

# Genetic population structure of the blue sea star *Linckia laevigata* in the Visayas (Philippines)

DIANA SR ALCAZAR<sup>1,2</sup> AND MARC KOCHZIUS<sup>1</sup>

<sup>1</sup>Marine Biology, Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussels, Belgium, <sup>2</sup>Current address: College of Education, Samar State University, Catbalogan, Philippines

*Coral reef associated marine invertebrates, such as the blue sea star *Linckia laevigata*, have a life history with two phases: sedentary adults and planktonic larvae. On the one hand it is hypothesised that the long pelagic larval duration facilitates large distance dispersal. On the other hand, complex oceanographic and geographic characteristics of the Visayan seascape could cause isolation of populations. The study aims to investigate the genetic diversity, genetic population structure and gene flow in *L. laevigata* to reveal connectivity among populations in the Visayas. The analysis is based on partial sequences (626 bp in length) of the mitochondrial cytochrome oxidase I gene (COI) from 124 individuals collected from five localities in the Visayas. A comparative analysis of these populations with populations from the Indo-Malay Archipelago (IMA) published previously is also presented. Genetic diversity was high ( $h = 0.98$ ,  $\pi = 1.6\%$ ) and comparable with preceding studies. Analyses of molecular variance (AMOVA) revealed a lack of spatial population differentiation among sample sites in the Visayas ( $\Phi_{ST}$ -value = 0.009;  $P > 0.05$ ). The lack of genetic population structure indicates high gene flow among populations of *L. laevigata* in the Visayas. Comparative analysis with data from the previous study indicates high connectivity of the Visayas with the central part of the IMA.*

**Keywords:** Coral triangle, Southeast Asia, starfish, phylogeography, conservation

Submitted 22 October 2014; accepted 2 June 2015; first published online 11 August 2015

## INTRODUCTION

In the Philippines, *Linckia laevigata*, together with other marine species, is exploited for the marine ornamental and aquarium trade on a local and international scale. The Visayan seas are one of the biggest and most productive fishing grounds and are also a prime collection site for marine ornamental products in the Philippine Archipelago (PA) (Floren, 2003). The Philippines are the second largest exporter of marine invertebrates, after Indonesia. *Linckia laevigata* is the most commonly exported sea star for aquarium trade, which accounted for 3% of the total international trade in invertebrates. The main importing countries from 1998–2003 were the USA, European Union and other Asian countries such as Japan, Hong Kong and Taiwan (Wabnitz *et al.*, 2003). Five Visayan islands are identified as major shell collection sites (Cebu, Bohol, Negros, Masbate and Samar; Floren, 2003) and are also possible sites for the collection of *L. laevigata*. The blue sea star is not included in the list of threatened and endangered species, thus no direct efforts on protection are imposed.

The marine ornamental fisheries need to be managed in such a way that they are biologically sustainable. Management tools, such as marine protected areas (MPAs), are considered important to reduce exploitation and to

enhance fish catch and biodiversity for the sustainable use of marine resources (Palumbi, 2001). Suggested conservation efforts should be focused on establishing interconnected MPA networks to protect threatened populations and areas of rich marine biodiversity. It is essential that life history and dispersal capability of target species are understood and taken into consideration in designing the MPA networks (Palumbi, 2003; Kochzius & Nuryanto, 2008; Timm & Kochzius, 2008; Timm *et al.*, 2012). Knowledge on the phylogeographic structure of taxa is also important to define conservation priorities (Féral, 2002). Connectivity among populations is an important factor for natural processes, such as metapopulation and community dynamics, community structure, and genetic diversity, but also plays an important role for the resilience of populations to anthropogenic impacts. The dispersal capability of a species coupled with oceanographic processes influences connectivity among marine populations (Cowen *et al.*, 2007).

Understanding the genetic population structure of a species can give insight into the connectivity of populations. A long pelagic larval duration (PLD) gives high probability of larval exchange among populations, leading to high gene flow and low genetic structuring among populations. Conversely, a short PLD limits gene flow and leads to genetic differentiation among populations (Bossart & Prowell, 1998; Hedgecock *et al.*, 2007).

Past and present oceanographic and geological processes clearly influence the genetic structure of marine populations, e.g. in the Indo-Malay Archipelago (IMA). During Pleistocene glaciations the sea-level dropped by up to 120 m,

### Corresponding author:

M. Kochzius

Email: marc.kochzius@vub.ac.be

exposing the shallow shelf areas of the region. This process formed land bridges and reduced shallow water habitats by exposing the Sunda and Sahul shelves, narrowing the Balabac Strait between Palawan, Borneo and Sulawesi, restraining water mass exchange among the South China Sea and Pacific Ocean as well as the Indian Ocean (Voris, 2000). Studies on the population structure of the giant clams *Tridacna crocea* (Kochzius & Nuryanto, 2008) and *T. maxima* (Nuryanto & Kochzius, 2009), the anemonefish *Amphiprion ocellaris* (Timm & Kochzius, 2008; Timm *et al.*, 2012) and the mushroom coral *Heliofungia actiniformis* (Knittweis *et al.*, 2009) showed a complex genetic population structure, characterized by genetic breaks between the western, central and eastern IMA.

