

Potential of Microsatellite Markers for Genetic Assessment of Scalloped Spiny Lobster (*Panulirus homarus*) Populations in Al Sharqiyah Governorate and their Application in Specie's Fishery Management

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إمكانات علامات التوابع الدقيقة في التقييم الوراثي لتجمعات جراد البحر الشوكي (*Panulirus homarus*) في محافظة الشرقية وتطبيقها في إدارة مصائد الأسماك

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ABSTRACT. Spiny lobster is one of the highest-priced seafood worldwide. Its fishery contributes well to the country's income and is considered one of the important export items to other countries. However, its annual catch declined sharply. Studying the stock is important for proper fishery management because stock responds differently to overexploitation and adaptation, therefore different stocks need different conservation plan. In this study, the genetic diversity and population differentiation of 50 *P. homarus* (the most present spiny lobster subspecies in Oman) from two regions (Al-Ashkharah and Masirah) of Al Sharqiyah governorate were studied using 49 microsatellite markers. The results showed that *P. homarus* from the two sites are genetically similar based on low *Fst* (0.009), AMOVA test (1% variation). In addition, they are admixed as it is shown by DAPC (Discriminate Analysis of Principal Component) and by the phylogenetic tree. In conclusion, *P. homarus* from the two sites can be considered as a single stock, therefore the same fishery management and conservation strategy can be used to manage the spiny lobster fishery in Al Sharqiyah governorate. More studies with a larger sample size are necessary to support our findings.

KEYWORDS: *P. homarus*, microsatellites, Al Sharqiyah, stock.

الخلاصة: يعتبر جراد البحر الشوكي من المأكولات البحرية الأعلى سعرا في جميع أنحاء العالم. كما تساهم مصائدها بشكل جيد في دخل البلاد، وتعتبر أحد أهم العناصر المصدرة إلى الدول الأخرى. ولكن كمية الإنزال السنوية لهذا النوع انخفضت بشكل حاد. ومن المعلوم ان دراسة المخزون المرتبط بأي نوع مهم جدا للحصول على إدارة سمكية ناجحة وذلك لان كل مخزون يتأثر بشكل مختلف بالاستغلال المفرط ويتأقلم بشكل مختلف في البيئات الجديدة وبالتالي قد يحتاج إلى خطة حفظ مختلفة. في هذه الدراسة تمت دراسة التنوع الجيني والتمايز السكاني لخمسعين عينة من جراد البحر الشوكي (*P. homarus*) النوع الأكثر شيوعا من جراد البحر الشوكي في عمان باستخدام 49 تابعا دقيقاً (mi-crosatellite markers). وبالنظر الى النتيجة المنخفضة لمعامل التميز الجيني ($Fst=0.009$) ونسبة الاختلاف بين جراد البحر الشوكي من المنطقتين (1%) وأيضا بالنظر الى النتائج الأخرى من باقي البرامج ومختلف حزم برنامج R ومن شجرة النشوء والتطور فقد تبين ان هناك تشابه جيني كبير لجراد البحر من الموقعين. نستنتج أن ليس هناك هيكلية واضحة لجراد البحر الشوكي من المنطقتين وبالتالي فهي تنتمي الى نفس المخزون وبالتالي نفس إدارة المصائد ممكن ان تتبع لإدارة مصايد جراد البحر في الشرقية ككل. هناك الحاجة إلى مزيد من الدراسات ذات العدد الأكبر من العينات لدعم هذه النتيجة

