Genetic structure of *Culcita sp.* pincushion seastar in the Coral Triangle

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Abstract. From the Coral Triangle, which is the global centre of marine biodiversity, species richness tends to decrease eastward across the Pacific Ocean and westward across the Indian Ocean. However, this region is severely threatened by both human and natural disturbances, calling for urgent conservation efforts. Conservation and management decisions inevitably require definition of the limits of population structure of each species as well as mechanisms that produce high biodiversity in the Coral Triangle. In this study we sequenced the mitochondrial COI region of the pincushion seastar, *Culcita sp.*, a common coral reef seastar whose life history resembles other well-studied coral reef seastars. We found a large genetic difference between Indian (*Culcita schmideliana*) and Pacific Ocean (*Culcita novaeguineae*) lineages. All the populations (except from Thailand) had Pacific mtDNA haplotypes, while central and western Indonesian populations had both Indian and Pacific haplotypes, showing a sign of sympatric distribution of these two genetic lineages around these areas. Overall gene flow of *Culcita sp.* in the Coral Triangle was strong and resembled that of *Acanthaster planci* with similar life history traits. Further analysis using nuclear marker might reveal possible hybridization between the distinct genetic lineages of the species in this region.

Key words: Coral Triangle, *Culcita* sp, mitochondrial DNA, CO1, speciation

Introduction

The Coral Triangle, bounded by the Philippines, the Malay Peninsula, and Papua New Guinea, is the global centre of marine biodiversity and species richness is known to decrease from this region eastward across the Pacific Ocean and westward across the Indian Ocean. However, this region is severely threatened from both human and natural disturbances, calling for urgent conservation efforts. Because most marine species have pelagic larval dispersal, conservation management decisions inevitably require defining the limits of population/species structure in the Coral Triangle. Hence, phylogeographic and population genetic analysis using mitochondrial DNA sequences are regarded as one of the most powerful tools (Carpenter et al. 2011) towards these objectives.

A number of genetic studies were conducted using mitochondrial DNAin the Coral Triangle in the last decade and some concordant phylogenetic breaks are identified in some taxa (Carpernter et al. 2011). However there is still lack of concordant phylogeny in other marine invertebrates, especially in echinoderm species. *Holothuria nobilis* showed broad connectivity across the Indo-Pacific (e.g. (Uthicke and Benzie 2003) while *Acanthaster planci* showed clear differentiation between Indian and Pacific lineages in the Coral Triangle (Vogler et al. 2008; Yasuda et al. 2011). *Linckia laevigata* also shows Indian and Pacific lineages (Williams 2000), however, unlike *A. planci* two lineages of *L .laevigata* are mixed in the Coral Triangle (Crandall et al.2008) Given these discrepancies, more studies on echinoderm species with known larval ecology for comparative phylogeography will help to infer what kinds of biological and ecological traits govern the patterns of gene flow in in the Coral Triangle.

Culcita sp. is a good candidate for examining the phylogeography in this area because we have knowledge about larval ecology (Yamaguchi 1977) and it has similar ecological traits with well-studied species *A. planci*, the notorious coral-eating seastar that sometimes causes population outbreaks (Birkeland and Lucas 1990). The habitat of *Culcita sp* is similar to that of *A. planci*, Both species have a similar summer spawning period (Yasuda et al. 2010, Ohta et al. 2011) and similar larval ecology (16 days for *A. planci* and 18 days for *Culcita novaeguineae*

(Yamagushi 1977). The largest ecological difference between the two is that *A. planci* has higher fecundity and sometimes causes population outbreaks while *Culcita* sp. does not (Birkeland and Lucas 1990).

In order to gain further insights on gene flow in the Coral Triangle, we conducted a phylogeographic study of *Culcita sp.* using partial mitochondrial Cytochrome Oxidase subunit I (COI) region collected from the Coral Triangle as well as in its neighboring countries including Thailand, Japan and Palau.

