CHAPTER 6

Phylogeography of a widely distributed unicornfish species, *Naso vlamingii*

6.1 Introduction

A phylogeographic study of the broadly distributed species *Naso vlamingii* was undertaken to: (a) determine the extent of genetic differentiation between populations, from which levels of gene flow can be inferred within and (b) amongst ocean basins, and (c) to determine whether the suggested long evolutionary history of *Naso vlamingii* (approx. 17 to 20 MY old lineage, Chapter 4) is reflected in the population genetic architecture (haplotype and nucleotide diversities).

Naso vlamingii (Cuvier & Valenciennes 1835) is widely distributed throughout the tropical Indo-Pacific Ocean. It occurs from the west Indian Ocean (east African coast) to the central Pacific Islands (French Polynesia), including the West Pacific (GBR, New Caledonia, Society Islands, and Japan to the north) and apparently has been observed in the eastern Pacific Ocean (Galapagos Isl. see Randall 2002). Although *N. vlamingii* is a reef-associated species, it is semi-pelagic as an adult and occurs in open water near drop-offs of outer reefs (Randall 2002). It is a generalist utilising different habitats, and it is an omnivore feeding on pelagic and benthic matter as well as on faeces of pelagic fish (coprophagy see Robertson 1982; Choat et al. 2002; Randall 2002). This species is long-lived, living for up to 40 years (Choat and Robertson 2002), and reproduces by broadcast spawning (gonochoristic). It probably has a long pelagic larval duration (PLD) of 1.5 to 3 months, as

has been recorded for a number of *Naso* species (Wilson and McCormick 1999). The rapid and early development of sensory systems in acanthurids (Leis and Richards 1984; Leis and McCormick 2002), coupled with their ability to swim continuously for extended periods at an early stage, as well as an extended PLD, provides them with the capacity to disperse extensively (Leis and Carson-Ewart 1997; Stobutzki 1997).

The lineage giving rise to *N. vlamingii* arose during the Miocene (estimates range between 19.8 and 16.5MY, chapter 4). This was a period of drastic sea level fluctuations (Haq et al. 1987) associated with recurring glaciations and oscillating sea temperatures (Hallam 1984; Pickering 2000; Zachos et al. 2001a). Modern coral reef systems, cooler ocean circulations with increased ocean productivity were already established at this time, restricting coral reefs to lower latitudes (Zachos et al. 2001a).

Given these features, I would predict this species to have (a) high levels of gene flow inferred from little or no genetic differentiation between populations within and (b) possibly amongst ocean basins. (c) Due to high connectivity among populations, I would predict a shallow population structure within the deeply divergent lineage of *N. vlamingii*. Here, I use sequences of the rapidly evolving mt d-loop region to determine the level of genetic differentiation between populations of *N. vlamingii*, and to test these predictions by calculating haplotype and nucleotide diversity indices.

6.2 Materials & Methods

6.2.1 Sampling

Overall, 77 individuals of *N. vlamingii* were collected either by spearing from a wide geographic range or acquired from fish markets (Philippines) (Figure 6.1).