الكلمات الرئيسية: جراد البحر الشوكي، التوابع الدقيقة، الشرقية، المخزون

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Introduction

Understanding of population structure is needed for effective management and preservation of marine organisms (Kerr et al., 2017). Individuals from different gene pool may exist in the same species which need to be considered as different stock (Gaeta et al., 2020). Stocks respond differently to overexploitation and management strategies (Was et al., 2010). Different studies show that ignoring population structure and stock identification can lead to overexploitation, less productivity and therefore fishery collapse of that species (Kerr et al., 2017). Population structure studies needs more effort because different factors have an effect on the structuring of marine organisms. Oceanographic influences (e.g.: current, eddies and temperature), bio-physical factors (e.g. : larval dispersal and swimming pattern) play a role in the structuring of marine organisms (Cetina-Heredia et al., 2015; Kerr et al., 2017). Spiny lobster (*P. homarus*) has long planktonic duration (PLD) which may extend to 24 months and this enhances larval dispersal by ocean current (Abrunhosa et al., 2008; Butler et al., 2011) resulting in mixing of individuals from different populations and therefore evolving of different species and stocks (Cowen et al., 2006). Spiny lobster is highly priced seafood in Oman and worldwide. However, lobster fishery in Oman dropped from 2000 tons in 1980 to only 950 tons in 2020 (Fishery statistics book, 2020). Ministry of Agriculture and Fisheries wealth set some rules (e.g. size of lobster, catch season, no egg bearing females are allowed to be caught) to manage the catch. However, still there is little recovery in the catch. Different studies have shown the potential of genetic markers for studying the population structure and biodiversity of marine species (Port et al., 2016) which help in the restoration and conservation plan (Young et al., 2015). More studies done worldwide showed that newly discovered stocks were discovered using genetic markers (Cadrin et al., 2010; Clark et al., 2016; Garcia et al., 2012). Microsatellites markers are

polymorphic and are expressed co-dominantly and therefore can be used in such studies (Dao et al., 2015). In this study, the population structure of *P. homarus* from Al-Sharqiyah Governorate (Oman) is studied using microsatellite markers. Eight microsatellites were developed by Dao et al. (2013) from a taxonomically closely related species *Panulirus orantus* by Roch 454 whole-genome sequencing, and then successfully cross-species amplified for *P. homarus*. The remaining microsatellites were developed for *P. homarus* by using the same technique (Delghandi et al., 2015; Delghandi et al., 2016a; Delghandi et al., 2016b).

Materials and Methods

Sampling and DNA Extraction

A part of the second leg (Pereiopod) from twenty and thirty *P. homarus* was collected from fishermen from two regions (Al-Ashkarah and Masirah Island respectively) of Al-Sharqiyah governorate and preserved in 90% ethanol until extraction. DNA from those samples were extracted using modified CTAB (Cetyltrimethylammonium bromide) with some modifications.

Genotyping

Forty-nine microsatellites containing di, tri and tetra nucleotide repeats were used in this study. Microsatellites were amplified in 14 multiplexes based on their allele size, PCR condition and annealing temperature. For each multiplex a mixture of 6.25 μ l 1 \times Type –it master mix (Qiagen), specific concentration of each primer (based on the multiplex condition) and distilled water were mixed (supplement: table 1-4). Four different PCR conditions were designed for the total microsatellites. The 3130 Genetic Analyzer (Applied Biosystems) capillary electrophoresis and GeneMapper software were used to size and genotype the studied microsatellites.

Genetic Diversity

Table 1. Genetic diversity of *P. homarus* from the two studied sites. N: Sample Size, Na: Number of alleles, Ar: Allelic richness, Heterozygosity (observed (Ho) and expected (He)), Np: Private allele, Fis: Inbreeding coefficient.

	N	PIC	Na	Ar	Ho	He	Np	F _{is}
Al-Ashkharah	20	0.510	5.612	4.878	0.371	0.561	1.082	0.372
Masirah Island	30	0.482	6.020	5.504	0.395	0.526	1.490	0.275

Quality of microsatellite markers like presence of stuttered and null alleles and their frequency were examined using Micro-checker v.2.2.3 (Van Oosterhout et al., 2004) and FreeNA software (Chapuis and Estoup, 2006). The deviation of the markers from Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD) was tested by Arlequin V.3.5.2 software. Genetic diversity indices like average number of alleles, observed (Ho)- and expected (He)- heterozygosity, allelic richness and average Polymorphic Information Content (PIC) of the studied microsatellites are calculated using FSTAT v.2.9.3.2 software (Goudet, 1995). Average number of private alleles and number of migrants (Nm) were calculated using Genepop v.4.2 software.

Genetic Structure

Different software (Micro-Checker V.2.2.3, FreeNA, Genepop v.4.2, Arlequin v.3.5.2, FSTAT v.2.9.3.2, GenAlix v.6.5) along with some R packages were used for genetic differentiation of *P. homarus* from Al-Ashkharah and Masirah Island. Structure v.2.3.4 (Pritchard et al. 2000) software was used to study the possible genetic structures within samples. Mega 7 software was used for constructing the phylogenetic tree. Multiple R packages were run for the same purpose, e.g. adegenet (Jombart, 2008), Netview (Steinig et al., 2016), and divMigrate (Keenan et al., 2013).

