



# Genetic population structures of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina*

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**ABSTRACT:** Comparative analyses of the genetic population structure of hosts and parasites can be useful to elucidate factors that influence dispersal, because common ecological and evolutionary processes can lead to congruent patterns. We studied the comparative genetic population structure based on partial sequences of the mitochondrial cytochrome oxidase I gene of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina* in order to elucidate evolutionary processes in the Indo-Malay Archipelago. AMOVA revealed a low fixation index but significant genetic population structure ( $\phi_{ST} = 0.03$ ) in *L. laevigata*, whereas *T. crystallina* showed panmixing ( $\phi_{ST} = 0.005$ ). According to a hierarchical AMOVA, the populations of *L. laevigata* could be assigned to the following groups: (1) Eastern Indian Ocean, (2) central Indo-Malay Archipelago and (3) Western Pacific. This pattern of a genetic break in *L. laevigata* between the Indian and Pacific Ocean, congruent to studies on other marine species in the Indo-Malay Archipelago, is likely due to allopatry caused by Pliocene and Pleistocene glacial sea level low stands.

**KEY WORDS:** COI · Coral Triangle · Coral reef · Phylogeography · Population expansion · Southeast Asia

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## INTRODUCTION

Comparative analyses of the genetic population structure of a host and its parasite can be used to elucidate factors that influence dispersal (Criscione 2008), because common ecological and evolutionary processes can lead to congruent patterns (Bermingham & Moritz 1998, Avise 2000). The blue starfish *Linckia laevigata* (Ophidiasteridea; Chao 1999) is widely distributed on Indo-Pacific coral reefs, from the Western Indian Ocean across the Indo-Malay Archipelago to southeastern Polynesia (Yamaguchi 1977). It is frequently parasitised by the obligate and strictly specific ectoparasitic gastropod *Thyca crystallina* (Eulimidae;

Warén 1980, Janssen 1985), which penetrates the radial hemal and perihemal system of *L. laevigata* with its proboscis to obtain nutrients (Egloff et al. 1988). *T. crystallina* seems to be co-distributed with its host, ranging from the Western Indian Ocean across the Indo-Malay Archipelago and northeastern Australia to Samoa and Fiji (Sloan et al. 1979, Warén 1980). Infection rates of *L. laevigata* with *T. crystallina* vary among populations, ranging from 14.3% in Fiji (Egloff et al. 1988), 15.7% (Troncoso & Van Goethem 1998) and 22.3% (Bouillon & Jangoux 1984) in eastern New Guinea to 62.0% in the Moluccas (Elder 1979).

Here we compare the genetic population structure of the host *Linckia laevigata* and its parasite *Thyca*

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*crystallina* in the Indo-Malay Archipelago. This highly dynamic region in terms of biodiversity, ecology, geology and oceanography is the centre of marine shallow water biodiversity (Briggs 1999, Hoeksema 2007). Molecular phylogenetic studies have shown a break between the Indian and Pacific Oceans, e.g. in lionfishes (Kochzius et al. 2003), clownfishes (Timm et al. 2008), and damselfishes (Froukh & Kochzius 2008). Additionally, a growing number of population genetic studies in the Indo-Malay Archipelago show a complex pattern of divergent lineages and restricted gene flow, e.g. in a clownfish (Timm & Kochzius 2008), giant clams (Kochzius & Nuryanto 2008, Nuryanto & Kochzius 2009), and a mushroom coral (Knittweis et al. 2009).

Plate tectonic movements in the Indo-Malay Archipelago and global fluctuation of sea level during multiple Pliocene and Pleistocene glaciations are the primary hypothesised triggers for this genetic separation of the 2 ocean basins. Most of the islands in the Indo-Malay Archipelago did not exist or were not at their current position about 30 million years ago. During that time, water masses of the Pacific South Equatorial Current (SEC) entered the Indian Ocean via the so-called Indonesian seaway. This current pattern started to change about 25 million years ago, due to the development of Sulawesi by the amalgamation of several fragments and the northward movement of New Guinea, the Bird's Head Peninsula, and Australia (Hall 1998). The Indonesian seaway was finally closed about 5 million years ago by the northward displacement of New Guinea (Cane & Molnar 2001). Since then, the major exchange of water masses between the 2 oceans has been facilitated by the Indonesian throughflow (ITF), which originates from the northern Pacific (Gordon & Fine 1996, Gordon 2005).

Additionally, multiple glaciations in the Pliocene and Pleistocene caused global fluctuations in sea level with low stands of up to 120 m below present sea level (Krantz 1991, Rohling et al. 1998, Siddall et al. 2003). Shallow shelf areas such as the Sunda shelf were exposed, and ocean basins were separated (Voris 2000; Fig. 1). Molecular clock estimates support the view of allopatric speciation in separate ocean basins during the Pliocene and Pleistocene in some species (Kochzius et al. 2003, Timm et al. 2008).

