Bandelt, Forster and Röhl 1999). Conformation to neutral expectations was tested using Tajima's D (Tajima 1989) and Fu’s Fs (Fu 1997) in DnaSP v.5.10 (Rozas and Rozas 1999). Significant deviations from neutrality were assessed using the coalescent simulations method in DnaSP. Genetic diversity indices were computed in ARLEQUIN v.3.1 (Excoffier, Laval and Schneider 2005). Several measures of diversity were calculated including: (h) number of haplotypes, (s) polymorphic sites, (Hd) haplotype diversity, (π) nucleotide diversity (mean number of pairwise nucleotide differences). To describe the partitioning of genetic variation at different hierarchical levels (within/among populations), AMOVA (Analysis of Molecular Variance) was used as implemented in ARLEQUIN (Excoffier, Laval and Schneider 2005). Statistical significance of P.

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radiata population pairwise Φst estimates were calculated based on 10,000 iterations of a non-parametric permutation process.

3.4 Results

Analysis of genetic diversity in three wild P. radiata populations at three sites in Qatar based on mtDNA (COI) sequences indicated that no significant structure was evident and the three populations formed a single monophyletic clade but there was obvious evidence for population expansion. This implies that gene flow has been ongoing among the sampled sites. When the same data were compared with haplotypes of P. radiata sampled previously from UAE waters in the Arabian Gulf, this also confirmed the absence of distinct P. radiata clades suggesting no spatial variation at this geographical scale (approximately 300km. Ecological studies indicate that many marine species have high potential to disperse (including many oyster, sea urchin and starfish taxa) due to possession of a planktonic larval phase (e.g. pelagic eggs or larvae) that is manifested by essentially passive movement (dispersal) in water currents for time intervals from several days to weeks or even longer time frames (Bohonak 1999). Potentially this can result in dispersal over large open ocean distances (even between oceans) and consequently, can homogenize populations when compared with equivalent sedentary species that lack a pelagic larval phase (; Freeland 2005). The general pattern for P. radiata appears to reflect extensive dispersal among all sites examined here.

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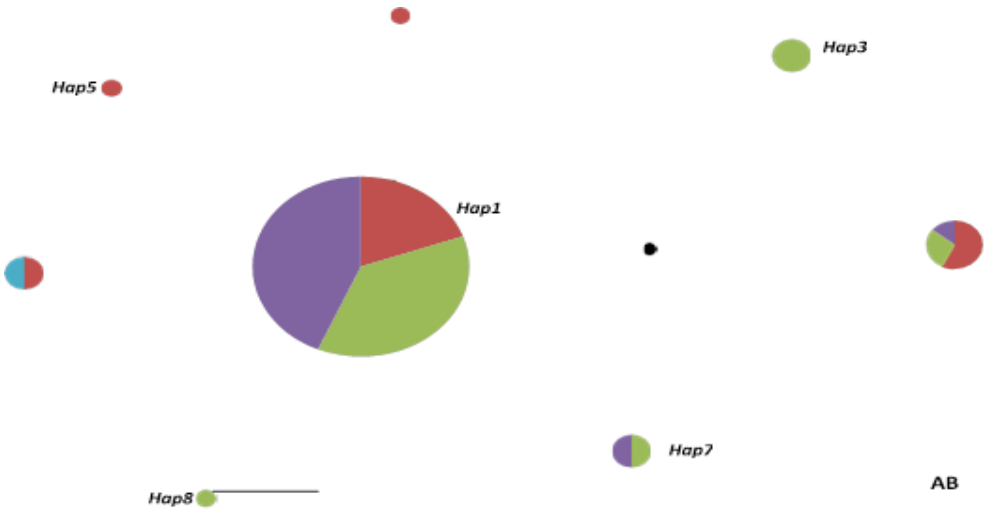

Figure 2: Median-Joining Network of phylogenetic relationships between sampled P. radiata haplotypes and haplotypes sampled from GenBank. Coloured circles represent individual haplotypes, size of circles indicates relative haplotype frequencies, lines between haplotypes indicate single mutational changes and small black circle represents missing haplotype.