Results

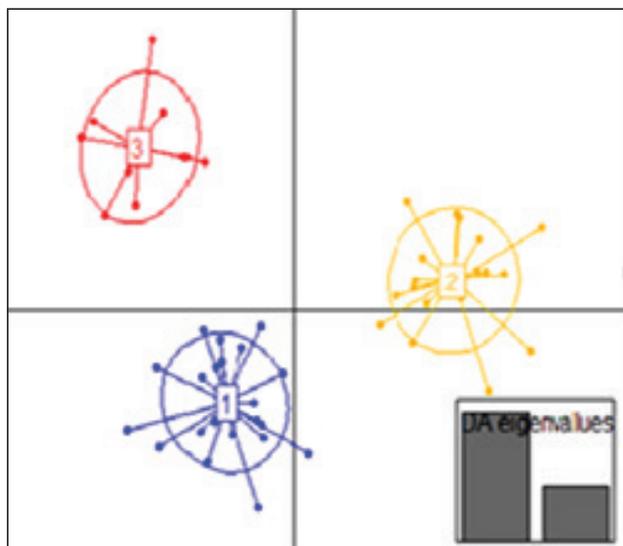
Genetic Diversity

Genetic diversity of *P. homarus* from Al-Sharqiyah was studied using 49 microsatellites. Ten loci had null alleles above 20%, however, the null alleles do not change the *Fst* value significantly, (*Fst* before ENA correction= 0.009, *Fst* after ENA correction=0.016). Hence, all 49 loci were included in further analyses. From the total number of loci only 14 loci were deviated from HWE after Bonferroni correction ($p < 0.001$), Linkage Disequilibrium (LD) was detected only in five pairs from the total 1176 loci pairs at ($p < 0.001$). Only one locus (Pho-G02) was found to be selective by using Bayescan v.2.1 (Follm 2012) software, Pho-G02 found to be a directional outlier using five FDRs (False Discovery Rate: (0.01, 0.05, 0.1, 0.15, and 0.2)). Polymorphic Information Content is an indicator for the polymorphism of loci to be used for genetic differentiation between populations. Allelic richness of loci is another indication for the polymorphism of loci and can be known by calculating the number of alleles each locus has. In this study, the loci had a PIC = 0.505 and an average allelic richness = 6.44. Genetic diversity statistics of the studied 50 samples from the two sites using the 49 microsatellites are summarized in Table 1. *P. homarus* population from the two sites has similar genetic diversity indices.

Genetic Structure

Population differentiation between *P. homarus* individuals from the two sites were measured by the fixation index (F_{st}) value. F_{st} between locations was calculated to be 0.009, which is low. The number of migrants entering the population (N_m) was found to be 2.946. AMOVA test also showed that variation between the two sampling sites is only 1.1% (F_{st} at 95% confidence interval (0.016), F_{st} is low and not significant in differentiating between populations. In population differentiation, study $F_{st} > 0.15$ is considered as significant to differentiate between two populations (Frankham et al., 2002). Results from DAPC (Discriminate Analysis of Principal Component) and Structure v.2.3.4 (Figures 1 and 2 respectively) showed that *P. homarus* individuals from the two sites are grouped into three groups.

Figure 1. Discriminate Analysis of Principal Component (DAPC) showing the distribution of *P. homarus* (dots) into three groups (by number: 1-3)



The phylogenetic tree using Mega 7 was constructed to examine the genetic relationship between individuals. Figure 3 shows that *P. homarus* individuals are belonging to three different groups (three internal nodes) which matches the result from Structure and DAPC. However,

Figure 2. Structure analysis ($k=3$) of *P. homarus* from the two sites (x-axis). Color bars represent a percentage contribution of individuals to the genetic cluster (y-axis).