The present study aims to elucidate if common ecological and evolutionary processes lead to congruence in the genetic structure of *Linckia laevigata* and *Thyca crystallina* populations from the Indo-Malay Archipelago. The genetic marker used for both species in the present study is the cytochrome c oxidase I gene (COI), which is suitable to investigate the genetic population structure of starfishes (e.g. Harley et al. 2006) and snails (e.g. Reid et al. 2006).

## MATERIALS AND METHODS

**Sampling.** Tissue samples from 270 specimens of the blue starfish *Linckia laevigata* and 324 specimens of its ectoparasite, the snail *Thyca crystallina*, were collected during several field trips from 2004 to 2007 at 24 sample sites across the Indo-Malay Archipelago (Fig. 1A,C, Table 1). Several colour morphs of *L. laevigata* are known, such as blue, orange, green, and violet. Since genetic studies indicated that colour variation is congruent to genetic variation (Williams & Benzie 1998) and that species boundaries in *Linckia* are difficult to define (Williams 2000), only blue colour morphs were analysed. Tissue samples were preserved in >96% ethanol and later stored at 4°C.

**DNA extraction, amplification and sequencing.** Extraction of genomic DNA from both species was done with the Chelex method, following the protocol of Walsh et al. (1991). A fragment of the mitochondrial COI gene was used for both species as molecular marker and amplified with the primers from Folmer et al. (1994). PCR was conducted in a volume of 50 µl and contained 2 µl DNA template, 10 mM Tris-HCl (pH 9), 50 mM KCl, 4 mM MgCl<sub>2</sub>, 0.4 µM of each primer, 0.2 mM dNTPs, 2 µl BSA (2 mg/ml) and 1 U *Taq* polymerase. The following temperature profile was used for the PCR: 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. The PCR products were purified using the QIAquick spin column PCR purification kit (Qiagen), following the manufacturer's protocol. Sequencing was done using the DyeDeoxy terminator chemistry (PE Biosystem) and an automated sequencer (ABI PRISM 310 and 3100, Applied Biosystems). New primers were designed for cycle sequencing in *Linckia laevigata* (*Linckia* Seq Frw [forward]: 5'-AAA ATC AGA ATA AGT GCT GGA-3'; *Linckia* Seq Rev [reverse]: 5'-TTT GGA GCT TGA GCT GGA ATG-3') and *Thyca crystallina* (*Thyca* Seq Frw [forward]: 5'-TAT TGT AAC TGC TCA TGC TTT TG-3').

**Genetic diversity.** Sequences were edited with the programmes Sequence Navigator (version 1.0.1, Applied Biosystems) or Seqman (version 4.05, DNASTar). They were translated to amino acids with the program Bioedit (version 7.0.9.0, Hall 1999) in order to exclude mistakes in sequencing and to verify if a functional mitochondrial DNA sequence was obtained and not a nuclear pseudogene. Multiple alignment was done using Clustal W (Thompson et al. 1994) as implemented in the software Bioedit. Haplotype diversity *h* (Nei 1987) and nucleotide diversity (Nei & Jin 1989) were calculated with the programme Arlequin (<http://cmpg.unibe.ch/software/arlequin3>, version 3.11; Excoffier et al. 2005).