The PA is part of the Coral Triangle (CT) in the IMA, which is the area of the highest marine biodiversity. The PA is a biodiversity and endemism hotspot (Roberts *et al.*, 2002) and the central PA shows a higher concentration of marine shore fishes than other areas in the IMA (Carpenter & Springer, 2005). Despite this high biodiversity, studies on the genetic population structure and gene flow in marine taxa of the PA are rather rare. The few studies conducted investigated *L. laevigata* and *T. crocea* in Palawan (South China Sea and Sulu Sea; Magsino *et al.*, 2002; Juinio-Meñez *et al.*, 2003), *T. crocea* at the Western Pacific coast of the Visayas (Ravago-Gotanco *et al.*, 2007), the anemonefish *Amphiprion clarkii* in Cebu (Pinsky *et al.*, 2010), rabbitfishes along the eastern coast (Magsino & Juinio-Meñez, 2008) and the sea urchin *Tripneustes gratilla* (Malay *et al.*, 2000). Hence, information on connectivity of marine population within the PA and their connectivity to other regions in the IMA is limited.

The asteroid *L. laevigata* is widely distributed in the Indo-West Pacific (IWP), from Eastern Africa across South-east Asia and Australia to Samoa and Fiji. It is a conspicuous coral reef associated benthic species from the family Ophidiasteridae. The adults are sedentary and have a limited dispersal potential, because they belong to the slow-moving macrobenthos, creeping in corals and rocky crevices and migrating between reef patches for grazing on incrusting microscopic organisms and fine detritus. *Linckia laevigata* is a broadcast spawner and has a PLD of 22 days (Yamaguchi, 1977), potentially enabling large-scale dispersal by ocean currents.

This view of large-scale dispersal in *L. laevigata* is supported by genetic studies in the Great Barrier Reef (Williams & Benzie, 1993) and the central IMA (including Cebu, Visayas; Kochzius *et al.*, 2009), showing panmixia over distances of more than 1000 km. Only on an ocean-wide scale, could a significant differentiation between populations of the Indian and Pacific Oceans be detected (Williams & Benzie, 1997; Crandall *et al.*, 2008; Kochzius *et al.*, 2009). However, investigations on a much smaller scale in Palawan (PA) revealed a low but significant differentiation (Juinio-Meñez *et al.*, 2003), indicating that probably local oceanographic and geographic conditions can limit connectivity among populations. In the Visayas, other taxa such as the anemonefish *A. clarkii* (Cebu) and the giant clam *T. crocea* (Eastern Philippine seaboard) also show restricted gene flow (Ravago-Gotanco *et al.*, 2007; Pinsky *et al.*, 2010).

In order to elucidate the pattern of connectivity within the Visayas and of this region to the IMA, this study investigates the genetic population structure of *L. laevigata*. In order to

compare the results with a previous study on connectivity in the IMA (Kochzius *et al.*, 2009), the analysis is based on partial sequences of the mitochondrial cytochrome oxidase subunit I gene (COI).

## MATERIALS AND METHODS

### Sample collection

Samples were collected from five sites around Samar and Leyte (Eastern Visayas), representing the Pacific side and the waters among the islands (Figure 1 and Table 1). This area is facing the fast flowing western boundary currents that split from the North Equatorial Current (NEC): the Kuroshio to the North and the Mindanao current to the South (Qu & Lukas, 2003; Qiu & Chen, 2010). As the NEC bifurcates at the Philippine coast, it significantly contributes to regional water mass transport into the Samar Sea, Gulf of Leyte, and Camotes Sea through the San Bernardino Strait in the North of Samar and Surigao Strait between Leyte and Mindanao in the South (Figure 1; Wyrтки, 1961).

Tissue samples were taken by cutting off about 1 cm of the tip of an arm from 124 *L. laevigata* specimens. Sampling is not lethal and the starfish can regenerate the lost tissue. Among the three observed colour morphs (blue, orange and violet) only the blue morph was sampled and analysed, because species boundaries in *Linckia* are difficult to define (Williams, 2000) and genetic studies indicated that colour variation is congruent to genetic variation (Williams & Benzie, 1998). Collected samples were preserved in absolute ethanol and stored at 4°C.