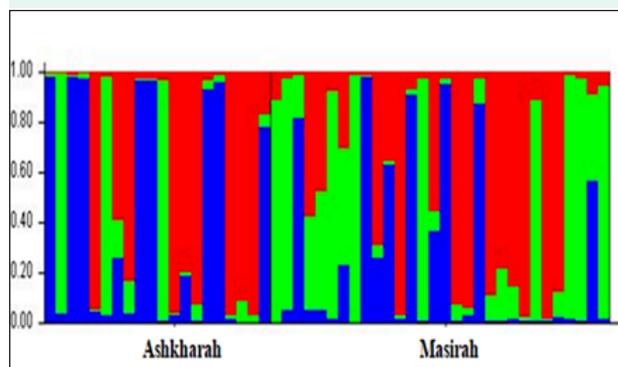
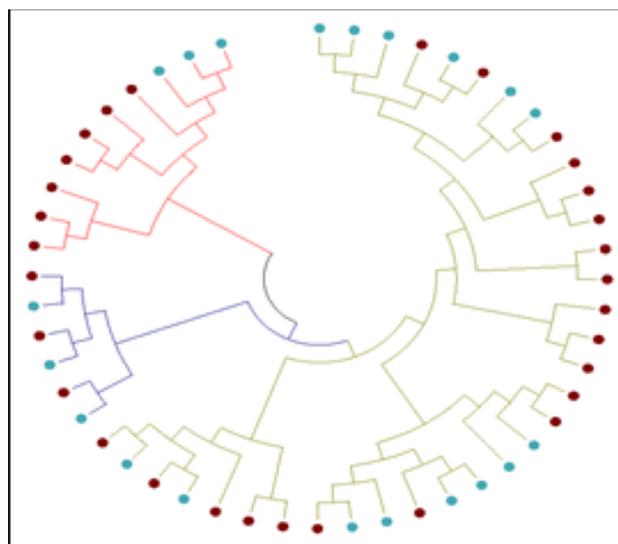
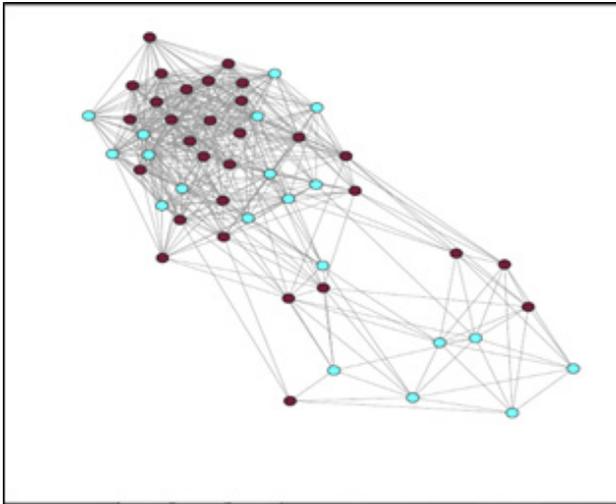


Figure 3. Phylogenetic tree for *P. homarus* from the two sites showing as dots (Blue for Al Ashkharah; violet red for Masirah Island).



individuals from the three groups are admixed confirming the results obtained by Netview R package (Figure 4). For further supporting the results, a migration network diagram was constructed by running divMigrate R package. Figure five shows that 100% of *P. homarus* individuals are migrating from Masirah to Al-Ashkharah, backward migration rate is also high (62%). This may cause the observed admixture of *P. homarus* from the two sites and is in accordance with the results obtained by other R packages mentioned before.

Figure 4. Population structure of *P. homarus* from Al AShkharah and Masirah regions (blue and violet) using Netview R package (k=25)



Discussion

Genetic Diversity

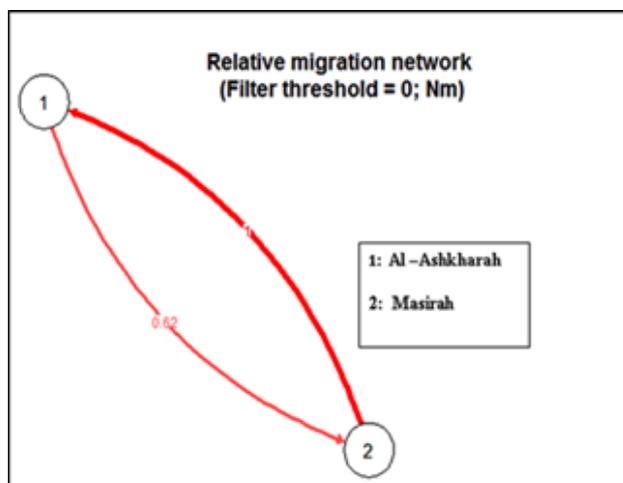
Studying the genetic diversity of marine organisms is important for the conservation and fishery management. The microsatellite markers used in this study to examine the population structure of *P. homarus* along Al Sharqiyah governorate are polymorphic as it is indicated by the average PIC (0.505). It is mentioned by Cao et al. (2016) that microsatellites markers of $PIC > 0.5$ are considered polymorphic, those with $(0.3 < PIC < 0.5)$ are moderately polymorphic and can be used in population genetic studies. Ten microsatellite loci are deviated from HWE at $P < 0.001$, the reason behind this deviation either because of homozygote excess (in 8 loci) or heterozygote excess (in 2 loci). Small sample size may be the reason behind the homozygote excess (Yan et al., 2020). However, it is mentioned by Hale et al. (2012) that small sample size around 25-30 individuals are sufficient for population studies. Homozygote excess result from different factors like selection, present of null alleles, which leads to genotyping errors and other reasons. However, null alleles are commonly present in marine invertebrates (Dailianis et al., 2011; Roterman