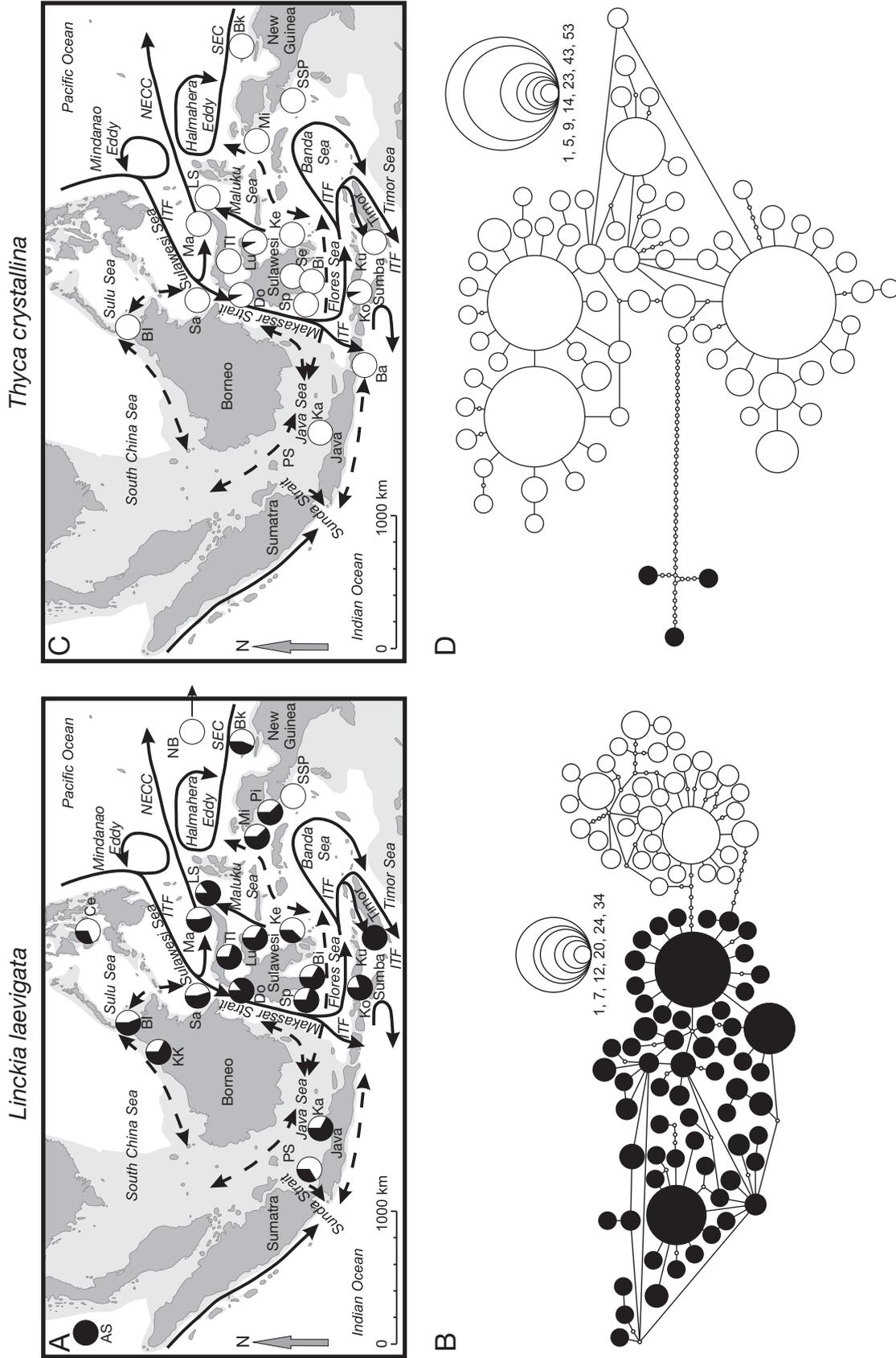


Fig. 1. *Linckia laevigata* (A, B) and *Thyca crystallina* (C, D). (A, C) Indo-Malay Archipelago with sample sites (for abbreviations see Table 1) as well as oceanographic patterns with dominant (solid lines) and seasonally changing (dashed lines) currents (Wyrki 1961, Gordon & Fine 1996, Gordon 2005). ITF: Indonesian throughflow; SEC: Southern Equatorial Current; NECC: Northern Equatorial Counter Current. Pleistocene maximum sea level low stand of 120 m is indicated by the light grey area (Voris 2000). Pie charts represent the proportion of clades defined in the network at the different sample sites. (B, D) Networks of mitochondrial cytochrome c oxidase I haplotypes. Large circles represent haplotypes and connecting lines between them one mutational step. The size of the circles is proportional to haplotype frequency. Small circles indicate missing intermediate haplotypes

Table 1. *Linckia laevigata* and *Thyca crystallina*. Sample sites in the Indo-Malay Archipelago, number of sequences (n), number of haplotypes (N<sub>hp</sub>), number of haplotype diversity (h), nucleotide diversity (π), Tajima's D, Fu's Fs, sum of square deviation (SSD) and Harpending's raggedness index (HRI). \*: 0.05 ≥ p ≥ 0.01; \*\*: 0.01 > p ≥ 0.001; \*\*\*: p < 0.001; ns: not significant, -: not determined