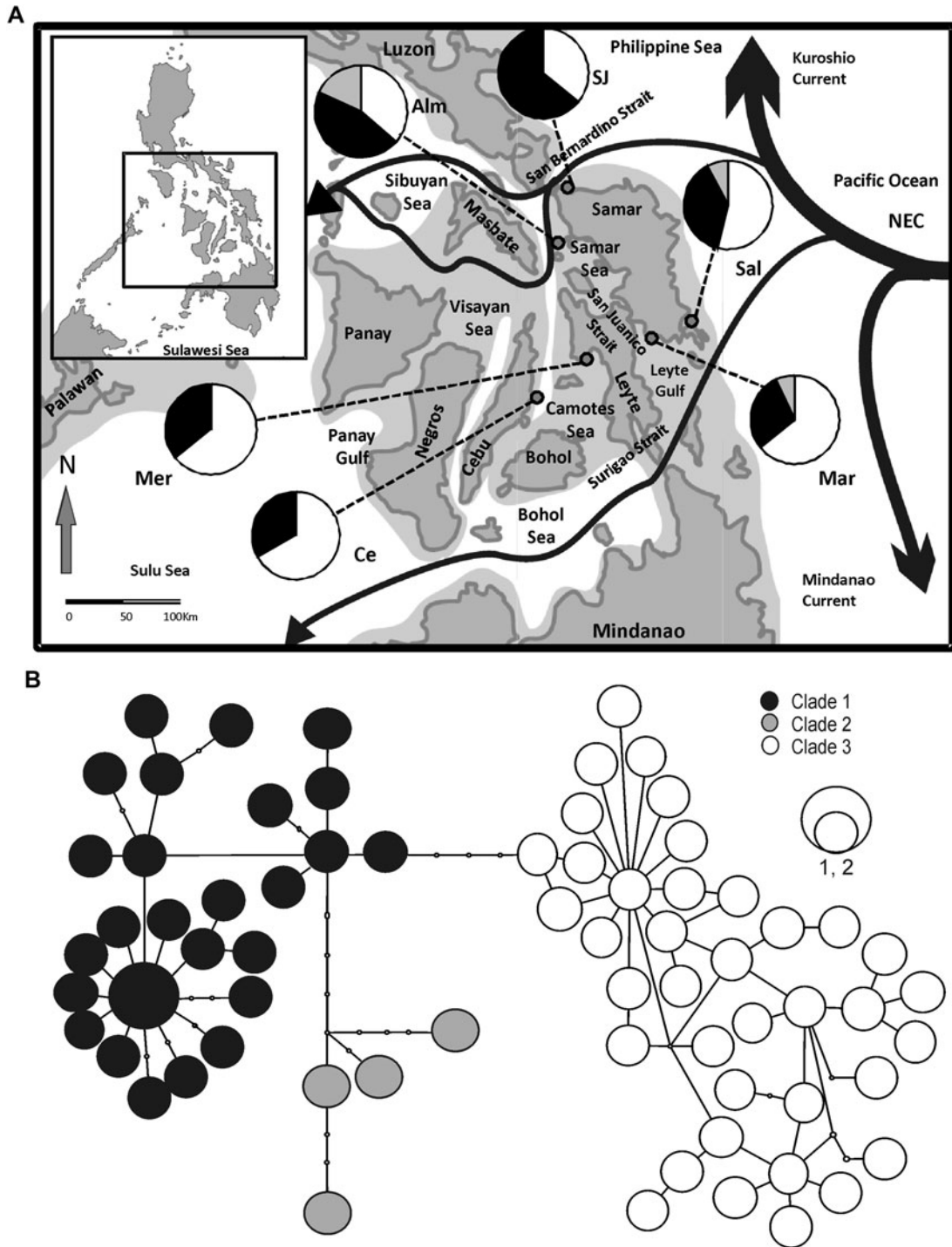
### DNA extraction, amplification and sequencing

DNA from tissue samples was extracted using the NucleoSpin® Tissue extraction kit (Macherey Nagel) following the manufacturer's protocol. Amplification of the COI fragment was conducted with the universal DNA primers described by Folmer *et al.* (1994) through PCR in a 50 µl reaction volume. The PCR reaction contained 2 µl DNA template, 10 mM Tris-HCl (pH 9), 50 mM KCl, 0.2 mM dNTPs, 2 µl BSA (2 mg mL<sup>-1</sup>), 1U Taq polymerase, 0.4 µM of each primer and 1.5–2 mM MgCl<sub>2</sub>. PCR was conducted with a temperature profile of 94°C for 5 min, followed by 35 cycles of 1 min at 94°C, 1.5 min at 45°C and 1 min at 72°C. Final extension was conducted at 72°C for 5 min (Kochzius *et al.*, 2009). Sequencing of both strands was done using the DyeDeoxy terminator chemistry (PE Biosystems) and an automated sequencer (ABI PRISM 3100, Applied Biosystems).

For the analysis of the genetic population structure in the Visayas, COI sequence data of a previous study (Kochzius *et al.*, 2009) from Cebu were utilized. In order to infer connectivity of the Visayas to the IMA, this dataset was compared to COI sequences of 21 populations from the IMA (Table 2; Kochzius *et al.*, 2009).