The (MJ) network (Figure 2) generated for P. radiata shows evidence for a population expansion in Qatar waters because a star like pattern was evident with common haplotypes (H1 and H2) in the centre. All haplotypes were very closely related (including rare haplotypes) and connected to central haplotypes (H1 and H2) by only a small number of base pairs. When P. radiata COI haplotypes from GenBank were included, one of the UAE haplotypes (P. radiata 4) was shared with the derived haplotypes (H4) from Qatar and both Qatari and UAEs' P. radiata haplotypes shared a common ancestor (H1). Sheppard (1993) reported that the Arabian Gulf region experienced a complete drying out during the late Pleistocene. Thus, the region now is characterized by relatively low biological marine fauna and flora biodiversity due to the fact that marine conditions there are only recent and are very stressful (high temperatures and hypersaline) (Sheppard 1993). As a result of the water current circulation pattern in the Arabian Gulf (anti-clockwise rotation) this would tend potentially to increase gene diversity and hence genetic variation towards the south as

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alleles move passively downstream in marine currents (Sheppard et al. 2010). The RF site however, showed only moderate haplotype diversity (0.278) compared with other sites. This less than expected level of diversity could be affected by presence of the largest desalination plant in Qatar at RF. This desalination plant has been reported to adversely affect the local marine biota and the general ecology of the local environment there. High water temperatures and salinity affect the growth and health of benthic invertebrates in the vicinity of the desalination plant, which as a consequence shows reduced benthic community abundance.

Low water quality has likely changed the species composition and diversity. The lowest haplotype diversity (Hd=0.094) and nucleotide diversity (π=0.00032) estimates were found at the Doha Harbour site. A nchorages and to fishing boat harbours that all can be a major source of leaded gasoline used as fuel. The Doha Harbour showed lowest mtDNA diversity of all three sites examined here, and this site is also the site most impacted by human activities. The most prominent physical characteristics of the Arabian Gulf region are its semi-enclosed, shallow, hyper-arid setting where evaporation is extreme and flushing rates are low. Thus, it would appear that both natural and anthropogenic factors may have contributed significantly to shaping the contemporary P. radiata population diversity in the region.

Analysis of Molecular Variance (AMOVA) analysis results showed only 5.39% of the variation was present among P. radiata sites while 94.61% of the variation was present within sites and the mean fixation index (Φst) among sites in Qatar was not significantly different from zero. The results, although limited in scale, suggest that it is unlikely real divergent wild populations of P. radiata are present in the Qatari region and suggest that P. radiata populations are essentially homogenous there. Low levels of genetic differentiation among sampled sites in Qatar can be attributed largely to high levels of gene flow suggesting relative high exchange of migrants among sites where the species occurs naturally.

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The pattern of population structure observed here for P. radiata showed no evidence for genetic differentiation among populations within the Qatari region and also when populations were included from another geographical area in the Gulf (UAE). Low levels of population differentiation have also been reported in several pearl oyster species and other marine organisms. Thus, data are clear and indicate that P. radiata populations sampled from the Qatari coast are panmictic and therefore should be considered as a single management unit. At a larger spatial scale (approximately 300km) in the Arabian Gulf, there was no significant genetic differentiation between Qatar's P. radiata stock and that present in UAE's based on mtDNA (COI). Thus, the pearl oyster fishery in the Gulf should probably consider these populations as a single stock for management purposes until wild populations that occur between the two regions have been evaluated to determine if they are genetically differentiated.

4. SSR Marker Development and Genome Survey Sequence (GSSs) in P. radiate

4.1 Introduction

Microsatellites or Simple Sequence Repeats (SSRs) are repeating fragments of DNA. The recurrent forms range in length from 1 to 6 nucleotide bases and are inherited as two-parent dominant common markers. They are found at high frequency across the nuclear genomes of most varieties of eukaryotes and in general, exhibit relatively high levels of allelic polymorphisms (Selkoe and Toonen 2006; Jarne and Lagoda 1996; Chistiakov, Hellemans and Volckaert 2006). The development of SSR tags using the traditional approach can be problematic due to cost, time, and labor intensive requirements. The major limitation is the need to pre-define sequences with SSR iteration to design specific primers for amplification. The second limitation is the requirement to create the library by isolating genomic clones that contain duplicate SSRs, followed by sequencing each one. A third problem could arise when designing

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optimal prefixes for a single polymorphic micro-satellite position. Collectively, high cost requirements, high experience, and significant effort are needed to produce usable SSR tags for target species using the conventional approach. These problems have often been limited in developing SSR markers for new species especially when appropriate molecular research laboratories have limited or no facilities available.