Sample site	<i>Linckia laevigata</i>						<i>Thyca crystallina</i>												
	Code	n	N <sub>hp</sub>	h	π (%)	Tajima's D	Fu's Fs	SSD	HRI	Mismatch distribution	n	N <sub>hp</sub>	h	π (%)	Tajima's D	Fu's Fs	SSD	HRI	Mismatch distribution
Andaman Sea	AS	3	3	1.00	1.4	0.00 <sup>ns</sup>	0.59 <sup>ns</sup>	0.37 <sup>ns</sup>	1.11 <sup>ns</sup>	-	-	-	-	-	-	-	-	-	-
Bali	Ba	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bira, Sulawesi	Bi	13	9	0.94	1.5	0.27 <sup>ns</sup>	-1.00 <sup>ns</sup>	0.03 <sup>ns</sup>	0.04 <sup>ns</sup>	15	8	0.88	0.6	1.05 <sup>ns</sup>	-2.43 <sup>ns</sup>	0.01 <sup>ns</sup>	0.03 <sup>ns</sup>	0.03 <sup>ns</sup>	0.03 <sup>ns</sup>
Bangi Islands, Borneo	BI	15	10	0.92	1.6	0.30 <sup>ns</sup>	-1.07 <sup>ns</sup>	0.01 <sup>ns</sup>	0.02 <sup>ns</sup>	14	6	0.80	0.6	1.33 <sup>ns</sup>	-0.75 <sup>ns</sup>	0.03 <sup>ns</sup>	0.03 <sup>ns</sup>	0.13 <sup>ns</sup>	0.13 <sup>ns</sup>
Biak, New Guinea	Bk	9	9	1.00	1.8	0.10 <sup>ns</sup>	-3.44*	0.05 <sup>ns</sup>	0.07 <sup>ns</sup>	29	17	0.95	0.9	-0.72 <sup>ns</sup>	-9.44***	0.01 <sup>ns</sup>	0.03 <sup>ns</sup>	0.03 <sup>ns</sup>	0.03 <sup>ns</sup>
Cebu, Visayas	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>	3	2	0.67	0.2	0.00 <sup>ns</sup>	0.20 <sup>ns</sup>	0.09 <sup>ns</sup>	0.09 <sup>ns</sup>	0.56 <sup>ns</sup>	0.56 <sup>ns</sup>
Donggala, Sulawesi	Do	11	6	0.84	0.8	-0.89 <sup>ns</sup>	0.06 <sup>ns</sup>	0.05 <sup>ns</sup>	0.11 <sup>ns</sup>	-	-	-	-	-	-	-	-	-	-
Karimunjawa, Java	Ka	10	9	0.98	1.9	-0.40 <sup>ns</sup>	-2.16 <sup>ns</sup>	0.10 <sup>ns</sup>	0.06 <sup>ns</sup>	23	13	0.91	2.8	-2.01*	-1.71 <sup>ns</sup>	0.01 <sup>ns</sup>	0.02 <sup>ns</sup>	0.02 <sup>ns</sup>	0.02 <sup>ns</sup>
Kendari, Sulawesi	Ke	11	8	0.93	1.9	-0.55 <sup>ns</sup>	-0.24 <sup>ns</sup>	0.02 <sup>ns</sup>	0.06 <sup>ns</sup>	2	2	1.00	1.1	0.00 <sup>ns</sup>	1.39 <sup>ns</sup>	-	-	-	-
Kota Kinabalu, Borneo	KK	6	6	1.00	2.1	-0.27 <sup>ns</sup>	-1.16 <sup>ns</sup>	0.09 <sup>ns</sup>	0.31 <sup>ns</sup>	17	9	0.89	0.7	-0.57 <sup>ns</sup>	-3.00*	0.02 <sup>ns</sup>	0.02 <sup>ns</sup>	0.06 <sup>ns</sup>	0.06 <sup>ns</sup>
Komodo	Ko	9	8	0.97	1.8	-0.50 <sup>ns</sup>	-1.65 <sup>ns</sup>	0.02 <sup>ns</sup>	0.05 <sup>ns</sup>	-	-	-	-	-	-	-	-	-	-
Kupang, Timor	Ku	8	7	0.96	0.9	-0.33 <sup>ns</sup>	-2.26 <sup>ns</sup>	0.03 <sup>ns</sup>	0.11 <sup>ns</sup>	21	13	0.93	2.8	-2.08**	-2.18 <sup>ns</sup>	0.01 <sup>ns</sup>	0.03 <sup>ns</sup>	0.03 <sup>ns</sup>	0.03 <sup>ns</sup>