### Genetic diversity

The software ChromasPro (version 1.5; Technelysium) was used to edit the sequences. The alignment was done with ClustalW (Thompson *et al.*, 1994) as implemented in the programme BioEdit (version 7.0.9; Hall, 1999). The sequences were translated into amino acid sequences using the



**Fig. 1.** (A) Map of the Visayas (Philippines) showing the sample sites (for abbreviations see Table 1) as well as major ocean currents (Wyrтки, 1961) (NEC: North Equatorial Current). Pie charts represent the portion of clades at the different sampling sites as defined in the haplotype network. Pleistocene maximum sea-level low-stand of 120 m is indicated by the light grey area (Vorіs, 2000). (B) Network of mitochondrial cytochrome oxidase I gene haplotypes. Data for Cebu are taken from Kochzius *et al.* (2009). Large circles represent haplotypes and lines are mutational steps. Small circles between connecting lines indicate missing intermediate haplotypes.

programme Squint Alignment Editor (version 1.02; Goode & Rodrigo, 2007) to ensure that functional mitochondrial DNA was obtained and not a nuclear pseudogene. The sequence dataset was reduced to haplotypes with the programme DNA Collapse (FaBox; Villesen, 2007). Genetic diversity was analysed by calculating haplotype (Nei, 1987) and nucleotide diversity (Nei & Jin, 1989) with the programme Arlequin (version 3.5; <http://cmpg.unibe.ch/software/arlequin3.5>; Excoffier & Lischer, 2010).

### Historical demography

The programme Arlequin was used to test the hypothesis of neutral evolution of the marker, using the Tajima’s D test (Tajima, 1989) and Fu’s *F<sub>s</sub>* (Fu, 1997). Mismatch distribution analysis and model of sudden population expansion (Rogers, 1995) was tested by estimation of sum of square deviation (Rogers & Harpending, 1992) and Harpending’s raggedness index (Harpending, 1994).

**Table 1.** Sample sites, number of sequence ( $n$ ), number of haplotypes (Nhp), haplotype diversity ( $h$ ) nucleotide diversity ( $\pi$ ), Tajima's D, Fu's  $F_s$ , sum of square deviation (SSD), and Harpending's raggedness index (HRI) for *Linckia laevigata* in the Eastern Visayas, Philippines. Data for Cebu are taken from Kochzius *et al.* (2009).

Sample sites	Code	$n$	Nhp	Genetic diversity		Neutrality tests		Mismatch distribution	
				$h$	$\pi$ (%)	Tajima's D	Fu's $F_s$	SSD	HRI
Almagro, western Samar	Alm	19	18	0.99	2.0	-0.71 <sup>ns</sup>	-7.06 <sup>**</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>
Cebu	Ce	10	8	0.96	1.7	0.38 <sup>ns</sup>	-1.04 <sup>ns</sup>	0.02 <sup>ns</sup>	0.03 <sup>ns</sup>
Marabut, western Samar	Mar	28	26	0.99	1.5	-0.58 <sup>ns</sup>	-13.55 <sup>***</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>
Merida, Leyte	Mer	16	14	0.98	1.4	0.07 <sup>ns</sup>	-4.68 <sup>*</sup>	0.04 <sup>ns</sup>	0.03 <sup>ns</sup>
Salcedo, eastern Samar	Sal	25	19	0.98	1.5	-0.81 <sup>ns</sup>	-5.4 <sup>*</sup>	0.01 <sup>ns</sup>	0.02 <sup>ns</sup>
San Jose, northern Samar	SJ	36	28	0.98	1.8	-1.66 <sup>*</sup>	-10.39 <sup>**</sup>	0.03 <sup>ns</sup>	0.01 <sup>ns</sup>

\*0.05  $\geq$   $P \geq$  0.01; \*\*0.01  $>$   $P \geq$  0.001; \*\*\* $P <$  0.001; ns, not significant.

## Genetic population structure

The programme Arlequin was used to conduct the analysis of molecular variances (AMOVA; Excoffier *et al.*, 1992) to test for significant population structure. PAUP (version 4.0b10; Swofford, 1998) and Modeltest (version 3.7; Posada & Crandall, 1998) were utilized to determine the appropriate evolutionary model and gamma shape parameter for the dataset used in statistical analysis: Kimura two parameter model (K2P) with a gamma value of 0.365. A hierarchical AMOVA was conducted to elucidate genetic variance among groups of populations, considering the oceanography and geography of the IMA. A haplotype network was calculated with the programme TCS (version 1.21; Clement *et al.*, 2000).

## RESULTS

### Genetic diversity

A COI sequence alignment of 626 bp from 124 specimens of *L. laevigata* collected from five sample sites in the Visayas was obtained (Table 1). Among 124 samples, 81 haplotypes were observed, showing 51 polymorphic sites (8.2%) and 53 substitutions. The lowest number of haplotypes was observed in Merida and the highest in San Jose. Substitutions included 41 transitions and 12 transversions. Genetic diversity within each population showed a high level of haplotype and nucleotide diversity. Haplotype diversity was homogeneous for the entire dataset at a range of 0.98–0.99, whereas nucleotide diversity ranged from 1.4% in Merida to 2% in Almagro with an average of 1.6% (Table 1).

### Historical demography

Based on the result of the Tajima's D test, the null hypothesis of neutral evolution of the COI marker could not be rejected for all sample sites, except for San Jose. However, Fu's  $F_s$  rejected the null hypothesis of neutral evolution at all sample sites (except Cebu), which could also indicate population expansion. Population expansion was supported by a mismatch distribution analysis and Rogers' test of sudden population expansion (Table 1).