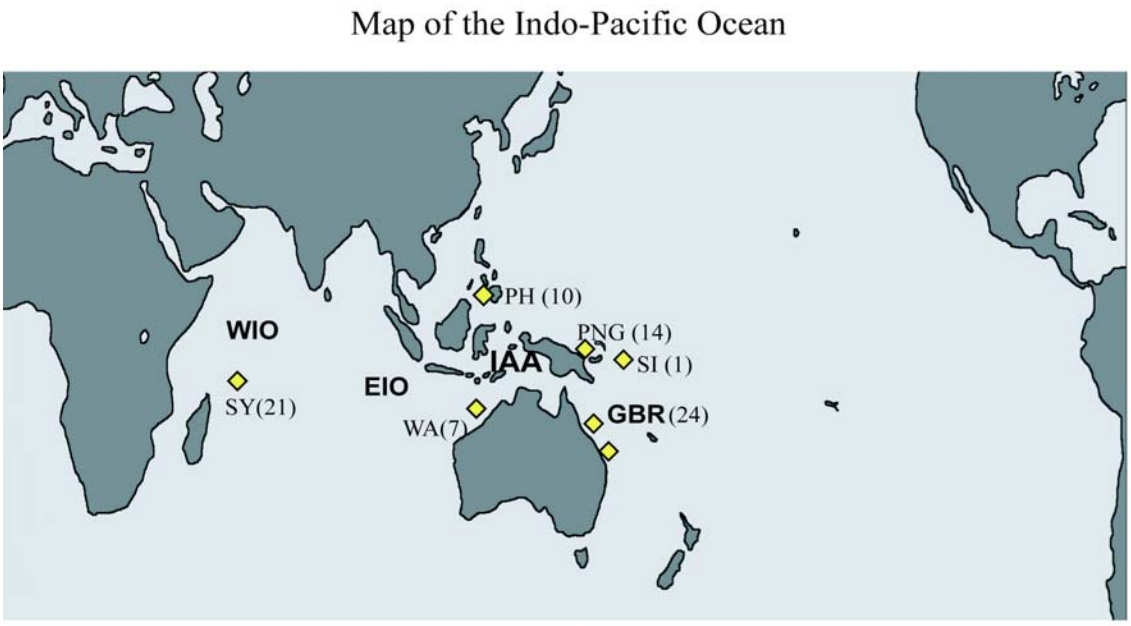

Figure 6.1: Collection sites of 77 *N. vlamingii* individuals. **IAA**: Indo-Australian archipelago (northern PNG- Kimbe Bay in Papua New Guinea, PH- Apo Island in the Philippines, and SI- Solomon Islands). **GBR**- Great Barrier Reef (north: Lizard Island and south: One Tree Island). **EIO**: East Indian Ocean (WA- Western Australia). **WIO**: West Indian Ocean (SY- Amirante Plateau in the Seychelles). Numbers in brackets represent sample sizes. Sample from the Solomon Islands (1 individual) is grouped with samples from PNG; GBR samples are grouped together (22 individuals from Lizard Island, 2 from One Tree Isl.)

6.2.2 Laboratory methods

DNA extraction procedures followed the protocol outlined previously (Chapter 2). The mt control region (d-loop) was amplified with *Naso* specific primers as described previously (Chapter 2, Table 2.1).

6.2.3 Population analysis

Sequences were edited using Sequencher (Gene codes corporation) and aligned using ClustalX (Thompson et al. 1997). To infer relationships between individual specimens (from all localities sampled), a phylogenetic analysis was performed using maximum likelihood approaches (ML) implemented in PAUP* version 4.10b (Swofford 1998). The best substitution model for the data was selected using a likelihood approach implemented in Modeltest 3.06 (Posada and Crandall 1998). The subtree-pruning-regrafting (SPR) branch-swapping algorithm was used in the maximum likelihood analysis with 50 bootstrap replicates. The tree was rooted with *N. unicornis* as an outgroup. Due to the size of the data set and limited capacity of desktop computers, a supercomputer (UNIX platform) was utilised. A time limit of 1 hour per bootstrap replicate was enforced in the command line settings for ML.

In addition, a haplotype tree was constructed to illustrate the relatedness of individuals in the population and to explore geographic partitioning of the data, which was also examined using AMOVA (Analysis of Molecular Variance) (Weir and Cockerham 1984; Excoffier et al. 1992) implemented in Arlequin Version 2.01 (Schneider et al. 2000). *Naso vlamingii* was divided into five populations corresponding to following regions, (1) GBR (Great Barrier Reef), (2) Papua New Guinea (PNG) and Solomon Islands, (3) the Philippines, (4) EIO the East Indian Ocean (Western Australia), and (5) WIO (Seychelles) for AMOVA analyses. PNG, Solomon Islands and Philippine samples were combined as IAA (Indo-Australian archipelago) for the ML phylogram and haplotype tree. The haplotype tree should reveal how many haplotypes are shared and show the number of substitutions that occurred between haplotypes. Few substitutions between haplotypes would suggest a

shallow population structure. Many substitutions between haplotypes would suggest a deep population structure.

Fixation indices (Φ_{ct}, Φ_{sc} and Φ_{st}) and population pairwise F_{st} values were obtained from AMOVA analyses implemented in Arlequin V. 2.01 (Schneider et al. 2000) to infer what levels of gene flow occur between populations. A *P*-value with standard deviation (S.D.) is generated by a nonparametric permutation approach (Excoffier et al. 1992). This *P*-value indicates the "probability of having a more extreme variance component and Φ-statistics than the observed values by chance alone" (Excoffier et al. 1992).

Isolation by distance (using a Mantel test) for population pairs (using pairwise F_{st}-values as a function of geographic distance) was implemented in IBD V. 1.4 (Bohonak 2002). Distances between populations (*ie*. from specific geographic locations) were measured using the shortest possible distance by sea from one location to the other. The program runs through a set of calculations, starting with the non-transformed distance matrix (km) and pairwise F_{st} matrix and finally log transforming both data matrices (see Slatkin 1993; Bohonak 2002). The pairwise comparison among populations (based on locations) should indicate if isolation by distance has any effect on the genetic structure (distinct geographic pattern) of *N. vlamingii*.