Lembbeh Strait, Sulawesi	LS	12	10	0.97	1.4	-0.98 <sup>ns</sup>	-2.88 <sup>ns</sup>	0.01 <sup>ns</sup>	0.02 <sup>ns</sup>	21	12	0.90	0.8	-0.49 <sup>ns</sup>	-4.69**	0.01 <sup>ns</sup>	0.03 <sup>ns</sup>	0.03 <sup>ns</sup>	0.03 <sup>ns</sup>
Luwuk, Sulawesi	Lu	9	8	0.97	1.8	-0.23 <sup>ns</sup>	-1.62 <sup>ns</sup>	0.05 <sup>ns</sup>	0.18 <sup>ns</sup>	11	8	0.95	1.1	-0.29 <sup>ns</sup>	-1.99 <sup>ns</sup>	0.04 <sup>ns</sup>	0.15 <sup>ns</sup>	0.15 <sup>ns</sup>	0.15 <sup>ns</sup>
Manado, Sulawesi	Ma	13	10	0.95	2.2	0.44 <sup>ns</sup>	-1.09 <sup>ns</sup>	0.01 <sup>ns</sup>	0.03 <sup>ns</sup>	14	10	0.95	3.9	-1.83*	-0.76 <sup>ns</sup>	0.02 <sup>ns</sup>	0.04 <sup>ns</sup>	0.04 <sup>ns</sup>	0.04 <sup>ns</sup>
Misool, Moluccas	Mi	16	13	0.97	2.1	-0.51 <sup>ns</sup>	-3.12 <sup>ns</sup>	0.04 <sup>ns</sup>	0.06 <sup>ns</sup>	7	7	1.00	0.9	-0.61 <sup>ns</sup>	-3.86**	0.18*	0.73*	0.73*	0.73*
New Britain, New Guinea	NB	2	2	1.00	1.0	0.00 <sup>ns</sup>	1.39 <sup>ns</sup>	-	-	19	12	0.94	0.9	-1.39 <sup>ns</sup>	-5.04**	0.01 <sup>ns</sup>	0.04 <sup>ns</sup>	0.04 <sup>ns</sup>	0.04 <sup>ns</sup>
Pisang, New Guinea (NG)	Pi	13	11	0.96	1.7	-0.14 <sup>ns</sup>	-3.12 <sup>ns</sup>	0.02 <sup>ns</sup>	0.02 <sup>ns</sup>	-	-	-	-	-	-	-	-	-	-
Pulau Seribu, Java	PS	9	8	0.97	2.3	0.67 <sup>ns</sup>	-1.13 <sup>ns</sup>	0.03 <sup>ns</sup>	0.07 <sup>ns</sup>	-	-	-	-	-	-	-	-	-	-
Sangkalaki, Borneo	Sa	11	9	0.96	1.8	-0.32 <sup>ns</sup>	-1.45 <sup>ns</sup>	0.01 <sup>ns</sup>	0.02 <sup>ns</sup>	40	21	0.93	0.9	-1.44 <sup>ns</sup>	-11.2***	0.01 <sup>ns</sup>	0.06 <sup>ns</sup>	0.06 <sup>ns</sup>	0.06 <sup>ns</sup>
Sembilan, Sulawesi	Se	-	-	-	-	-	-	-	-	19	14	0.96	0.7	-1.29 <sup>ns</sup>	-10.2***	0.002 <sup>ns</sup>	0.005 <sup>ns</sup>	0.005 <sup>ns</sup>	0.005 <sup>ns</sup>
Spermonde, Sulawesi	Sp	52	30	0.95	1.7	-1.05 <sup>ns</sup>	-11.88**	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>	19	15	0.97	0.9	-0.76 <sup>ns</sup>	-10.1***	0.003 <sup>ns</sup>	0.003 <sup>ns</sup>	0.003 <sup>ns</sup>	0.003 <sup>ns</sup>
Sebakor/Sanggala/Papisol, New Guinea	SSP	10	9	0.98	0.9	-1.49 <sup>ns</sup>	-4.30**	0.04 <sup>ns</sup>	0.08 <sup>ns</sup>	40	20	0.91	0.8	-1.48 <sup>ns</sup>	-12.4***	0.01 <sup>ns</sup>	0.04 <sup>ns</sup>	0.04 <sup>ns</sup>	0.04 <sup>ns</sup>
Togian Islands, Sulawesi	TI	20	13	0.91	1.7	-0.31 <sup>ns</sup>	-2.10 <sup>ns</sup>	0.03 <sup>ns</sup>	0.06 <sup>ns</sup>	10	7	0.93	0.8	-0.61 <sup>ns</sup>	-2.01 <sup>ns</sup>	0.02 <sup>ns</sup>	0.02 <sup>ns</sup>	0.02 <sup>ns</sup>	0.02 <sup>ns</sup>