4.2 Methods

Pearl oyster samples, Pinctada radiata, were collected from mantle tissue from the following two locations; Doha Harbour and Ras Bu Fontas in Qatar. Species identification was verified initially in the field through examination of morphological traits then confirmed via mtDNA (COI) sequence analysis. Fresh mantle tissue samples were first rinsed with 95% ethanol then stored in 99% ethanol in the Biotechnology lab in Qatar before being transported to QUT (Molecular Genetics Research Facility) for genomic DNA (gDNA) extraction using a NucleoSpin Tissue extraction kit (MACHEREY-NAGEL). A quick and effective method to develop a large number of SSR markers rapidly was trialled using next generation sequencing (NGS). Fresh mantle tissues were obtained to overcome the low DNA quality issue.

Two different kits: (1) Ion Plus Fragment Library and (2) Ion Xpress Plus Fragment Library (Life Technology) were used to obtain high quality genomic libraries from gDNA following the manufacture's protocols. Two gDNAs of high quality were obtained and pooled at equimolar concentrations. Pooled gDNAs were then fragmented to a selected size of (250bp). Ion adaptors were used then to ligate the blunt- ends of DNA fragments. Subsequently, Ion-compatible adapters and nick repair were used as a linkage ring between the DNA inserts and adaptors. To quantify gDNA yields, a Quant-iT RiboGreen fluorometer (Invitrogen) was used. Bioanalyzer (Aglient) was then used to determine the average sequence length reads. Analysis of aliquots (1 µl) on a Bioanalyzer was also used to determine average sequence length. Next, the ligated library underwent size selection to produce an optimal target read

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length of 250 bp. All successful libraries were amplified following the manufacturer's protocol (Life Technology) for the Ion Xpress Template kit and the automated Ion OneTouch System on Sphere particles. After successful amplification and ligation, pooled gDNAs were sequenced using a 314 semiconductor chip and PGM chemistry according to (Life Technology) the manufacturer's protocol.

4.3 Data Analysis

After obtaining sequence reads from the PGM, all reads were run via the Ion Torrent server in order to remove any poor sequences and sequencing adaptors. Data were then captured from the three different chips (Ion Chip Sequencing protocol). Default parameters in the Software SeqMan NGen 10.1(DNASTAR) were used to assemble (de novo) trimmed sequences. Both singleton and contig datasets were generated after quality filtering in SeqMan NGen. All genome survey sequences (GSSs) for P. radiata were captured and then analysed further.

Predicting functions for GSSs was completed using Blast2GO software (Götz et al. 2008) in order to assign Gene Ontology (GO) terms (The Gene Ontology Consortium 2008). The Kyoto Encyclopaedia of Genes and Genomes (KEGG) (Kanehisa et al. 2006) were used to identify metabolic pathways. InterProScan tool was utilised to interrogate all translated sequences against the InterPro database (http://www.ebi.ac.uk/Tools/pfa/iprscan/) to predict protein domains. Quantification and drawing of each GO term for all annotated contigs was done using Blast2GO (Götz et al. 2008). Msatcommander (Faircloth 2008) was then employed to search all P. radiata GSS sequences for SSR motifs. To detect different SSR nucleotide motifs (di, tri, tetra, penta and hexa), default parameters were applied (Rozen and Skaletsky 2000).

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4.4 Results

Ion Torrent and contig assembly were used to obtain a GSS dataset from gDNA isolated from P. radiata. A mean of 201 bp GSS read length retrieved was expected because 200 bp library preparation kits were utilized here. The total average GSS read length of 201 bp was similar however to previous studies that have reported NGST runs for ESTs in non-model species including for Glanville fritillary (197 bp Roche 454) European hake (206 bp Roche 454), but lower than for giant freshwater prawn (311 bp Roche 454) (Jung et al. 2011) and channel catfish (292 bp Roche 454) (Jiang et al. 2011). In bivalves, average EST read lengths include; 413 bp in 454 pyrosequencing of Meretrix meretrix (Huan, and Liu 2012), 313 bp in 454 run of Patinopecten yessoensis (Hou et al. 2011), 234 bp in Roche 454 pyrosequencing of P. margaritifera (Joubert et al. 2010), 347 ~ 361 bp in Roche 454 run of P. fucata , and 286 ~ 341 bp in 454 run and 50 bp in Illumina run of P. fucata (Takeuchi et al. 2012).