### Genetic population structure

Since the published sequences by Kochzius *et al.* (2009) were shorter than the one obtained in this study, the sequence

alignment had a length of 473 bp, resulting in a reduced number of haplotypes. The haplotype network of *L. laevigata* from the Visayas shows the evolutionary relationship among the 69 haplotypes, which can be divided into three clades (Figure 1B). Clades were separated by three to four mutational steps and clade 1 is characterized by a star-like structure. Clade 1 and 3 were present in all sites and the latter was dominant at the majority of sample sites: Cebu, Merida, Marabut and Salcedo, whereas clade 1 showed a higher percentage in San Jose and Almagro. Clade 2 occurs only in Marabut, Salcedo and Almagro.

Analysis of molecular variance revealed genetic homogeneity among *L. laevigata* populations in the Visayas, with a  $\Phi_{ST}$ -value of 0.009 ( $P >$  0.05). The hierarchical AMOVA including sequences from Kochzius *et al.* (2009) revealed the highest significant  $\Phi_{CT}$ -value (0.083;  $P =$  0.004) for the following grouping: Eastern Indian Ocean, Central Indo-Malay Archipelago (including Visayas) and Western Pacific (Table 2).

**Table 2.** Samples sites with COI sequences from Kochzius *et al.* (2009) that were included in the study on gene flow in the Indo-Malay Archipelago. CIMA, Central Indo-Malay Archipelago; EIO, Eastern Indian Ocean; WP, Western Pacific.

Sample site	Region	$n$
Andaman Sea	EIO	3
Bira, Sulawesi	CIMA	13
Banggi Islands, Borneo	CIMA	15
Biak, New Guinea	WP	9
Cebu, Visayas	CIMA	10
Donggala, Sulawesi	CIMA	11
Karimunjawa, Java	CIMA	10
Kendari, Sulawesi	CIMA	11
Kota Kinabalu, Borneo	CIMA	6
Komodo	CIMA	9
Kupang, Timor	EIO	8
Lembah Strait, Sulawesi	CIMA	12
Luwuk, Sulawesi	CIMA	9
Manado, Sulawesi	CIMA	13
Misool, Moluccas	CIMA	16
New Britain, New Guinea	WP	2
Pisang, New Guinea (NG)	WP	13
Pulau Seribu, Java	CIMA	9
Sangkalaki, Borneo	CIMA	11
Spermonde, Sulawesi	CIMA	52
Sebakor/Sanggala/Papisol, New Guinea	CIMA	10
Togian Islands, Sulawesi	CIMA	20

## DISCUSSION

### Genetic diversity

The populations of *L. laevigata* sampled from sites in the Visayas showed a high genetic diversity. Levels of haplotype and nucleotide diversity (0.98–0.99; 1.4–2.0%) are comparable to the previous studies on *L. laevigata* conducted in the IMA using the same genetic marker (Crandall *et al.*, 2008; Kochzius *et al.*, 2009). Similar values for COI haplotype diversity were observed in other invertebrates in the IWP, e.g. the starfish *Protoreaster nodosus*, the shrimp *Periclimenes sorror*, the ectoparasitic snail *Thyca crystallina* and the giant clams *Tridacna crocea* and *T. maxima* (Crandall *et al.*, 2008; DeBoer *et al.*, 2008; Kochzius & Nuryanto, 2008; Nuryanto & Kochzius, 2009; Kochzius *et al.*, 2009). However, nucleotide diversity was usually higher in *L. laevigata* than in the other taxa mentioned.

### Historical demography

While the results of Tajima's *D* neutrality test rejected the null hypothesis of neutral evolution at only one sample site, Fu's *F<sub>s</sub>* rejected it for all of them, except Cebu. This could indicate purifying selection or demographic expansion of *L. laevigata* populations in the study area. A mismatch distribution analysis and Rogers' test of sudden population expansion (Rogers & Harpending, 1992; Rogers, 1995) suggested that the population passed through a demographic expansion. This change in population size could be explained by Pleistocene glacial cycles, which caused changes in sea-level. In this period, a large area of the shelf in the PA was exposed. All the Visayan Islands were connected by land bridges with each other, as well as to Luzon in the North and Mindanao in the South (Figure 1; Voris, 2000), thus reducing the habitat area. In the interglacial period, the rising sea-level opened an opportunity for new habitats to be colonized, which enabled growth of the reduced populations. This was also observed in populations of *L. laevigata* (Kochzius *et al.*, 2009) and other species in the IMA, e.g. giant clams (Kochzius & Nuryanto, 2008; Nuryanto & Kochzius, 2009), anemonefish (Timm & Kochzius, 2008; Timm *et al.*, 2012) and the parasitic snail *Thyca crystallina* (Kochzius *et al.*, 2009).