The haplotype diversity index (*h*) was calculated using the equation: $h = n(1-\Sigma x_i^2)/(n-1)$ (Nei 1987) and the nucleotide diversity index (π) was calculated in Arlequin V. 2.01 (Schneider et al. 2000). The first index (*h*) provides information about the number of shared haplotypes and if these haplotypes are grouped by geographic location (category I or III,

according to Avise 2000). For example, are there haplotypes shared by several individuals of a particular location, e.g. WA, but which are not seen at other locations? The second index (π) should reveal whether a population has a deep (many substitutions between haplotypes) or shallow (few substitutions between haplotypes) population structure (category II or IV respectively, according to Avise 2000).

6.3 Results

In total 307 base pairs (bp) from the 3' end of the tRNA-Pro gene and the 5' end of the control region were analysed in this study. The transition to transversion ratio was 2.4:1. There were 204 polymorphic sites, 142 of which were parsimony-informative. Sequences were A-T rich (69%) as is usual for mtDNA of marine fish species (McMillan and Palumbi 1997). All 77 individuals examined in this study had unique haplotypes.

The optimal substitution model for these data, HKY + I + Γ, was used in the ML analysis. A total of 53 ML trees was obtained, of which 7 had the same, best log likelihood score (-lnL 4903.428), one of which is illustrated (Figure 6.2). There was no geographic subdivision (Figure 6.2) of samples with only a few clusters having high bootstrap support.

The haplotype tree (Figure 6.3) supports the result of the ML analysis. Individuals from different geographic regions do not segregate into distinct clades. Rather, individuals from all locations are dispersed throughout the haplotype tree (Figure 6.3). Not a single haplotype is shared by any individuals.

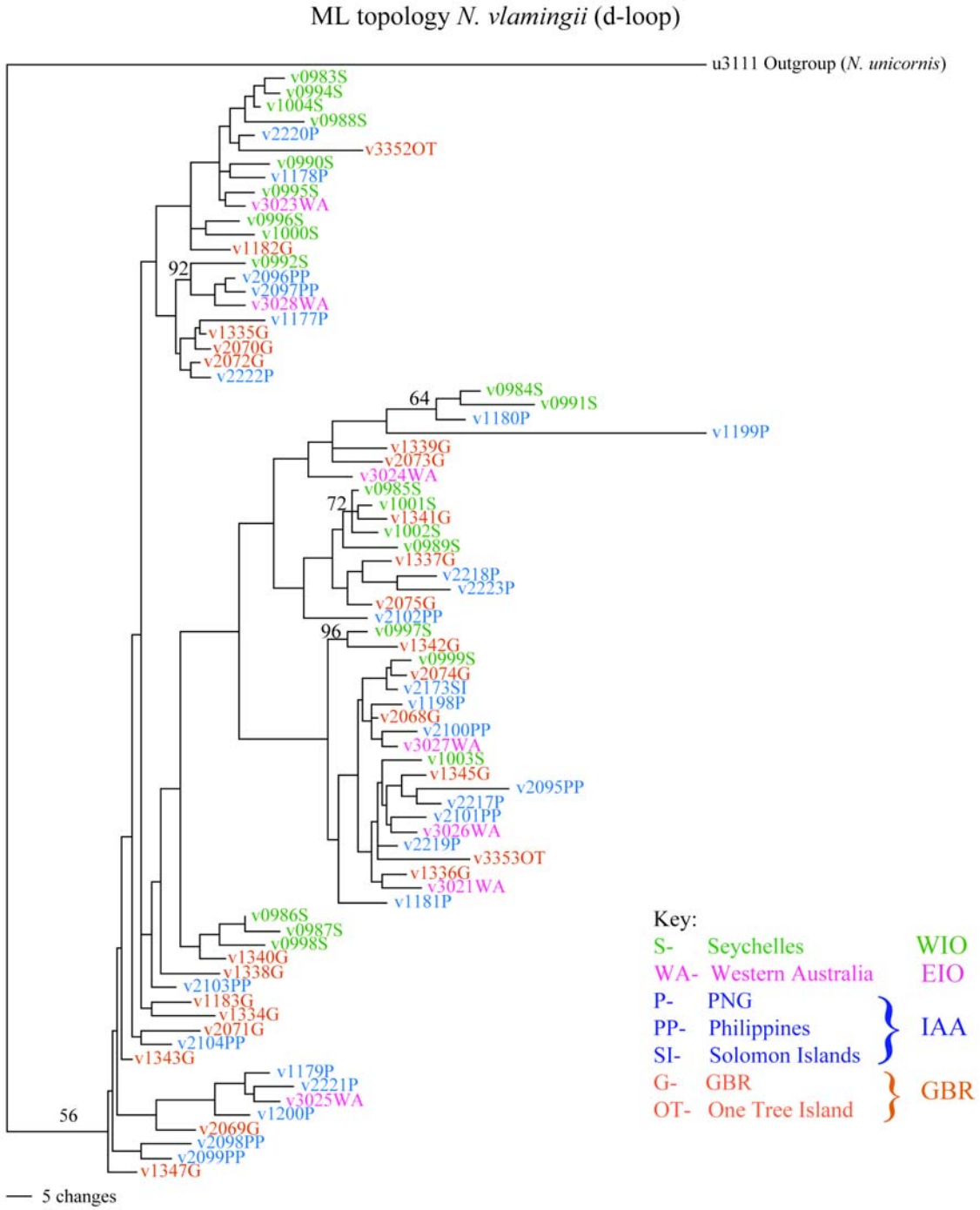

Figure 6.2: Rooted topology of best max. likelihood tree (lnL -4903.428). Individuals are colour-coded by region (see Key). Bootstrap values (> 50 obtained from 50 replicates) are indicated.