In particular, the Ion-Torrent is the most cost-effective approach because it is less time consuming and labour intensive compared with other commercialised NGSTs. When the 400 bp library preparation kit is available for Ion Torrent, the disadvantages associated with relative short read lengths should be reduced significantly. A total of 782 microsatellite motifs were detected among the P. radiata GSS sequences. Most of the SSRs identified in the P. radiata genome were dinucleotide repeats (37.2%). This result concord in particular with reports from other pearl oyster species and previous studies of aquatic organisms in general (Jung et al. 2011). A total of 98 SSR primer sets were designed successfully for P. radiata that included 37.7% dinucleotide repeat primers, 23.4% trinucleotide repeat primers, 35.7% tetranucleotide repeat primers, and 3.06% penta/hexanucleotide repeat primers. This number is however, relatively small compared with some previous outcomes from NGST studies (Jones, Zenger and Jerry 2011). While the number of SSRs detected in P. radiata contig sequences was relatively low, the majority of SSRs detected and primers designed were in singletons suggesting that P. radiata GSS sequences mostly are present in non-coding regions

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which embraced many polymorphic sites or as a consequence of homopolymer issues which are common in Ion-Torrent sequencing.

A low rate of gene identification is common for mollusc databases, which usually range from only 15 to 40% (Craft et al. 2010; Clark et al. 2010). The majority of contigs matched in the current study however, were from metagenomic sequences. Considering the filter feeding behaviour of oysters, discovering a large number of bacterial sequences in the current study was not a surprise. In the case of Pinctada species, P. fucata was identified as the major species match for P. radiata sequences largely due to availability of many sequences in the public databases. GSSs identified here did not match any P. radiata published sequences because of the low number of sequences available for P. radiata in the NCBI database (only a few mtDNA sequences). Based on Gene Ontology functions for the P. radiata GSSs many were related to both binding and structural molecular activity. These sequences constitute either enzyme regulators or molecular transducers. Biological process assignments showed that many hits were likely involved in metabolic processes and cellular process, while those coding sequences assigned to cellular components were mostly assumed to contribute to both macromolecular complexes and cell functions.

KEGG pathways identified that GSS coding sequences from P. radiata were largely confined to; purine metabolism, alcohol dehydrogenase, oxidative phosphorylation, and nitrogen metabolism. The highest number of GSS sequences identified were assigned to purine metabolism, a process that plays an important role in many cellular and biochemical pathways and contributes potentially to glutamine synthetase that is required mostly during embryonic development as well as during embryogenesis and oogenesis in oysters. Purine metabolic pathways are potentially involved in interactions with GTP levels and ATP when there is a cellular stress (e.g. oxidative stress or oxygen depletion) (Mommsen and Hochachka 1988; Hardie and Hawley 2001).

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Protein domains identified in P. radiata GSSs in general included; reverse transcriptase, ribonuclease H-like, integrase catalytic core. Reverse transcriptase (RVT) is considered as a cellular gene that plays a critical role in polymerizing DNA on RNA templates (Gladyshev and Arkhipova 2011). Cellular genes (RVTs) are found in all major taxonomic groups and were also identified among the P. radiata contigs (12) in the current study. Ten domains containing ribonuclease H-like were also recovered in the P. radiata GSSs. In addition, a total of three ATP-binding cassette (ABC) transporter-like domains were identified. ABC transporter-like genes are a type of transporter that participate in microcystin transportation (Pearson et al. 2004) and toxin localization in thylakoids or toxin extrusion under some growth conditions (Kaebernick et al. 2000). ABC transporters must be considered as a primary candidate gene due to their significance in bivalves' gills as an active physiological and selective-permeability barrier that act as a defence mechanism against environmental toxicants (Kingtong, Chitramvong and Janvilisri 2007).