### Genetic population structure

The populations of *L. laevigata* in the Visayas did not show significant population structure. This suggests high gene flow among the sample sites, due to the high dispersal capability of the species. Preceding studies on *L. laevigata* have shown a shallow genetic population structure in the IWP based on RFLP data (Philippines, Indonesia and Western Australia; Williams & Benzie, 1997) and in the IMA using COI sequences (Crandall *et al.*, 2008; Kochzius *et al.*, 2009). Other studies on invertebrates in the PA based on allozyme variation revealed homogeneity as well as fine scale genetic differentiation in the sea urchin *Tripneustes gratilla* (Malay *et al.*, 2000) and the giant clam *T. crocea* (Ravago-Gotanco *et al.*, 2007), which has a PLD of only 9 days. A study on the anemone fish *Amphiprion clarkii* based on microsatellite loci did not reveal a significant genetic structure among populations from Cebu and Leyte (Pinsky *et al.*, 2010). The long

PLD of *L. laevigata* of about 4 weeks (Yamaguchi, 1977) probably facilitates substantial gene flow and the lack of population structuring. For fish and invertebrate species having a planktonic phase of more than 2 days, over 50% of the variation in genetic dispersal estimates can be explained by the PLD (Kinlan & Gaines, 2003).

The haplotype distribution illustrates strong mixing in the region. Clades 1 and 3 were observed in all stations with highest frequencies. The region in the East is characterized by offshoots of the large bifurcating NEC, which significantly contribute to the inflow into the Visayan seas through the San Bernardino Strait in the North of Samar and Surigao Strait in the South of Leyte (Qu & Lukas, 2003). Fast flowing tidal currents in San Bernardino Strait and Surigao Strait are connected with several passages to feed the water exchange in the region (Wyrcki, 1961). These water transports could facilitate dispersal of propagules, which give rise to high genetic affinity of distant sample sites. The Visayas are composed of several adjacent islands, which possibly provide a more or less continuous habitat that is important for recruitment success. However, clade 2 was only found in the eastern part of the Visayas and did not occur in the central part (Cebu and Merida), indicating some differentiation. It is possible that the sample size was not sufficient to detect a significant genetic differentiation in the AMOVA. Further sampling would be necessary to verify if there is indeed a separation of populations from the central and eastern part of the Visayas.

The general structure of the haplotype network for populations of the Visayas was concordant with that of populations from the IMA (Kochzius *et al.*, 2009), showing two major clades. This could be due to ancestral haplotype clades that had the opportunity for long-distance dispersal over several generations and therefore tend to be frequently occurring at large geographic range (Barber *et al.*, 2006). A star-like structure with a haplotype in a central position and radiating connections to other haplotypes is a characteristic of an ancestral haplotype (Horne *et al.*, 2008), which is evident in the present study. Since clade 2 occurs at only three sites in relatively low frequency, these haplotypes might have evolved recently. However, it is also possible that these haplotypes originate in the Pacific to the east of the PA and are transported by the NEC. This clade was not detected in the previous study (Kochzius *et al.*, 2009).

The significant structuring of populations in the IMA (including Visayas) and EIO is consistent with previous findings (Kochzius *et al.*, 2009). The populations from the Visayas and IMA have a high affinity, which is supported by the higher  $\Phi_{CT}$ -value of the grouping that includes the Visayas in the group of the central IMA. This suggests high connectivity of the Visayas and the central IMA. A similar pattern was also observed in the black tiger prawn, showing no significant structure among western Pacific populations (*Penaeus monodon*; Duda & Palumbi, 1999).

The long PLD and prevailing hydrographic and geographic characteristics of the region explain the low level of genetic differentiation. The major drivers of water transport in this region are the seasonal surface water currents that are induced by monsoon winds and the Indonesian Throughflow (Wyrcki, 1961; Carpenter & Springer, 2005), facilitating dispersal. Since these waterways were greatly reduced during Pleistocene sea-level lowstand (Voris, 2000), mixing of clade 1 and clade 3 might have only re-occurred after the rise of the sea-level.

According to Kochzius *et al.* (2009), clade 1 can be assigned to the Indian Ocean, whereas clade 3 originates in the Pacific Ocean.

## Conservation and management implications

In order to protect a part of the *L. laevigata* population from exploitation, the establishment of a MPA network in the Visayas is recommended, because the extent of connectivity and potential of re-colonisation from other regions of the IMA is not quantified. Such MPAs can serve as a source for new recruits in other areas of the Visayas, where *L. laevigata* is collected for the marine ornamental trade. Since *L. laevigata* shows high connectivity in the central Visayas, no special requirements for the spatial design of a MPA network have to be considered. Since some populations in the eastern Visayas show a higher genetic diversity in regard to clades (Almagro, Marabut and Salcedo), the establishment of MPAs in this region should be prioritised to protect the adaptive potential of *L. laevigata* in the Visayas.

## ACKNOWLEDGEMENTS

We thank the institutions and individuals that have made this study possible: Office of the Municipal Agriculturist, Marabut, Western Samar; Office of the Municipal Agriculturist, Merida, Leyte; Carangian Fisherfolks Development Cooperative, San Jose, Northern Samar; Mr Emilio Cebu, Office of Provincial Agriculturist, Catbalogan, Western Samar; Marine Laboratory Institute of Tropical Ecology, Visayas State University, Baybay, Leyte, Philippines for support during sampling.