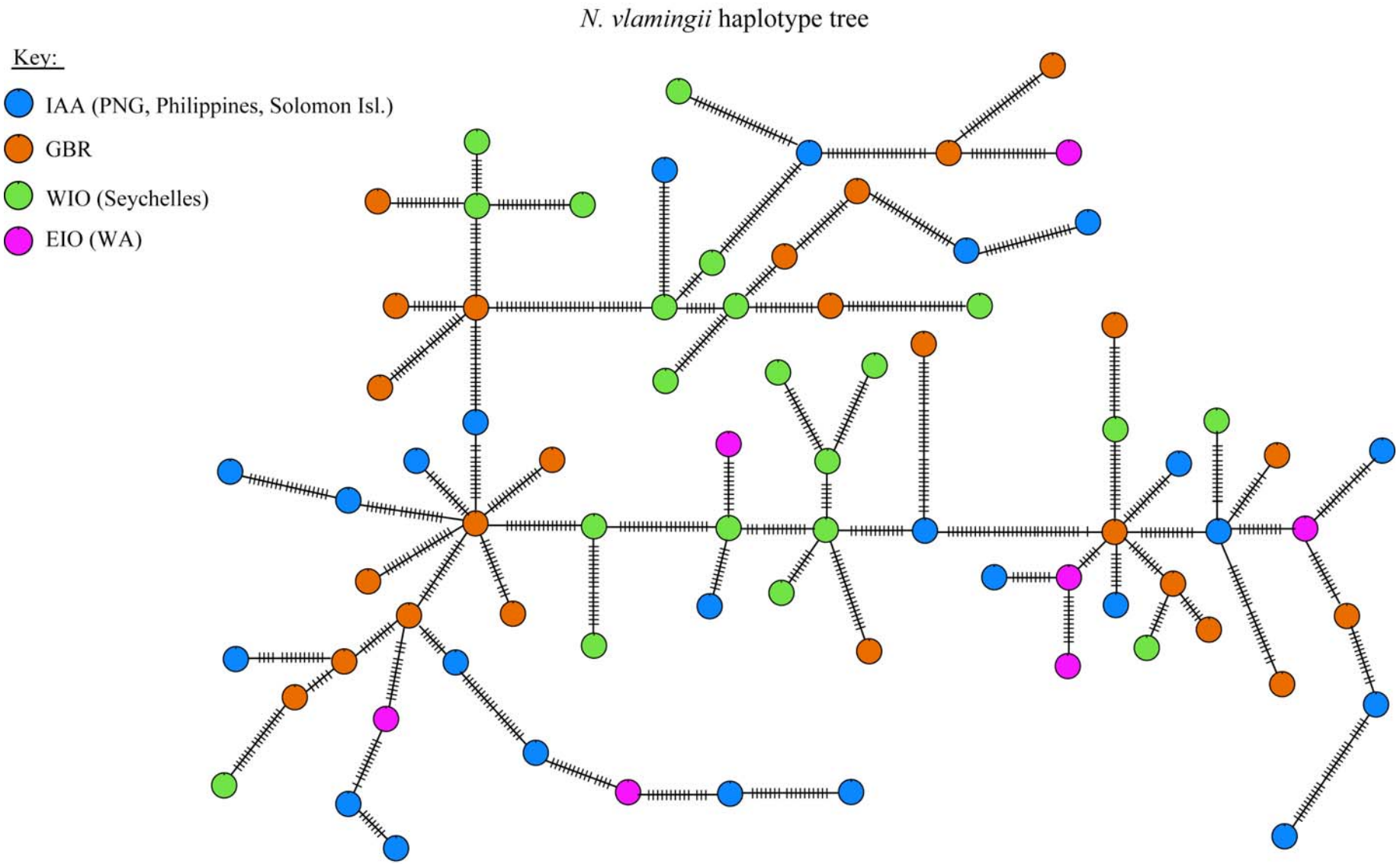

Figure 6.3: Haplotype tree of 77 samples of *N. vlamingii*, all samples are colour-coded based on 4 regions (see Key). In this study no two individuals had the same haplotype. Crossbars on the connecting lines indicate the number of base changes between haplotypes.
Note: For ease of illustration (fewer substitutions) 7 hyper-variable sites were excluded and the haplotype tree was drawn based on 300 bp.