Glycoside hydrolase (GH), which belongs to the family 18 chitinases and is widely expressed in prokaryotes and eukaryotes, has a fundamental role in physiological processes including T-cell mediation via allergenic reactions and inflammation responses (Funkhouser and Aronson 2007; Elias et al. 2005). GHs act as key players in the degradation of polysaccharides. Glycosylation is an important chemical reaction for compound modification and in biological activities. Biotechnologically, glycoside hydrolases are considered to be attractive enzymes that can be useful components in synthetic reactions in order to assemble glycosidic linkages. Zinc finger proteins exist abundantly in eukaryotic genomes and have varied functions including; transcriptional activation, protein folding, DNA recognition, lipid binding, RNA packaging and protein assembly (Laity, Lee and Wright 2001). Cation transport atpase is responsible for anion exchange in bivalves and crustaceans in the gills when there is cellular incorporation of many ions (Bianchini and Wood 2002). An organic cation transporter, which is a major facilitator superfamily, has been suggested to be

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involved in regulating cell growth and differentiation in Drosophila (Herranz, Morata and Milán 2006) and also in osmoregulatory ion transport across crustacean gills.

Laminarinase and α-amylase are two important digestive enzymes in bivalves. They are involved in carbohydrase activity as well as hydrolysis of (α-1,4) and (β-1,3) internal glucosidic bonds in starch and laminaran. α-amylase is considered to be a key enzyme in molluscs due to its capability for assimilating carbohydrate in their digestive tracts. CSPs are evolutionarily conserved proteins from archaea to eukaryotes and are known to be involved in diverse cellular processes including cellular growth, cellular division, nutrient stress adaptation to low temperature, and the stationary phase (Kumari Garapati and Suryanarayana 2012).

Alkaline phosphatase (ALP) can be used as an early indicator of cell differentiation and in osteogenic lineages. In addition, ALP has been involved in bivalve immunity responses and various important metabolic activities including; growth, shell formation, protein synthesis, molecule permeability and steroidogenesis (Chen et al. 2005). Glucose-6-phosphate G6P enzymes have also been reported to function as oxidative stress biomarkers in bivalves under heavy metal exposure and temperature change (Viselina and Luk'yanova 2000).

NADH dehydrogenase, ATP synthase and cytochrome C oxidase are play key roles in ion transport and energy metabolism because they are engaged in energy production and consumption (Liu et al. 2007). Metabolic depression in marine bivalves can cause a reduction in ATP consumption or turnover as a result of shifting to anaerobiosis that occurs as a consequence of hypoxia (Anestis et al. 2007). A reduction in the expression of the encoded gene for ATP synthase will decrease the stored energy required for growth and consequently disrupt energy pathways (production and conversion), suggesting that a large amount of the energy has been diverted as a response to metal stress. The data in combination also constitutes in all

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possibility the largest single genome resource for any pearl oyster species, and so has broader applications in this important mollusc group.

5. Conclusion

The paper concluded with the identification of the natural levels and patterns of genetic diversity in the Arabian Gulf of pearl oyster P. radiata and the external factors (such as water temperature, wave action, and prevailing wind change and water currents) that could affect any terrestrial structure detected via a natural distribution. The study was able to generate a huge data set of GSS for the target species P. radiata primarily to identify SSR markers that have the potential to be applied in future population genetic studies of this species. The results of the GSS analysis extended beyond characterization of the SSRs and also identified some putative gene fragments in the GSS dataset that may be affected by environmental stresses and may influence the growth of P. radiata. The results here provide valuable information about the natural levels of genetic diversity and stock structure for the most common pearl oyster, P. radiate in the Arabian Gulf in the Indian Ocean. This information will be important to the long-term sustainability of P. radiata across the region and to wild fisheries management. Anthropogenic factors and natural stresses (including high temperatures and extreme salinity) could play important roles in shaping the local natural demographics of P. radiata across the geographic range of the present study. Obviously, these factors combined with a continuous historical genetic flow were sufficient to hinder any potential for local population differentiation. These genetic sequences could be useful in future studies looking at the effects of human factors on the stocks of wild pearl oysters, which are important local invertebrates, as well as the partial genome dataset of P. Radiata could have applications in biotechnology and pearl oyster culture in the future..

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ISSN: 2616-9185

Reference

Al-Khayat, J. A. and M. A. Al-Ansi. 2008. "Ecological features of oyster beds distribution in Qatari waters, Arabian Gulf." *Asian Journal of Scientific Research* 1 (6): 544-561.

Arbogast, B. S. and G. J. Kenagy. 2001. "Comparative phylogeography as an integrative approach to historical biogeography." *Journal of Biogeography* 28 (7): 819-825.