Material and Methods

We sampled a total of 273 individuals of *Culcita sp.* by diving from 15 locations in Southeast Asia and West Pacific regions collaborating with 5 different countries between 2009 and 2011 (Fig.1). Abbreviations of sampling sites are shown in Table 1. There are two species in this region, Pacific species, *C. novaeguineae* and Indian Ocean species, *Culcita schmideliana*. In this paper, we use the term *Culcita sp*. including both two species because this study does not include any detailed morphological analysis. All specimens were photo-vouchered and were returned to their original habitats after collecting a few tube feet per individual. The tube feet were preserved in micro tubes filled with 99.5% ethanol until they are used for genetic analysis. We amplified and sequenced partial cytochrome c oxidase subunit I gene (COI) from genomic DNA extracted using 5 % Chelex (Bio-rad, Hercules, CA) (Walsh et al. 1991) via polymerase chain reaction (PCR) using CO1EF and CO1ER primers (Arndt et al. 1996) with thermocycling parameters of 35 cycles of 94 ^{o}C/30 sec, 45 ^{o}C/30 sec, 72 ^{o}C/30 sec. One microliter of PCR product were cleaned using one unit of exonuclease, and then incubated 37 ^{o}C for 15 min and 80 ^{o}C for 15 min. Cleaned PCR products were sequenced on an ABI 3130xl automated sequencer using BigDye ver.3 (Applied Biosystems, Foster City,CA) terminator chemistry. Forward and Reverse sequences were proofread and subsequently aligned on GENETIX version 10 and then trimmed to 650 base pairs.

We calculated haplotype diversity *h* and nucleotide diversity π (Nei 1987) with the program Arlequin ver. 3.5. The null hypothesis of neutral evolution of the marker was tested using Tajima's *D* test (Tajima 1989) and Fu's *Fs* test (Fu 1997). Mismatch distribution and the model of sudden population expansion (Rogers 1995) were also analyzed. We conducted all the sequence divergence and neutrality tests with 20,000 permutations as implemented in the Alrequin 3.5 (Excoffier and Lischer 2010). To investigate the genetic structure of *C. novaeguineae* in the Coral Triangle region, we first draw median-joining haplotype network (Bandelt et al. 1999) using NETWORK ver 4.6.1.0 with default setting. Pairwise comparisons between *Culcita novaeguineae* populations, Wright's F_{ST} and an analogue of Wright's F_{ST} (Φ_{ST}), which incorporates the model of sequence evolution, were calculated only for populations with N > 9 in ARLEQUIN.

Results

We obtained a 650 bp segment of mitochondrial COI in 273 individuals yielding 97 haplotypes with a total of 110 segregating sites. The basic polymorphism parameters for each population, including, haplotype diversity (h) and nucleotide diversity (π) are provided in Table 1. Overall nucleotide diversity in *Culcita sp.* was 0.012 and corresponding haplotype diversity was 0.880. The highest nucleotide diversity 0.03 was found in KES, whereas the lowest value 0.0008 was found in CAL. The highest haplotype diversity 1.0 was found in THI and BOT and the lowest value 0.464 was found in BOL.