**Historical demography.** The null hypothesis of neutral evolution of the marker was tested using Tajima's *D*-test (Tajima 1989) and Fu's *F<sub>s</sub>*-test (Fu 1997). Negative Tajima's *D*-values can indicate selection, but also population bottlenecks or population expansions (Tajima 1989). The historical demography was analysed by mismatch distribution (Schneider & Excoffier 1999) of the sum of square deviation (Rogers & Harpending 1992) and Harpending's raggedness index (Harpending 1994), thus testing the model of sudden population expansion (Rogers 1995). The mismatch distribution, which is the distribution of the observed differences between haplotypes, is multimodal in populations under a demographic equilibrium and unimodal if a recent and fast demographic expansion of the population has taken place. All tests were conducted with 10 000 permutations as implemented in the programme Arlequin.

**Genetic population structure and connectivity.** Analysis of molecular variance (AMOVA; Excoffier et al. 1992) and pairwise  $\phi_{ST}$ -values were used to test for significance of population structure. Both statistical calculations were carried out using the software Arlequin, applying the Tamura-Nei substitution model and gamma shape parameters of 0.3 (*Linckia laevigata*) and 0.2 (*Thyca crystallina*), based on the result obtained with the programmes PAUP (version 4.0b10; Swofford 1998) and Modeltest (version 3.7; Posada & Crandall 1998). Several groupings of *L. laevigata* and *T. crystallina* populations were tested in a hierarchical AMOVA  $\phi_{CT}$ , considering the geography of the region.

Since the coverage of sample sites for the 2 species was different, AMOVA was also conducted with reduced data sets, containing only sites for which samples of both species were available. This was done in order to test if differences in the genetic population structure were due to unequal sampling. Based on an analysis with Modeltest, a gamma shape parameter of 0.2 and the Tamura-Nei substitution model were applied for both species.

Haplotype networks were calculated with the programme TCS (version 1.21; Clement et al. 2000). Clades were defined by the highest number of mutational steps found in a network.

## RESULTS

### Genetic diversity

Sequence alignments of the COI fragment of 473 and 401 bp lengths were obtained from 270 individuals of *Linckia laevigata* and 324 individuals of *Thyca crystallina*, respectively (Table 1). The observed number of haplotypes was 101 in *L. laevigata* and 68 in *T. crystal-*

*lina*. The sequences of these haplotypes are available at the EMBL sequence database under the accession numbers FN392698-FN392798 (*L. laevigata*) and FN392799-FN392866 (*T. crystallina*).

Genetic diversity within each population of *Linckia laevigata* showed high levels of haplotype and nucleotide diversity, ranging from 0.84 in the population from Donggala (Sulawesi) to 1.00 in Kota Kinabalu (Borneo), New Britain, and Biak (both New Guinea). Nucleotide diversity was between 0.8% in Donggala and 2.3% in Pulau Seribu (Java Sea) (Table 1).

In *Thyca crystallina*, haplotype diversity was similar to *Linckia laevigata*, but nucleotide diversity was generally lower in most populations. Haplotype diversity in *T. crystallina* ranged between 0.80 in Bira (Sulawesi) to 1.00 in Karimunjawa (Java Sea) and Manado (Sulawesi). Nucleotide diversity was between 0.2 in Donggala and 3.9% in Luwuk (Sulawesi) (Table 1).

### Historical demography

The null hypothesis of neutral evolution of the COI marker in *Linckia laevigata* was not rejected for all sample sites based on Tajima's *D*-test, whereas the results of Fu's *F<sub>s</sub>*-test rejected the null hypothesis for 3 sites. In *Thyca crystallina*, Tajima's *D*-test rejected the null hypothesis of neutral evolution in 3 cases; Fu's *F<sub>s</sub>*-test rejected the null hypothesis in half of the cases. However, this could indicate population expansion in both species, which is supported by the mismatch distribution analysis and Rogers' test of sudden population expansion, except in the population of *T. crystallina* from Manado (Sulawesi) (Rogers 1995; Table 1).

### Genetic population structure and connectivity

The evolutionary relationships among 101 *Linckia laevigata* haplotypes and 68 *Thyca crystallina* haplotypes are presented in networks, showing 2 clades in both *L. laevigata* (Fig. 1B) and *T. crystallina* (Fig. 1D). The distribution of clades across the Indo-Malay Archipelago is presented in Fig. 1A, C, respectively.

In *Linckia laevigata*, the 2 clades are separated by 6 mutational steps. The samples from the sites in the Andaman Sea and in Timor consisted only of haplotypes from the black clade, whereas the samples from New Britain and the southwestern coast of New Guinea were presented only by haplotypes of the white clade. All other sites showed haplotypes from both clades, >75% of them dominated by the black clade (Fig. 1A). AMOVA revealed a low fixation index, but significant genetic population structure ( $\phi_{ST} = 0.03$ ,  $p = 0.029$ ) across the Indo-Malay Archipelago. How-

ever, no significant genetic structure was found among sites in the Spermonde Archipelago ( $\phi_{ST} = 0.01$ ,  $p = 0.39$ ). Pairwise  $\phi_{ST}$ -values between sample sites revealed homogeneity among most of them; only 13% showed significant differences (data not shown). A hierarchical AMOVA with several groupings of sample sites was carried out, but only the following rejected the hypothesis of panmixing ( $\phi_{CT} = 0.07$ ,  $p = 0.003$ ): (1) Eastern Indian Ocean (Andaman Sea and Kupang), (2) Western Pacific (Biak, New Britain) and (3) all remaining sites in the central Indo-Malay Archipelago.