Avise, J. C. 2000. *Phylogeography: The History and Formation of Species*: Harvard University Press.

Balloux, F. and N. Lugon-Moulin. 2002. "The estimation of population differentiation with microsatellite markers." *Molecular Ecology* 11 (2): 155-165.

Bandelt, H. J., P. Forster and A. Röhl. 1999. "Median-joining networks for inferring intraspecific phylogenies." *Molecular Biology and Evolution* 16 (1): 37-48.

Beebee, T. J. C. and G. Rowe. 2008. *An introduction to Molecular Ecology*: Oxford University Press.

Bermingham, E. and C. Moritz. 1998. "Comparative phylogeography: concepts and applications." *Molecular Ecology* 7 (4): 367-369.

Bertozzi, T., K. L. Sanders, M. J. Sistrom and M. G. Gardner. 2012. "Anonymous nuclear loci in non-model organisms: making the most of high-throughput genome surveys." *Bioinformatics* 28 (14): 1807-1810.

Bohonak, A. J. 1999. "Dispersal, gene flow, and population structure." *The Quarterly Review of Biology* 74 (1): 21-45.

Bowen, R. L. 1951. "The pearl fisheries of the Persian Gulf." *The Middle East Journal* 5 (2): 161-180.

Bruford, M. W., D. G. Bradley and G. Luikart. 2003. "DNA markers reveal the complexity of livestock domestication." *Nature Reviews Genetics* 4 (11): 900-910.

Buhay, J. E. 2009. "“COI-like” sequences are becoming problematic in molecular systematic and DNA barcoding studies". *Journal of Crustacean Biology* 29(1): 96-110.

Chistiakov, D. A., B. Hellemans and F. A. M. Volckaert. 2006. "Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics." *Aquaculture* 255 (1): 1-29.

Multi-Knowledge Electronic Comprehensive Journal For Education And Science Publications(MECSJ)
Issues 77(2024)
ISSN: 2616-9185

Colgan, D. J. and W. F. Ponder. 2002. "Genetic discrimination of morphologically similar, sympatric species of pearl oysters (Mollusca: Bivalvia: *Pinctada*) in eastern Australia." *Marine and Freshwater Research* 53 (3): 697-709.

Doherty, P. J., S. Planes and P. Mather. 1995. "Gene flow and larval duration in seven species of fish from the Great Barrier Reef." *Ecology* 76 (8): 2373-2391.

Edwards, A. W. F. and L. L. Cavalli-Sforza. 1963. "The reconstruction of evolution." *Heredity*
18 (533): 104–105.

Excoffier, L., G. Laval and S. Schneider. 2005. "Arlequin (version 3.0): an integrated software package for population genetics data analysis." *Evolutionary Bioinformatics Online* 1: 47-50.

Faircloth, B. C. 2008. "Msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design." *Molecular Ecology Resources* 8 (1): 92-94.

Feral, J. P. 2002. "How useful are the genetic markers in attempts to understand and manage marine biodiversity?" *Journal of Experimental Marine Biology and Ecology* 268 (2): 121-145.

Folmer, O., M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates." *Molecular Marine Biology and Biotechnology* 3 (5): 294.

Frankham, R. 1995. "Conservation genetics." Annual Review of Genetics 29 (1): 305-327. Frankham, R. 2003. "Genetics and conservation biology." Comptes Rendus Biologies 326: 22-29. Frankham, R., J. D. Ballou and D. A. Briscoe. 2004. A primer of Conversation Genetics:
Cambridge University Press.

Freeland, J. R. 2005. Molecular Ecology: John Wiley & Sons.

Fu, Y. X. 1997. "Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection." *Genetics* 147 (2): 915-925.

Funkhouser, J. D. and N. N. Aronson. 2007. "Chitinase family GH18: evolutionary insights from the genomic history of a diverse protein family." *BMC Evolutionary Biology* 7 (1): 96.

Gardner, M. G., A. J. Fitch, T. Bertozzi and A. J. Lowe. 2011. "Rise of the machines– recommendations for ecologists when using next generation sequencing for microsatellite development." *Molecular Ecology Resources 11*(6): 1093-1101.

Gladyshev, E. A. and I. R. Arkhipova. 2011. "A widespread class of reverse transcriptase-related cellular genes." *Proceedings of the National Academy of Sciences* 108 (51): 20311- 20316.