The numbers of substitutions (mutations) separating haplotypes (Figure 6.3) were remarkably high; 75% of all haplotypes had 11 to 37 base changes separating them from neighbours, and only 25% of haplotypes had fewer base changes (≤10). The lack of distinct geographic structure or population subdivision was further supported by AMOVA analyses, which indicated no significant differences within population (relative to the total of the species), among populations within regions or among broader regions (Table 6.1). More than 98.1% of the observed sequence variation was within population relative to the total sample (Table 6.1). Accordingly, there was almost no genetic differentiation among regions or among populations within regions, as indicated by small, non-significant Φ-statistics (Table 6.1).

The only exception was a significant difference among regions when the WIO (Seychelles) samples were separated from the rest of the samples (EIO, IAA, GBR) Φ_{ct} =0.03, p≤0.001 (Table 6.1). Overall, there was a lack of distinct geographic subdivision in *N. vlamingii* populations, which was supported by an average Φ_{st} value of 0.005 (P=0.3±0.014, see also Table 6.4).

All population pairwise comparisons also produced low F_{st} values, except between the WIO (Seychelles) and the Philippines, which resulted in a marginally higher F_{st} value in comparison, but was not significant (p=0.07), suggesting a somewhat reduced gene flow (Table 6.2) between these locations.

Table 6.1: Hierarchical Analysis of Molecular Variance (AMOVA) of *N. vlamingii* populations

Individuals grouped into 5 populations: allocated into 2 ocean basins

(1) IO[a] vs. PO[b]	**d.f.**	**Sum of squares (SS)**	**Variance components**	**Percent variation (%)**	**Fixation indices Φ-statistics**	***P*-value ± S.D.**
Among regions	1	27.38	0.146	0.67	Φ_{ct} =0.0067	0.411±0.017
Among pop within regions	3	66.09	0.030	0.14	Φ_{sc} =0.0014	0.40±0.014
Within pop[¶]	72	1556.21	21.61	99.19	Φ_{st} =0.0081	0.3±0.014
Total	76	1649.69	21.79			

Individuals grouped into 5 populations: allocated into 3 broader regions

(2) IO vs. IAA[c] vs. GBR[d]	**d.f.**	**Sum of squares (SS)**	**Variance components**	**Percent variation (%)**	**Fixation indices Φ-statistics**	***P*-value ± S.D.**
Among regions	2	43.31	-0.22	-1.0	Φ_{ct} = -0.01	0.678±0.015
Among pop within regions	2	50.17	0.31	1.42	Φ_{sc} =0.0141	0.24±0.012
Within pop[¶]	72	1556.21	21.61	99.58	Φ_{st} =0.0042	0.3±0.014
Total	76	1649.67	21.70			

Individuals grouped into 5 populations: allocated into 3 broader regions

(3) WIO[e] vs. EIO[f] vs. rest	**d.f.**	**Sum of squares (SS)**	**Variance components**	**Percent variation (%)**	**Fixation indices Φ-statistics**	***P*-value ± S.D.**
Among regions	2	54.05	0.363	1.66	Φ_{ct} =0.017	0.395±0.018
Among pop within regions	2	39.42	-0.124	-0.57	Φ_{sc} =0.0058	0.541±0.019
Within pop[¶]	72	1556.21	21.61	98.91	Φ_{st} =0.011	0.3±0.014
Total	76	1649.67	21.85			

Individuals grouped into 5 populations: separating WIO from the rest (EIO, IAA, GBR)

(4) WIO vs. rest	**d.f.**	**Sum of squares (SS)**	**Variance components**	**Percent variation (%)**	**Fixation indices Φ-statistics**	***P*-value ± S.D.**
Among regions	1	36.37	0.612	2.78	**Φ_{ct} =0.03**	**0.00±0.00***
Among pop within regions	3	57.10	-0.198	-0.90	Φ_{sc} =-0.009	0.64±0.013
Within pop[¶]	72	1556.21	21.61	98.12	Φ_{st} =0.019	0.3±0.014
Total	76	1649.69	22.03			

IO[a] (Indian Ocean): includes WIO[e] (Seychelles), and EIO[f] (Western Australia).
PO[b] (Pacific Ocean): includes P.N.G., Philippines (IAA[c]: Indo-Australian archipelago) and the GBR[d] (Great Barrier Reef).
* significant at p≤0.05 [¶] within population relative to the whole of the species

Table 6.2: Pairwise F_{st} comparisons of populations for 5 regions (below diagonal) and the p-values ± SD (above diagonal).