The 2 clades in *Thyca crystallina* were separated by 35 mutational steps (Fig. 1D). All sample sites were dominated by the white clade, and haplotypes of the black clade were only present at sample sites from the central Indo-Malay Archipelago (Komodo, Donggala and Luwuk) (Fig. 1C). AMOVA revealed a very low and non-significant fixation index ( $\phi_{ST} = 0.005$ ,  $p = 0.24$ ) across the Indo-Malay Archipelago. Similar to *Linckia laevigata*, most pairwise  $\phi_{ST}$ -values were not significant in *T. crystallina* and only 10% showed restricted gene flow (data not shown). A hierarchical AMOVA did not show any significant groupings of sample sites.

AMOVA of data sets reduced to sites for which samples of both species were available did not show significant population structures for *Linckia laevigata* ( $\phi_{ST} = 0.02$ ,  $p = 0.09$ ) or *Thyca crystallina* ( $\phi_{ST} = 0.002$ ,  $p = 0.33$ ).

## DISCUSSION

### Genetic diversity

Most populations of the starfish *Linckia laevigata* and its ectoparasite *Thyca crystallina* showed high levels of haplotype diversity. These values are comparable to other studies on invertebrate species in the Indo-Malay Archipelago using COI as a marker, such as the giant clams *Tridacna crocea* (DeBoer et al. 2008, Kochzius & Nuryanto 2008) and *Tridacna maxima* (Nuryanto & Kochzius 2009), as well as the mantis shrimp *Haptosquilla pulchella* (Barber et al. 2002). Nucleotide diversity was generally  $>1\%$  in *L. laevigata*, but  $<1\%$  in *T. crystallina*, a pattern also observed in another study (Crandall et al. 2008a). Levels of nucleotide diversity  $<1\%$  were also detected in giant clams (DeBoer et al. 2008, Kochzius & Nuryanto 2008, Nuryanto & Kochzius 2009) and mantis shrimp (Barber et al. 2002).

### Historical demography

The null hypothesis of neutral evolution was rejected by Tajima's  $D$  and Fu's  $F_s$  neutrality tests at some sample sites (Table 1), but these tests cannot distinguish be-

tween selection and changes in population size. Demographic growth was indicated by mismatch distribution analysis and Rogers' test of sudden population expansion (Rogers & Harpending 1992, Rogers 1995). Since large shelf areas, such as the Sunda shelf between Java and Borneo, fell dry during several Pliocene and Pleistocene glacial sea level low stands, the extent of coral reef habitats in the Indo-Malay Archipelago and, consequently, population sizes were reduced (Fig. 1). Because of rising sea level in interglacial periods, new habitats could be colonised, resulting in a demographic and spatial population expansion. Signals of population expansion in the Indo-Malay Archipelago have also been observed in another study on *Linckia laevigata* and *Thyca crystallina* (Crandall et al. 2008a), as well as giant clams (Kochzius & Nuryanto 2008, Nuryanto & Kochzius 2009) and the clownfish *Amphiprion ocellaris* (Timm & Kochzius 2008).

### Genetic population structure and connectivity

*Linckia laevigata* showed a rather weak, but significant population structure ( $\phi_{ST} = 0.03$ ) across the Indo-Malay Archipelago. However, analysis of the reduced data set indicated panmixing ( $\phi_{ST} = 0.02$ ). This is probably due to the fact that populations from the margins of the study area, such as the Eastern Indian Ocean, Eastern New Guinea and the Philippines, have been removed. This indicates that there are high levels of gene flow in the central part of the Indo-Malay Archipelago and that populations at the margin are less well connected to the centre. Several studies based on allozyme data also showed low levels of genetic heterogeneity in *L. laevigata* on different geographic scales, ranging from panmixing (Williams & Benzie 1993, 1996) to significant but shallow genetic structures (Williams & Benzie 1998, Magsino et al. 2000, Juinio-Meñez et al. 2003). On large geographic scales in the Indo-West Pacific, PCR-restriction fragment length polymorphism analysis of mitochondrial DNA revealed a more prominent genetic population structure in *L. laevigata* (Williams & Benzie 1997, 1998) compared to the present study. Other invertebrate species, such as giant clams (DeBoer et al. 2008, Kochzius & Nuryanto 2008, Nuryanto & Kochzius 2009) and mantis shrimp (Barber et al. 2002), showed much higher  $\phi_{ST}$ -values for the COI marker than *L. laevigata* in the present study. The less prominent genetic population structure in *L. laevigata* could be due to substantial gene flow resulting from its high dispersal potential (pelagic larval duration [PLD] = 22 d; Yamaguchi 1973). In contrast, the PLD for giant clams is only 9 d (Lucas 1988). A much stronger genetic population structure was also revealed based on other genetic

markers in clownfish ( $\phi_{ST} = 0.24$ ; Timm & Kochzius 2008) with a PLD of 8 to 12 d (Fautin & Allen 1994), and a mushroom coral ( $\phi_{ST} = 0.26$ ; Knittweis et al. 2009) with a PLD of 3 d (Abe 1937).