Multi-Knowledge Electronic Comprehensive Journal For Education And Science Publications(MECSJ)
Issues 77(2024)
ISSN: 2616-9185

Götz, S., J. M. García-Gómez, J. Terol, T. D. Williams, S. H. Nagaraj, M. J. Nueda, M. Robles,
M. Talón, J. Dopazo and A. Conesa. 2008. "High-throughput functional annotation and data mining with the Blast2GO suite." *Nucleic Acids Research* 36 (10): 3420-3435.

Hardie, D. G. and S. A. Hawley. 2001. "AMP-activated protein kinase: the energy charge hypothesis revisited." *Bioessays* 23 (12): 1112-1119.

Harrington, R., C. Anton, T. P. Dawson, F. de Bello, C. K. Feld, J. R. Haslett, T. Kluvánkova- Oravská, A. Kontogianni, S. Lavorel and G. W. Luck. 2010. "Ecosystem services and Hooper, D. U., F. S. Chapin Iii, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D.
M. Lodge, M. Loreau and S. Naeem. 2005. "Effects of biodiversity on ecosystem functioning: a consensus of current knowledge." *Ecological Monographs* 75 (1): 3-35.

Hou, R., Z. Bao, S. Wang, H. Su, Y. Li, H. Du, J. Hu and X. Hu. 2011. "Transcriptome sequencing and de novo analysis for yesso scallop (*Patinopecten yessoensis*) using 454 GS FLX." *PloS One* 6 (6): e21560.

Huan, P., H. Wang and B. Liu. 2012. "Transcriptomic analysis of the clam *Meretrix meretrix* on different larval stages." *Marine Biotechnology* 14(1): 69-78

Hunter, J. R. 1986. "The physical oceanography of the Arabian Gulf: a review and theoretical interpretation of previous observations." The First Arabian Gulf Conference on Environmental and Pollution. Alden Press, Kuwait.

IUCN, SA. 1987. "An assessment of biotopes and management requirements for the Arabian Gulf." International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

Jarne, P. and P. J. L. Lagoda. 1996. "Microsatellites, from molecules to populations and back."*Trends in Ecology & Evolution* 11 (10): 424-429.

Jiang, Y., J. Lu, E. Peatman, H. Kucuktas, S. Liu, S. Wang, F. Sun and Z. Liu. 2011. "A pilot study for channel catfish whole genome sequencing and *de novo* assembly." *BMC Genomics* 12 (1): 629.

Joubert, C., D. Piquemal, B. Marie, L. Manchon, F. Pierrat, I. Zanella-Cléon, N. Cochennec- Laureau, Y. Gueguen and C. Montagnani. 2010. "Transcriptome and proteome analysis of *Pinctada margaritifera* calcifying mantle and shell: focus on biomineralization." *BMC Genomics* 11 (1): 613.

Jung, H., R. E. Lyons, H. Dinh, D. A. Hurwood, S. McWilliam and P. B. Mather. 2011. "Transcriptomics of a giant freshwater prawn (*Macrobrachium rosenbergii*): *de novo* assembly, annotation and marker discovery." *PloS One* 6 (12): e27938.

Kaebernick, M., B. A. Neilan, T. Börner and E. Dittmann. 2000. "Light and the transcriptional response of the microcystin biosynthesis gene cluster."

Multi-Knowledge Electronic Comprehensive Journal For Education And Science Publications(MECSJ)
Issues 77(2024)
ISSN: 2616-9185

Applied and Environmental Microbiology 66 (8): 3387-3392.

Kimura, M. 1980. "A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences." *Journal of Molecular Evolution* 16 (2): 111-120.

Kingtong, S., Y. Chitramvong and T. Janvilisri. 2007. "ATP-binding cassette multidrug transporters in Indian-rock oyster *Saccostrea forskali* and their role in the export of an environmental organic pollutant tributyltin." *Aquatic Toxicology* 85 (2): 124-132.

Kumari Garapati, U. and T. Suryanarayana. 2012. "Isolation of two strong poly (U) binding proteins from moderate halophile *Halomonas eurihalina* and their identification as cold shock proteins." *PloS One* 7 (4): e34409.