	WIO	EIO	GBR	PNG	Philippines
WIO	-	0.2±0.01	0.14±0.01	0.31±0.01	0.07±0.01
EIO	0.023	-	0.46±0.01	0.94±0.01	0.32±0.01
GBR	0.018	-0.008	-	0.50±0.01	0.58±0.01
PNG	0.005	-0.051	-0.006	-	0.34±0.01
Philippines	0.042	0.007	-0.013	0.005	-

The Mantel test, which tested if the pairwise F_{st} matrix is correlated with the pairwise geographic distance (km) matrix produced a non-significant p-value (0.1 with r =0.58, R^2=0.344). Both matrices were also log transformed (see Slatkin 1993; Bohonak 2002), which resulted in a marginally significant p-value (0.047, r =0.73, R^2=0.533) indicating a very small effect of isolation by distance (Figure 6.4).

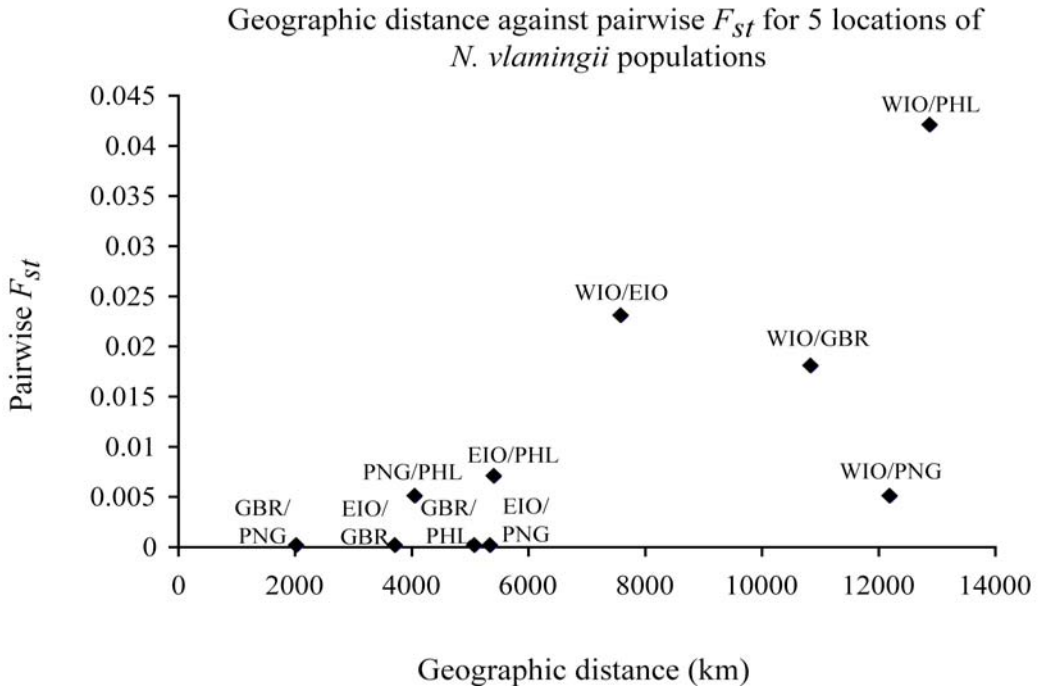

Figure 6.4: Pairwise comparison of geographic distance (km) against F_{st}. Negative F_{st} are set to 0.0001 (as per IBD V. 1.4). Sample locations are: GBR- Great Barrier Reef, PNG- Papua New Guinea (including 1 sample from Solomon Islands), PHL- Philippines, EIO-

East Indian Ocean (Western Australia samples), WIO- West Indian Ocean (Seychelles samples).

Haplotype (h = 1.0) and nucleotide diversities (π = 13.9% – 15.1%) were extremely high for all regions individually and combined (Table 6.3).

Table 6.3: *Naso vlamingii*, haplotype and nucleotide diversities per region and overall.

Geographic regions	n[a]	n_h[b]	Haplotype diversity h	% Nucleotide diversity $\pi \pm$ S.D.
GBR	25	25	1.0	13.9 ± 6.9
PNG	14	14	1.0	15.1 ± 7.7
Philippines	10	10	1.0	14.1 ± 7.6
WA	7	7	1.0	14.7 ± 8.4
Seychelles	21	21	1.0	13.9 ± 7.0
Total	77	77	1.0	14.2 ± 6.9

[a] number of samples per region; [b] number of haplotypes per region
h: Haplotype diversity (Nei 1987);
π: Nucleotide diversity as per Arlequin Ver.2.01

For comparison, gene flow (F_{st} or Φ-statistics) and diversity indices from 2 studies examining coral reef fish species across the same distribution range as *N. vlamingii* are presented in Table 6.4.

Table 6.4: Comparative haplotype (*h*) and percent nucleotide (π) diversities, and F_{st} or Φ_{st} (as a measure of gene flow) for d-loop studies.