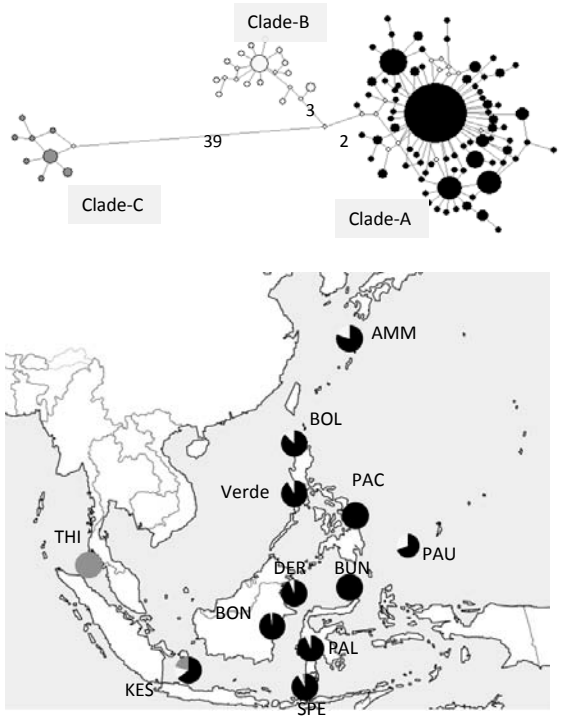

Figure 1: Haplotype network and geographic distribution of three genetic clades

The median-joining network revealed three genetic clusters of haplotypes (Fig. 1). The most distinct clade C was diverged from clades A and B by more than 40 substitutions. Clade C was only found in Thailand and two sites in western and central Indonesia (KES and SPE), corresponding to *Culcita schmideliana* sequences (Gustav Paulay unpublished data) while clade A and B were distributed throughout central Indonesia and the Pacific populations, corresponding

to *C. novaeguineae* (Fig. 1, Table 1). Pairwise Φ_{ST} and F_{ST} values showed PAU and KES are significantly differentiated (p<0.05) from the other populations in the Coral Triangle region (Table 2). Neutrality tests showed both Fu's *Fs* (-78.33, p <0.0001) and Tajima's D (-1.82, p <0.01).

Sampling sites	Abbr.	N_{ind}	N_{haplo}	π	h	F_S	Tajima's D
Amami Ohshima	AMM	5	4	0.0077	0.900	0.49	- 0.95
Verde Island Passage Anilao	BAT	4	4	0.0041	1.000	- 1.41	- 0.21
Verde Island Passage Caban	CAB	10	7	0.0030	0.867	***- 3.35***	***- 1.69***
Verde Island Passage Calatagan	CAL	4	2	0.0008	0.500	0.17	- 0.61
Verde Island Passage Maricaban	MAR	6	5	0.0142	0.933	0.68	0.66
Bolinao	BOL	8	3	0.0065	0.464	3.51	***- 1.81***
Bacon	PAC	6	4	0.0022	0.867	- 1.16	0.34
Palau	PAU	13	10	0.0119	0.949	- 1.58	0.41
Manado	BUN	30	16	0.0033	0.883	***- 10.78***	***- 2.05***
Derawan	DER	32	15	0.0054	0.802	***- 5.15***	***- 1.94***
Palu	PAL	28	14	0.0054	0.825	***- 4.54***	- 1.75
Bontang	BON	40	23	0.0046	0.915	***- 17.56***	***- 2.19***
Spermonde	SPE	34	21	0.0094	0.939	***- 7.65***	- 2.15
Pulau Pari	KES	48	27	0.0300	0.918	- 0.97	0.72
Puhket	THI	2	2	0.0046	1.000	1.10	0.00

Table 1: Summary of collected samples and statistical tests of *Culcita* sp. Haplotype diversity (h), nucleotide diversity (π), Fs, Tajima's D.

	CAB	PAU	BUN	DER	PAL	BON	SPE	KES
CAB		**0.153**	- 0.018	- 0.025	- 0.023	- 0.028	- 0.026	**0.094**
PAU	**0.154**		**0.236**	**0.129**	**0.113**	**0.192**	**0.080**	0.056
BUN	- 0.018	**0.238**		0.007	0.018	- 0.003	0.009	**0.156**
DER	- 0.025	**0.129**	0.008		- 0.013	- 0.013	- 0.013	**0.127**
PAL	- 0.023	**0.113**	0.018	- 0.013		- 0.003	- 0.006	**0.124**
BON	- 0.028	**0.194**	- 0.003	- 0.013	- 0.003		- 0.002	**0.154**
SPE	- 0.026	**0.079**	0.009	- 0.013	- 0.006	- 0.002		**0.089**
KES	0.095	0.056	**0.157**	**0.128**	**0.125**	**0.155**	**0.089**	

Table 2: Pairwise Φ_{ST} (below) and F_{ST} (above) values calculated using Arlquin 3.5. Significant pairs (p < 0.05) are shown in bold

Discussion

While the Coral Triangle harbors the richest marine biodiversity in the world, many species are now severely threatened due to global as well as local anthropogenic stresses. The knowledge of gene flow and comparative phylogeny to identify management units are necessary for conservation of the Coral Triangle. In this study, we examined the genetic structure of a common reef seastar, *Culcita sp.* which would provide a great opportunity for comparative phylogeographic study with other well-studied seastar species that have similar ecological characteristics.