## FINANCIAL SUPPORT

We thank VLIR-OUS for providing a ICP scholarship to D.S.R.A; and Vrije Universiteit Brussel (OZR2068BOF) for financial support.

## REFERENCES

- Barber P.H., Erdmann M.V. and Palumbi S.R. (2006) Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the Coral Triangle. *Evolution* 60, 1825–1839.
- Bossart J.L. and Prowell D.P. (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology and Evolution* 13, 202–206.
- Carpenter K.E. and Springer V.G. (2005) The center of the center of marine shore fish biodiversity: the Philippine Islands. *Environmental Biology of Fishes* 72, 467–480.
- Clement M., Posada D. and Crandall K.A. (2000) TCS: a computer program to estimate genealogies. *Molecular Ecology* 9, 1657–1659.
- Cowen R.K., Gawarkiewicz G.G., Pineda J., Thorrold S.R. and Werner F.E. (2007) Population connectivity in marine systems: an overview. *Oceanography* 20, 14–21.
- Crandall E.D., Jones M.E., Muñoz M.M., Akinrombi B., Erdmann M.V. and Barber P.H. (2008) Comparative phylogeography of two seastars and their ectosymbionts within the Coral Triangle. *Molecular Ecology* 17, 5276–5290.
- DeBoer T.S., Subia M.D., Ambariyanto Erdmann M.V., Kovitvongsa K. and Barber P.H. (2008) Phylogeography and limited genetic connectivity in the endangered boring giant clam across the Coral Triangle. *Conservation Biology* 2, 1255–1266.
- Duda T.F. Jr and Palumbi S.R. (1999) Population structure of black tiger prawn, *Penaeus monodon*, among western Indian Ocean and western Pacific populations. *Marine Biology* 134, 705–710.
- Excoffier L. and Lischer H.E.L. (2010) Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10, 564–567.
- Excoffier L., Smouse P.E. and Quattro J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131, 479–491.
- Féral 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, 121–145.
- Floren A.S. (2003) *The Philippine shell industry with specific focus on Mactan, Cebu. Coastal resource management project*. Philippines: DENR-USAID.
- Folmer O., Black M., Hoeh W., Lutz R. and Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3, 294–299.
- Fu X.Y. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147, 915–925.
- Goode M. and Rodrigo A.G. (2007) SQUINT: a multiple alignment program and editor. *Bioinformatics* 23, 1553–1555.
- Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41, 95–98.
- Harpending H.C. (1994) Signature of ancient population-growth in a low-resolution mitochondrial-DNA mismatch distribution. *Human Biology* 66, 591–600.
- Hedgecock D., Barber P.H. and Edmands S. (2007) Genetic approaches to measuring connectivity. *Oceanography* 20, 22–31.
- Horne J.B., Van Herwerden L., Choat J.H. and Robertson D.R. (2008) High population connectivity across the Indo-Pacific: congruent lack of phylogeographic structure in three reef fish congeners. *Molecular Phylogenetics and Evolution* 49, 629–638.
- Juinio-Meñez M.A., Ravago-Gotanco R.M., Magsino R. and Yu E.T. (2003) Genetic structure of *Linckia laevigata* and *Tridacna crocea* populations in the Palawan shelf and shoal reefs. *Marine Biology* 142, 717–726.
- Kinlan B.P. and Gaines S.D. (2003) Propagule dispersal in marine and terrestrial environment: a community perspective. *Ecology*, 84, 2007–2020.
- Knittweis L., Krämer W.E., Timm J. and Kochzius M. (2009) Genetic structure of *Heliofungia actiniformis* (Scleractinia: Fungiidae) populations in the Indo-Malay Archipelago: implications for live coral trade management efforts. *Conservation Genetics* 10, 241–249.
- Kochzius M. and Nuryanto A. (2008) Strong genetic population structure in the boring giant clam, *Tridacna crocea*, across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. *Molecular Ecology* 17, 3775–3787.
- Kochzius M., Seidel C., Hauschild J., Kirchhoff S., Mester P., Meyer-Wachsmuth I., Nuryanto A. and Timm J. (2009) Genetic population structures of the blue starfish *Linckia laevigata* and its