Species	*n*	n*h*	Region	*h*	% π	F_{st} or Φ_{st}	Source
Dascyllus trimaculatus	56	51	within Moorea	0.98	2.5	0.048	(Bernardi et al. 2001)
(damselfish)	62	56	French Polynesia	0.98	2.5	0.01	
	98	87	Indo-Pacific	0.99	5.1	0.72	
Chlorurus sordidus	31	29	Amirante, Seychelles	1.0-0.975	2.3-3.2		(Bay et al. online)
(parrotfish)	44	36	WA	0.98-0.99	2.6-3.2		
	42	36	GBR	0.98-0.99	2.5-3.0		
	43	40	PNG, Rota Island	0.99-1.0	2.6-3.6		
	25	17	Hawaii	0.94	2.8		
			WPO vs. Hawaii			0.2	
	185	158	Among Oceans	0.99	4.5	0.61	
Naso vlamingii	25	25	GBR	1.00	13.9		This study
(unicornfish)	14	14	PNG	1.00	15.1		
	10	10	Philippines	1.00	14.1		
	7	7	WA	1.00	14.7		
	21	21	Seychelles	1.00	13.9		
			Among Oceans			0.008	
			Seychelles vs. rest			0.019	
	77	77	All regions	1.00	14.2	0.005	

6.4 Discussion

The aim of the population genetic study was to: (a) determine the extent of gene flow within and (b) among ocean basins, to examine if known biogeographic breaks, such as the east Indian Ocean barrier are associated with intraspecific division, and (c) to ascertain if the population structure is shallow due to high levels of gene flow.

The overall lack of geographic subdivision among populations, even across a known biogeographic barrier (the EIO divide) indicates that life history traits, including larval swimming ability, maintain gene flow among all populations studied.
However, reduced gene flow does occur between *N. vlamingii* populations of the Seychelles in the West Indian Ocean and the rest, particularly the Philippines in the north-west Pacific Ocean. A plausible explanation for the marginally observed isolation by distance (only with log transformed matrices) may be palaeogeographical. During the last glaciations (Quarternary period), sea levels were about 150 - 200m below present day levels rendering the Sunda shelf into a "hard" land barrier, thereby halting dispersal across Indonesia until sea levels rose again, inundating the Sunda shelf and permitting gene flow once again (Nelson et al. 2000). However, the lack of sampling across the Indian Ocean may have contributed to this observation. Additional samples from the EIO (e.g. Cocos Keeling Island and Christmas Island) may clarify the matter.

In contrast to *N. vlamingii*, *Chlorurus sordidus* and *Dascyllus trimaculatus*, showed evidence of very low levels of gene flow at the larger spatial scale (amongst west Indian - and Pacific Oceans) (Bernardi et al. 2001; Bay et al. online). Population subdivision was observed between the ocean basins for the parrotfish *C. sordidus* ($\Phi_{st} = 0.61$) (Bay et al.

online) and for the damselfish, *D. trimaculatus* (F_{st} = 0.72) (Bernardi et al. 2001). It is now suggested that the latter (damselfish) actually consists of 3 cryptic species (Bernardi et al. 2003). This stands in strong contrast to *N. vlamingii*, which maintains gene flow across ocean basins (all regions Φ_{st} = 0.005), indicating that cryptic speciation is not observed in the *N. vlamingii* population sampled in this study. The observed high connectivity of *N. vlamingii* across ocean basins, though unusual, is not unique. For example, several species of sea urchins, *Diadema savignyi* and two *Tripneustes* species (*T. depressus* and *T. gratilla*) (Lessios et al. 2001; Lessios et al. 2003), display gene flow across two oceanic barriers (EPO- and EIO divide) over distances far greater then have been sampled for *N. vlamingii*. However, a marginal weak population structure across different ocean basins was observed in another marine fish species, *Aulostomus chinensis* (trumpetfish) (Φ_{st} = 0.093). The observed genetic differentiation of the trumpetfish is an order of magnitude greater than the genetic differentiation found for *N. vlamingii* (Φ_{st} = 0.005).

The overall result of the *N. vlamingii* phylogeographic study contrasts entirely with the hypothesis that high levels of gene flow would result in shallow gene trees (or divergences) with only few substitutions. Instead, *N. vlamingii* populations showed almost no genetic differentiation (high connectivity) across ocean basins with deep divergences between haplotypes, identified by extremely high nucleotide and haplotype diversities. This paradox of high levels of gene flow coupled with deep divergences is probably the result of a lengthy unstable evolutionary history of the species. (approx. 17 – 20 MY, see below). Grant and Bowen (1998) based their four categories on shallow phylogenies of marine fish species with deep lineages (e.g. sardines are approx. 20 MY old). However, their category 4 (chapter 5, Table 5.2) indicating either a long, stable evolutionary history or secondary

contact of (allopatrically) divergent lineages could fit *N. vlamingii*, if it were not for the deep divergences observed among *N. vlamingii* haplotypes. Therefore, category II of Avise (2000), which describes deep gene trees for broadly sympatric lineages seems to be a more appropriate classification of *N. vlamingii* phylogeography (see Chapter 5, Figure 5.1). Populations of this species evolved in allopatry repeatedly (~ 17 – 20 MY) with many interspersed periods of connectivity (see Figure 4.3 for pulses of sea level change), resulting in haplotype lineages that are broadly sympatric and co-distributed over a wide distribution range. According to Avise (2000), category II patterns probably also suggest a large effective population size, but at present there is no data to evaluate this for *N. vlamingii*.

