CHAPTER 8

General Conclusion

There were three major aims in this study. The first was to examine the evolutionary history of the genus *Naso* using phylogenetic analyses to establish the relationships amongst the 19 species and their pattern of descent. The second aim was to put this phylogenetic analysis into a temporal framework to estimate divergence dates (in MY) to establish the timing of important events in the evolution of this genus. This provided an opportunity to relate evolutionary events to significant tectonic and palaeoenvironmental changes in marine habitats over the relevant time frame. The third aim of the thesis was phylogeographic in nature and involved a detailed investigation of the genetic structure of selected individual taxa. The primary purpose of this aim was to establish the extent to which population structures reflected the evolutionary histories of the species and to infer modes of speciation in the genus.

The hierarchical structure of this study provided an appropriate framework for linking broad patterns of evolution within the genus to the histories of individual taxa. The key process in this linkage was the detailed analysis of the timing of divergences through knowledge of the fossil record and the estimation of ages (MY) and local rates. The capacity to relate lineage divergences to periods of important biogeographic, tectonic and palaeoenvironmental events provides additional support for the conclusions of this study.

The *Naso* phylogeny inferred from this molecular study overturned previously suggested hypotheses pertaining to the evolution of foraging modes. Until recently, it was believed that benthic foraging is the ancestral state and pelagic foraging is derived in *Naso* however, this hypothesis was rejected. Rather, the exact opposite scenario is supported, with pelagic foraging as the ancestral feeding mode and benthic foraging as derived. In addition, this study establishes that benthic foraging arose repeatedly and independently in the genus *Naso*. The scombriform morphology of pelagic foragers is retained in all clades, whilst other distinct morphological features (horns, protuberances) arose repeatedly and independently.

Here, I argue that the molecular phylogeny is more likely to reflect true evolutionary relationships between *Naso* species than the morphological phylogeny for two reasons. First, a much larger number of character states was available to infer the molecular phylogeny (1332 bp of sequence data from 2 linked and one unlinked gene region) compared to only 12 morphological character states (soft musculature, mainly from the feeding apparatus). Second, several morphological characters are functional and subject to selection, which may result in convergent evolution, *ie*. such traits may produce tree topologies that do not reflect "identity by descent", but rather "identity by state". Although, molecular data may also undergo convergence (due to saturation), it was shown in this molecular data set that there was no saturation evident.

Establishing the temporal pattern of diversification in the genus *Naso* allowed the role of historical biogeography in lineage diversification to be examined. The accumulation of lineages over time dates back to the Eocene, with proliferation of lineages during the Miocene. Relatively rapid radiations of *Naso* during the Miocene indicates that the majority of species in this genus are more than 10MY old, except for two Plio-Pleistocene-derived species that are less than 5MY old. Major climatic events, such as sea level- and temperature fluctuations, ocean circulation patterns and productivity, all of which influenced reef systems (algal or coral dominated), were probably important factors promoting vicariant and ecological diversification due to changes in habitat and available food sources. For example, the first benthic foraging species arose during a period of major sea level fluctuations, associated with major glaciations. During such times, sea levels and temperatures dropped, cooler ocean currents were prevalent and reef systems were dominated by algal -, rather than coral reefs. As a result, new habitats became available and some species of the genus *Naso* probably underwent ecological diversification in response to these changed environmental conditions.

Having determined the evolutionary history and established species relationships in the genus, more in-depth population genetic and comparative phylogeographic studies were required to understand modes of speciation in the genus. The examination of sister-species pairs allowed me to evaluate the importance of vicariance biogeography in this genus and the examination of a widely distributed species (*N. vlamingii*) indicated that extensive gene flow may counteract speciation. The last section of this study therefore indicated that population genetic structure is primarily influenced by historical events, in addition certain life history traits of particular species are important in maintaining this structure.
Naso species are characterised by extremely high dispersive abilities, relatively short generation times (compared to their age) and overlapping generations, which result in the co-occurrence of parental and *de novo* mutant offspring haplotypes. These traits together with the longevity of these species provide the opportunity for extensive dispersal and

reduced level of genetic differentiation, even across established biogeographic barriers, such as the East Indian barrier.

In the case of the sister-species pairs (*N. lituratus – N. elegans* and *N. tuberosus – tonganus*) studied here, it became clear that allopatric speciation has occurred in at least one of the species pairs (*N. lituratus – N. elegans*) despite the high dispersal abilities of these species. This is in contrast with the widely distributed species, *N. vlamingii*, which has not speciated across the same geographic range sampled, *ie.* there was no evidence of cryptic speciation across the sampling range of *N. vlamingii* in this study. It is important to note that despite the high connectivity among populations of *N. vlamingii*, this species follows the same pattern as *N. elegans* in the WIO. In both cases, the Seychelles populations are more divergent from the remaining Indo-Pacific populations.

All 5 species examined here displayed high levels of gene flow among populations, even across known biogeographic barriers as was the case for the limited samples of *N. vlamingii* (especially in the Indian Ocean). High nucleotide diversity indices indicate past deep divergences in all species, suggesting long evolutionary histories for all species. Furthermore, the lack of geographic structure (no distinct subdivision of populations) reflects the dispersal capabilities of *Naso* species especially for the more widely distributed species (*N. vlamingii*, *N. lituratus* and *N. elegans*). It is high levels of gene flow that repeatedly allow for secondary contact to be made between populations that have diverged repeatedly in isolation at times of low sea level.

The study of the sister-species pairs demonstrated that speciation can occur, despite the potential for long-distance gene flow across ocean basins. In these species, the more restricted habitat and dietary requirements of adults may prevent dispersive larvae from successfully populating distant locations to adulthood. This dispersive ability was illustrated by the *N. lituratus – N. elegans* species pair which now co-occur at Cocos Keeling Island in the East Indian Ocean. Despite their co-occurrence, the species of this pair still maintain their distinctiveness. They may have different dietary requirements, and are readily distinguishable morphologically and genetically.

Future studies may clarify additional questions, not addressed here. For example: how do nucleotide diversity indices of the most recently derived species of *Naso* (e.g. *N. hexacanthus*) compare to those species studied here? I would expect to see reduced nucleotide diversity indices relative to those obtained for species studied here, because of the shorter/abbreviated evolutionary history of younger species.

How do the ages of lineages of the genus *Naso* compare to those of another genus of the same family, for example *Acanthurus*? The species of the genus *Acanthurus* also display different foraging modes, but may have a different evolutionary history to *Naso*.

In this study I demonstrated that by combining phylogenetic, historic, temporal and phylogeographic approaches together with knowledge of the biology (life history traits), ecology and history of species, it was possible to ascertain what processes may have been instrumental in the diversification of this genus. A robust molecular data set has underpinned these findings and has resulted in an important re-evaluation of the evolutionary history of a relatively old group of reef fish.

The major conclusions are:

Pelagic foraging is the plesiomorphic state in the monophyletic genus *Naso*. The evolutionary history of the genus *Naso* differs from that of other tropical reef fish groups with most of the diversification occurring throughout the Miocene and not the Eocene or Plio-Pleistocene as seen in other groups. Most *Naso* species are relatively ancient. High levels of gene flow counteract local differentiation in the species studied.