- gastropod ectoparasite *Thyca crystallina*. *Marine Ecology – Progress Series* 396, 211–219.
- Magsino R.M. and Juinio-Meñez M.A.** (2008) The influence of contrasting life history traits and oceanic processes on genetic structuring of rabbitfish populations *Siganus argenteus* and *Siganus fuscescens* along the eastern Philippine coasts. *Marine Biology* 154, 519–532.
- Magsino R.M., Ravago R.G. and Juinio-Meñez M.A.** (2002) Genetic relationship of *Linckia laevigata* color morphs in the Kalayaan Islands Group, western Philippines: preliminary evidence. *Proceedings of the 9th International Coral Reef Symposium* 1, 113–120.
- Malay M.C.D., Junio-Menez M.A. and Villanoy C.J.** (2000) Population genetic structure of the sea urchin *Tripneustes gratilla* from selected sites in Western Luzon and Eastern Philippines. *Proceedings of the 9th International Coral Reef Symposium* 1, 107–111.
- Nei M.** (1987) *Molecular evolutionary genetics*. New York, NY: Columbia University Press.
- Nei M. and Jin L.** (1989) Variances of the average numbers of nucleotide substitutions within and between populations. *Molecular Biodiversity Evolution* 6, 290–300.
- Nuryanto A. and Kochzius M.** (2009) Highly restricted gene flow and deep evolutionary lineages in the giant clam *Tridacna maxima*. *Coral Reefs* 28, 607–619.
- Palumbi S.R.** (2001) The ecology of marine protected areas. In Bertness M.D., Gaines S.M. and Hixon M.E. (eds) *Marine community ecology*. Sunderland, MA: Sinauer Associates, pp. 509–530.
- Palumbi S.R.** (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications* 13, 146–158.
- Pinsky M.L., Montes H.R. and Palumbi S.R.** (2010) Using isolation by distance and effective density to estimate dispersal scales in anemonefish. *Evolution* 64, 2688–2700.
- Posada D. and Crandall K.A.** (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Qiu B. and Chen S.C.** (2010) Interannual-to-decadal variability in the bifurcation of the North Equatorial Current off the Philippines. *Journal of Physical Oceanography* 40, 2525–2538.
- Qu T.D. and Lukas R.** (2003) The bifurcation of the North Equatorial Current in the Pacific. *Journal of Physical Oceanography* 33, 5–18.
- Ravago-Gotanco R.G., Magsino M.A. and Juinio-Meñez M.A.** (2007) Influence of the North Equatorial Current on the population genetic structure of *Tridacna crocea* (Mollusca: Tridacnidae) along the eastern Philippine seaboard. *Marine Ecology – Progress Series* 336, 161–168.
- Roberts C.M., McClean C.J., Veron J.E.N., Hawkins J.P., Allen G.R., McAllister D.E., Mittermeier C.G., Schueler F.W., Spalding M., Wells F., Vynne C. and Werner T.B.** (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science* 295, 1280–1284.
- Rogers A.R.** (1995) Genetic evidence for a Pleistocene population explosion. *Evolution* 49, 608–615.
- Rogers A.R. and Harpending H.** (1992) Population-growth makes waves in the distribution of pairwise genetic-differences. *Molecular Biology and Evolution* 9, 552–569.
- Swofford D.L.** (1998) *PAUP\*: phylogenetic analysis using parsimony (and other methods)*. Version 4.ob10. Sunderland, MA: Sinauer Associates.
- Tajima F.** (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585–595.
- Thompson J.D., Higgins D.G. and Gibson T.J.** (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22, 4673–4680.
- Timm J. and Kochzius M.** (2008) Geological history and oceanography of the Indo-Malay Archipelago shape the genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*). *Molecular Ecology* 17, 3999–4014.
- Timm J., Planes S. and Kochzius M.** (2012) High similarity of genetic population structure in the False Clown Anemonefish (*Amphiprion ocellaris*) found in microsatellite and mitochondrial control region analysis. *Conservation Genetics* 13, 693–706.
- Villesen P.** (2007) FaBox: an online toolbox for fasta sequences. *Molecular Ecology Notes* 7, 965–968.
- Voris H.K.** (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography* 27, 1153–1167.
- Wabnitz C., Taylor M., Green E. and Razak T.** (2003) *From ocean to aquarium*. Cambridge: UNEP-WCMC.
- Williams S.T.** (2000) Species boundaries in the starfish genus *Linckia*. *Marine Biology* 136, 137–148.
- Williams S.T. and Benzie J.A.H.** (1993) Genetic consequences of long larval life in the starfish *Linckia laevigata* (Echinodermata, Asteroidea) on the Great Barrier Reef. *Marine Biology* 117, 71–77.
- Williams S.T. and Benzie J.A.H.** (1997) Indo-West Pacific patterns of genetic differentiation in the high-dispersal starfish *Linckia laevigata*. *Molecular Ecology* 6, 559–573.
- Williams S.T. and Benzie J.A.H.** (1998) Evidence of a biogeographic break between populations of a high dispersal starfish: congruent regions within the Indo-West Pacific defined by color morphs, mtDNA, and allozyme data. *Evolution* 52, 87–99.
- Wyrki K.** (1961) Physical oceanography of the Southeast Asian waters. *Naga Report, Volume 2, Scientific Results of Marine Investigations of the South China Sea and the Gulf of Thailand 1959–1961*. The University of California, Scripps Institution of Oceanography, La Jolla, 195 pp.

and

**Yamaguchi M.** (1977) Population structure, spawning, and growth of the coral reef asteroid *Linckia laevigata* (Linnaeus). *Pacific Science* 31, 13–30.

**Correspondence should be addressed to:**

M. Kochzius  
Marine Biology, Vrije Universiteit Brussel (VUB),  
Pleinlaan 2 1050 Brussels, Belgium  
email: [marc.kochzius@vub.ac.be](mailto:marc.kochzius@vub.ac.be)