For this study, I would therefore argue that both a long, unstable evolutionary history in combination with secondary contact between allopatrically divergent lineages is suggested for *N. vlamingii*. *Naso vlamingii* exhibits a population structure typical of marine pelagic fishes (Grant and Bowen 1998) and sea urchins (Lessios et al. 2001; Lessios et al. 2003), but the nucleotide diversities were much higher than previously recorded for any reef fishes (see Table 5.1 in chapter 5).

Naso vlamingii arose during the early to mid Miocene (19.8 – 16.5 MYA, chapter 4), a period characterised by extreme conditions with numerous glaciations, sea level fluctuations, and sea temperature changes (Hallam 1984; Pickering 2000; Zachos et al. 2001a). Ocean productivity also increased at this time (Pickering 2000; Zachos et al. 2001a). *Naso vlamingii* populations of the Miocene would therefore have experienced repeated periods of isolation, when genetic divergences accumulated in isolated pockets, followed by secondary contact due to elevated sea levels. From ~ 11 MYA, (mid-Miocene)

until the end of the Pleistocene (10,000 years ago) sea levels repeatedly dropped below present day sea levels (Hallam 1984; Haq et al. 1987). These conditions explain the deep divergences observed between haplotypes and the lack of distinct geographic subdivision between these divergent lineages.

Certain life history traits of *N. vlamingii* further contribute to the observed population structure and high genetic diversity. *Naso vlamingii* reaches reproductive maturity at 2-3 years of age (Choat pers. comm.), they broadcast-spawn and are long-lived (up to 40 years old) (Choat and Robertson 2002) with relatively short generation times for *Naso* species. Such a long life span coupled with relatively short generation times produces overlapping generations, which adds to the increased haplotypic diversity by the co-occurrence of parental and new haplotypes arising *de novo* in offspring. Furthermore, adults are semi-pelagic and larvae with strong swimming abilities (Stobutzki 1997) have a long pelagic larval duration (up to 3 months) (Wilson and McCormick 1999). Additionally, *N. vlamingii* is a generalist (omnivore) and is therefore, not restricted by dietary preferences to specific locations across reef systems. Cumulatively, these traits enable *N. vlamingii* to disperse and persist over vast distances relatively frequently and successfully.

Chlorurus sordidus (parrotfish) and *D. trimaculatus* (damselfish) exhibit similarly high haplotype diversities (h=0.99 for both species) but less extreme nucleotide diversities (π = 4.5% parrotfish, 5.1% damselfish) compared to *N. vlamingii* (Bernardi et al 2001; Bay et al. online). It appears that different life history traits underlie the differences in the population genetic structures observed. This is illustrated by comparing average numbers of substitutions (mutations) between *C. sordidus* and *N. vlamingii* haplotypes. *Chlorurus sordidus* haplotypes differed by 5 to 8 substitutions on average (Bay et al. online), whilst

N. vlamingii haplotypes differed by 15 to 20 substitutions on average. This suggests that *N. vlamingii* haplotype divergences are nearly 2.5 to 3 times deeper than that of *C. sordidus*. *Chlorurus sordidus* is not as long-lived (reaches 8 – 9 years Choat and Robertson 2002) as *N. vlamingii*. Neither do *C. sordidus* larvae have the same PLD (~ 25 – 30 days Chen 1999) as *N. vlamingii*.

It appears that high genetic connectivity amongst *N. vlamingii* populations has counteracted speciation. If anything, this long evolutionary history should have permitted allopatric speciation or at least cryptic speciation to occur in isolation (during co-occurring low sea levels), as was suggested for *D. trimaculatus* (Bernardi et al. 2002) and may be the case with *C. sordidus* (WIO population compared to the rest, Bay et al. online) (Table 6.4).

In summary, the deep divergences, high haplotype and nucleotide diversities and genetic homogeneity across ocean basins observed for *N. vlamingii* has so far not been recorded for any other coral reef fish. It is this observed genetic connectivity that has counteracted speciation and also prevented cryptic speciation at this (large) spatial scale studied.
In the following chapter (7), I explore similar issues as those explored here, but using *Naso* species that have different characteristics to *N. vlamingii*.