Molecular phylogeny, temporal patterns of lineage diversification and phylogeography of the surgeonfish genus *Naso* (Acanthuridae)

Thesis submitted by

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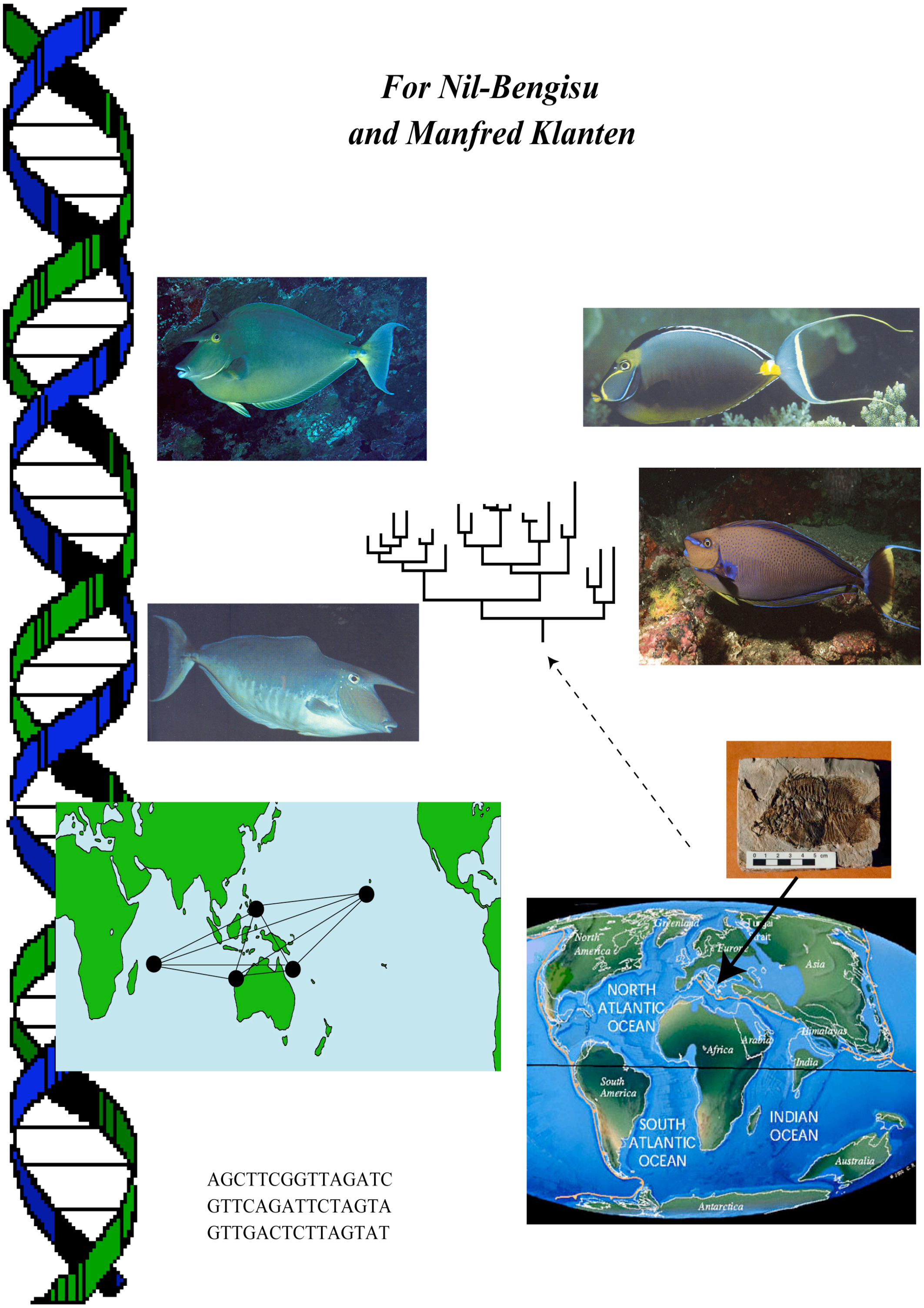
in November 2003

for the degree of Doctor of Philosophy
in Marine Biology
within the School of Marine Biology and Aquaculture
James Cook University

For Nil-Bengisu
and Manfred Klanten



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Publication list

Publications directly resulting from this thesis:

Chapter 2

Klanten, S.O., L. van Herwerden & J.H.Choat. (2003). Acquiring reef fish DNA sequences from formalin-fixed Museum specimens. *Bull. Mar. Sci*. (BMS4872)

Chapter 3 & 4

Klanten, S.O.,L. vanHerwerden, J.H.Choat & D. Blair (2004). Patterns of lineage diversification in the genus *Naso*. *Mol. Phylogent. Evol.* (doi:10.1016/j.ympev.2003.11.008)

Chapter 6

Klanten, S.O., L. vanHerwerden & J.H.Choat (MS). Lack of genetic structure in the widely distributed unicornfish *N. vlamingii. Mar. Biol.*

Chapter 7

Klanten, S.O., J.H.Choat, L. van Herwerden & R. Robertson (in prep.). Comparative phylogeography and modes of speciation for two sister-species pairs.