The significant genetic structure was more pronounced in the hierarchical analysis ( $\phi_{CT} = 0.07$ ) with the following grouping: (1) Eastern Indian Ocean, (2) central Indo-Malay Archipelago and (3) Western Pacific. This pattern of a discontinuity between the Indian and Pacific Oceans was more or less similar in the giant clams *Tridacna crocea* (DeBoer et al. 2008, Kochzius & Nuryanto 2008) and *Tridacna maxima* (Nuryanto & Kochzius 2009), the clownfish *Amphiprion ocellaris* (Timm & Kochzius 2008) and the mushroom coral *Heliofungia actiniformis* (Knittweis et al. 2009). In another study (Crandall et al. 2008a) on *Linckia laevigata*, a genetic break between grouped sites from the (1) Eastern Indian Ocean and (2) the central Indo-Malay Archipelago, as well as the Western Pacific, showed the same  $\phi_{CT}$ -value as the present analysis. Earlier large-scale studies using COI sequences also detected a genetic differentiation between the 2 ocean basins (Williams 2000, Williams et al. 2002). Such a break was also detected in the crown-of-thorns starfish *Acanthaster planci* based on allozymes (Benzie 1999) and COI sequences (Vogler et al. 2008).

This genetic break between the 2 ocean basins was also apparent in the distribution of the black and white clades in *Linckia laevigata*. Two populations in the Indian Ocean (Andaman Sea and Kupang) harboured only individuals carrying haplotypes of the black clade, whereas the populations in the Western Pacific either only consisted of white clade haplotypes (New Britain) or were dominated by them (Biak and Cebu). These clades showed a strong mixing in the central Indo-Malay Archipelago, but the black clade showed a higher frequency in most of the populations (Fig. 1). In contrast to other species that show a main route of dispersal from the Western Pacific into the Eastern Indian Ocean along the path of the ITF (Kochzius & Nuryanto 2008, Timm & Kochzius 2008, Knittweis et al. 2009, Nuryanto & Kochzius 2009), *L. laevigata* seems to enter the Western Pacific from the Eastern Indian Ocean against the ITF. This pattern can also be observed in other studies on *L. laevigata* (Williams et al. 2002, Crandall et al. 2008a). Dispersal against the strong ITF that transports up to 19 million m<sup>3</sup> of water per second from the Pacific to the Indian Oceans (Gordon & Fine 1996, Gordon 2005) might be facilitated by seasonally changing currents in Makassar Strait along the coast of Borneo, which are directed to the north in October (Wyrtki 1961; Fig. 1). This coincides with the spawning period of *L. laevigata* in October on Heron Island, Great Barrier Reef (Laxton 1974).

Neither an AMOVA nor hierarchical AMOVA indicated restricted gene flow in *Thyca crystallina* across

the Indo-Malay Archipelago. This species produces in extended breeding periods probably planktonic larvae (Elder 1979) with a high dispersal potential, which is supported by the shallow genetic population structure. This lack of a genetic break between the Indian and Pacific Oceans has also been shown for other species, such as the bigeye tuna *Thunnus obesus* (Alvarado Bremer et al. 1998, Chow et al. 2000), the swordfish *Xiphias gladius* (Chow et al. 1997), the tasslefish *Polynemus sheridani* (Chenoweth & Hughes 2003), the snails *Echinolittorina reticulata* (Reid et al. 2006) and *Nerita plicata* (Crandall et al. 2008b), as well as the sea urchin *Diadema savignyi* (Lessios et al. 2001). However, mechanisms that lead to genetic homogeneity across the Indo-Malay Archipelago are expected to be different among species.

Even though no significant genetic population structure was observed, *Thyca crystallina* showed 2 highly divergent clades that were separated by 35 mutational steps (Fig. 1D). This deep divergence might indicate a cryptic species, but this hypothesis should be verified in an integrative taxonomy approach, including genetic, morphological and ecological data. Such an approach revealed, for instance, a new species of giant clam *Tridacna costata* in the Red Sea (Richter et al. 2008). Similar deep divergences have been observed in the giant clams *Tridacna crocea* (DeBoer et al. 2008, Kochzius & Nuryanto 2008) and *Tridacna maxima* (Nuryanto & Kochzius 2009) across the Indo-Malay Archipelago. It is possible that these 2 divergent clades in *T. crystallina* were affiliated to the Indian and Pacific Oceans, respectively, caused by sea level low stands. After the rise in sea level, the 2 clades came into contact again and the black clade was replaced by the white clade. Even though *Linckia laevigata* and *Thyca crystallina* are codistributed due to a host-parasite relationship, they showed differences in their genetic population structure. This is probably caused by differences in their mode of reproduction, showing that it is not only common ecological and evolutionary processes that are important in shaping the genetic population structure of the 2 species.

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