Two *Culcita* species had a large genetic difference between the Indian (*Culcita schmideliana*) and Pacific Oceans (*Culcita novaeguineae*). As well as the other marine invertebrate species that showed large genetic difference between the Indian and Pacific Oceans such as *Linckia laevigata* and *Acanthaster sp.* (Williams et al. 2002; Vogler et al. 2008), Indo-Pacific lineages of *Culcita sp*. might have diverged during Pleistocene fluctuations in sea level that made land bridge and restricted gene flow between the Indian and Pacific Oceans. Over the last 700,000 years, sea level was lower by as much as 130m below the present level and limited gene flow of many coastal marine species between the Oceans (Voris 2000).

Neutrality tests showed either selection in form of genetic hitchhiking, or sudden population expansion have occurred in recent history. Given the scenario of a population reduction due to sea level fluctuations, following population expansion might cause this excess of low-frequency haplotypes. Similar population expansion is seen in other reef organisms such as reef fish and invertebrates in the Coral Triangle (Crandall et al. 2008; Timm and Kochzius 2008; Gaither et al. 2011).

This study also indicated sympatric distributions of deeply separated Indian and Pacific lineages in western (KES) and central (SPE) Indonesia, implying possible occurrences of hybridization. Alternatively these genetic patterns could be caused by incomplete lineage sorting. Further coalescent based analysis will be required to confirm this. In KES and SPE populations, all the different lineages coexist and their habitats overlap. However, eastern limit distribution of Indian Ocean lineage clade C was found in SPE (central Indonesia), suggesting that westward gene flow is stronger than eastward gene flow in the Java Sea. It is possible that continental shelf in Java Sea would partly prevent the Indonesian Throughflow from intruding westward during spawning period. This distribution pattern of Indian vs. Pacific lineages resemble another well-studied coral reef seastar, *Acanthaster planci* (Yasuda et al. 2011) with similar ecological traits such as spawning periods (Yasuda et al. 2010; Ohta et al. 2011) and larval duration period (Yamaguchi 1977).

The origins of two shallow Pacific lineages, clades A and B are not clear from this study. They diverged by only 5 substitutions in the CO1 sequences were found in both Indonesia and Pacific populations except for Thailand. Further sampling and analysis including nuclear DNA could possibly reveal the origins of two lineages.

Significant genetic differentiation observed between KES and other populations were possibly due to the admixture of distinct clade C lineages in KES (Table 1). Significantly differentiated pattern observed in PAU is also due to the higher proportion (about 30 %) of clade B lineages, suggesting that the North Equatorial Current is not strong enough to connect PAU population with the Coral Triangle populations. Similar genetic differentiation between the Philippines and Palau was observed in *A. planci* (Yasuda et al. 2009). Overall *Culcita. sp* exhibits quite similar genetic structuring patterns with *Acanthaster planci* (Yasuda et al 2011) that has similar larval ecological traits. Even though *Culcita* sp. and *A. planci* are strikingly different in the degree of demographic fluctuation, our result showed the

expected patterns of larval dispersal and demographic histories are quite similar to each other. This fact indicates only the number of successful recruitment per year is different between the two species. Therefore, it is also possible that causes of population outbreaks of *A. planci* are not only governed by larval dispersal but also deeply associated with successful fertilization and post-settlement survival rate. Significant genetic structure variations across the studied area (Φ_{ST} = 0.161, F_{ST} = 0.158 $p < 0.0001$) found in *Culcita sp.* is stronger than *Linckia laevigata* (Φ_{ST} = 0.068) and comparable with *Protoreaster nodosus* (Φ_{ST} = 0.166) (Crandall et al. 2008) which has a shorter larval duration than *C. novaeguineae*. Although high gene flow of *Culcita sp.* within the Coral Triangle was demonstrated, we need to further investigate the possible reproductive barriers or compatibility among lineages based on morphology and nuclear genetic markers.

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