Laity, J. H., B. M. Lee and P. E. Wright. 2001. "Zinc finger proteins: new insights into structural and functional diversity." *Current Opinion in Structural Biology* 11 (1): 39-46.

Liu, L., L. Xie, X. Xiong, W. Fan, L. Chen and R. Zhang. 2007. "Cloning and characterization of an mRNA encoding F_1-ATPase beta-subunit abundant in epithelial cells of mantle and gill of pearl oyster, *Pinctada fucata*." *Tsinghua Science & Technology* 12 (4): 381-388.

Mikkelsen, P. M., I. Temkin, R. Bieler and W. G. Lyons. 2004. "*Pinctada Lonisquamosa* (Dunker, 1852) (Bivalvia: Pteriidae), an unrecognized pearl oyster in the western Atlantic." *Malacologia 46*: 473-501.

Mommsen, T. P. and P. W. Hochachka. 1988. "The purine nucleotide cycle as two temporally separated metabolic units: a study on trout muscle." *Metabolism* 37 (6): 552-556.

Moritz, C. 1995. "Uses of molecular phylogenies for conservation." *Philosophical Transactions: Biological Sciences*: 113-118.

Palumbi, S. R. 1996. "What can molecular genetics contribute to marine biogeography? An urchin's tale." *Journal of Experimental Marine Biology and Ecology* 203 (1): 75-92.

Palumbi, S. R. 1997. "Molecular biogeography of the Pacific." *Coral Reefs* 16 (5): 47-52.

Planes, S., M. Parroni and C. Chauvet. 1998. "Evidence of limited gene flow in three species of coral reef fishes in the lagoon of New Caledonia." *Marine Biology* 130 (3): 361-368.

Rozas, J. and R. Rozas. 1999. "DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis." *Bioinformatics* 15 (2): 174-175.

Rozen, S. and H. Skaletsky. 2000. "Primer3 on the WWW for general users and for

Multi-Knowledge Electronic Comprehensive Journal For Education And Science Publications(MECSJ)
Issues 77(2024)
ISSN: 2616-9185

biologist programmers." *Methods Mol Biol* 132 (3): 365-386.

Saitou, N. and M. Nei. 1987. "The neighbor-joining method: a new method for reconstructing phylogenetic trees." *Molecular Biology and Evolution* 4 (4): 406-425.

Selkoe, K. A. and R. J. Toonen. 2006. "Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers." *Ecology Letters* 9 (5): 615-629.

Sheppard, C., M. Al-Husiani, F. Al-Jamali, F. Al-Yamani, R. Baldwin, J. Bishop, F. Benzoni, E. Dutrieux, N. K. Dulvy and S. R. V. Durvasula. 2010. "The Gulf: a young sea in decline." *Marine Pollution Bulletin* 60 (1): 13-38.

Sheppard, C. R. C. 1993. "Physical environment of the Gulf relevant to marine pollution: an overview." *Marine Pollution Bulletin* 27: 3-8.

Southgate, P. C. and J. Lucas. 2008. *The Pearl Oyster*: Elsevier Science Ltd.

Tajima, F. 1989. "Statistical method for testing the neutral mutation hypothesis by DNA polymorphism." *Genetics* 123 (3): 585-595.

Takeuchi, T., T. Kawashima, R. Koyanagi, F. Gyoja, M. Tanaka, T. Ikuta, E. Shoguchi, M. Fujiwara, C. Shinzato and K. Hisata. 2012. "Draft genome of the pearl oyster *Pinctada fucata*: a platform for understanding bivalve biology." *DNA Research* 19(2):117-130.

UN. 2010a. "Ecosystem services our natural capital: allowing their decline is 'like throwing money out the window'." Accessed on 27-10-2010 http://www.un.org/News/Press/docs/2010/sgsm13127.doc.htm.

Viselina, T. N. and O. N. Luk'yanova. 2000. "Cadmium-induced changes in the activity of carbohydrate metabolism enzymes in mollusks." Russian Journal of Marine Biology 26 (4): 289-291.

Wada, K. T. 1984. "Breeding study of the pearl oyster Pinctada fucata." Bulletin of National Research Institute of Aquaculture 6: 79–157.

Wallace, K. B and A. A Starkov. 2000. "Mitochondrial targets of drug toxicity." Annual Review of Pharmacology and Toxicology 40 (1): 353-388.