et al., 2016) and markers with null alleles < 0.2 are accepted to be used for population genetic studies (Dakin and Avise 2004). In our study, around five loci have null allele frequency > 0.2 .

Population Differentiation

Panulirus homarus from the two sampling sites are admixed as it is shown by the result of Structure v.2.3.4, Phylogenetic tree, DAPC and Netview R packages. Pairwise F_{st} between the two sites is low and not significant (F_{st} 95% confidence Interval: 0.001-.014) this is supported by low percent of variation (1%) from AMOVA test and high number of migrants ($N_m = 2.94$). The Number of migrants > 1 is high enough to cause genetic mixing of *P. homarus* from the two sites (Slaktin, 1993). Different oceanographic, biographic, physical, and biological factors influence larval dispersal and therefore have an effect on structuring of spiny lobsters. Spiny lobster larva have long pelagic phase which enhance their dispersal by ocean current to remote areas, however, it may retain to the shore by means of their vertical migration pattern (Singh et al., 2019). In addition there is a mechanism in marine environment called "Chaotic genetic patchiness" in which larvae may not enter their pelagic stage and related individuals aggregate in less than ten meters (Selwyn et al., 2016). Moreover, larva can tolerate a range of sea surface temperature (SST), and this affect its existence in the water. *Panulirus Homarus megalusculptus* (the most common subspecies in Oman) have multiple broods /per year and therefore they are expected to stay more in water (e.g., in Arabian sea) along the year if no barriers affect its dispersal (Al-Marzouqi et al., 2007; Al-Marzouqi et al., 2008). Omani coast is affected by two monsoons that influence current movement and eddies. Northeast monsoons start in mid of December until mid of March, currents move in anti-clockwise direction and from north to south, by a force larvae drift from Al-Ashkharah to Masirah. Southwest monsoons move the currents in clockwise direction, it starts in summer until fall, wave and winds are stronger during this monsoon and this may be the reason for a higher percentage of migrants from Masirah (south) to Al-Ashka-

Figure 5. The relative migration network constructed by the divMigrate R package shows the migration of *P. homarus*.



rah (North) (Figure 5) (Smith et al., 2020). Few studies were done to examine structuring of *P. homarus* along Oman. Some studies were done to investigate the structuring in Arabian Sea and West Indian Ocean (WIO). Using mitochondrial markers, Farhadi et al. (2013) found that there is little genetic structure of *P. homarus* along Arabian Sea (Oman and Iran) and more genetic structure between those two sites and Africa (Tanzania). Another study by Singh et al. (2017) showed that *P. homarus* from four sites of Oman (Al-Ashkharah, Duqm, Mirbat, Dalkut) were genetically similar. This result is further supported with the result found by Lavery et al. (2014) using phylogenetic tree that *P. homarus* from Oman (subspecies: *P.h.megasculpta*) and Iran are belonging to the same clade with *P. h. homarus* from Africa.

Conclusion

The forty-nine microsatellite markers used in this study are polymorphic and therefore can be used to differentiate between *Panulirus homarus* from different regions from Al-Sharqyah governorate. *Panulirus homarus* along Al-Sharqyah governorate are found to be admixed and should be considered as one stock, therefore same fishery management measures can be used to manage

the catch along Al- Sharqyah governorate. Studying connectivity between marine organisms especially those with long larval stage is important for proper fishery management, different stocks respond differently to the same fishery management strategy. Application of those microsatellite markers can be used to understand the genetic diversity of the other overexploited species that expose the same catch decline problem. More studies with a larger sample size from different regions of Al-Sharqyah and other Oman governorates are required to support the result of the study. In addition, including ecological, environmental and morphological data of spiny lobster when studying its genetic structure will support the result.

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