Publications that arose during the candidature of the thesis:

M.S. Prachett., N. Gust, G. Goby & **S.O. Klanten** (2001). Consumption of coral propagules represents a significant trophic link between corals and reef fish. *Coral Reefs* 20: 13-17.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my supervisors, Lynne van Herwerden, Howard Choat and David Blair. My thanks go in particular to Lynne, who introduced me to genetics and taught me the molecular techniques I needed. She was always available for questions and discussions, sometimes during her free time at her family's cost. Therefore, I'd like to extend my thanks to Carl, Caylene, Leon and Jaque for enduring the times I spent at their house. To Howard for providing and collecting most of the specimens from the Indo-Pacific, even though, he nearly lost a finger in the process on those nasty *Naso* knives. David who provided me with lab space at the start of my PhD.

I would like to extend my thanks to the following people at Museums around the world for supplying muscle tissue of specimens: Mark McGrouther from the Australian Museum (Sydney); Susan Jewett, Smithsonian National Museum of Natural History (Washington); Arnold Suzomoto and Jack Randall of the Bernice P. Bishop Museum (Hawaii); Rick Winterbottom and Marty, the Royal Ontario Museum (Canada); P. & E. Heemstra of the J.L.B. Smith Institute of Ichthyology, Durban, South Africa; the Western Australian Museum (Perth), J. Johnson the Commonwealth Scientific & Industrial Research Organisation (CSIRO, Brisbane); Aileen Maypa and A.Alcala from the Silliman University Angelo King Center for Research & Environmental Management, Philippines and Li-Shu Chen of the National Museum of Science & Technology, Taiwan.

Fresh tissue collections by: Will Robbins, John Ackerman, Geoff P. Jones, Maya Srinivasan, Mark McCormick, Jake Kritzer, Peter Wruck, Chris Fulton and Nicolai Konow (JCU). D.Ross Robertson (Smithsonian Panama), John Earle (Hawaii), Mark Meekan (AIMS, W.A), Kendall D. Clements (fresh *N. maculatus*, Hawaii), Edwin Grandcourt (Seychelles), Rick Hamilton (Solomon Islands), Alan Lo-Yat (samples from Tahiti), Aileen Maypa (Philippines).

Volunteers in the lab helping with extractions: Dean Richards, Jude & Tim Hatley, but in particular Lara Santana who helped with a large numbers of PCRs and sequencing.
For discussions with colleagues (apart from Lynne), Madelaine van Oppen, Lynn Atkinson, Phil Munday, Michelle Waycott, and friends in the molecular evolutionary field, Line Bay, Jacki Wolstenholme, Faye Christidis, Julia Gardiner and Chris Dudgeon.

The School of Marine Biology for infrastructure and support.
The finance office, Ros Burgess, Jan Nugent, Karen Woods; the teaching resource centre Jodie Wilson, Di McNamara, Savita Francis; the postgraduate officer, Jenny MacGregor.
The technical support staff, Gordon Bailey, Vince Pullela for PC, but especially Ainsley Calladine who introduced me to the world of Mac's and always helped along the way when I got stuck.

I am especially thankful for the support from my family, who believed in me, let me go this far away and financially supported me in the past years. I would not have been able to finish otherwise.
Friends, Bridget Green, Jackie Wolstenholme and Lynne who, especially during the last year, forever listened, your support cannot be measured.
My flatmates, John Ackerma, Sam Adams, Dan Barron, Heather Patterson, Will Robbins and Ailsa Kerswell who, stuck around over the past 4 years.
Most importantly, my two companions always happy and ready to go: Biber and Maccorah.

This work was supported by the The Queensland Governement - Smithsonian Institute Collaborative Research Program on Reef Fishes granted to J. Howard Choat and D. Ross Robertson; Australian Institute of Marine Science (Mark Meekan); The Seychelles Fishing Authority (Edwin Grandcourt, J.Robinson); MRG granted to H. Choat and L. van Herwerden; finally JCU internal funding, Doctoral merit research scheme (DMRS), Doctoral research scheme (DRS) and a write-up scholarship from JCU awarded to myself. JCU Ethics Approval No. A503.

Abstract

A species-level phylogeny was constructed for all described (19) *Naso* species (Family: Acanthuridae) using three markers in combination, one nuclear (ETS2) and two mitochondrial (16S rRNA and cytochrome *b*). A number of taxa representing species from all acanthurid genera were included to generate a genus-level (total of 38 species) phylogeny of the order Acanthuroidei (surgeonfishes). Maximum likelihood, maximum parsimony and Bayesian inference analyses produced similar tree topologies, which confirmed the previously proposed genus-level relationships and resolved for the first time inter-specific relationships of all species of the monophyletic genus *Naso*. Species of the genus *Naso* segregated into four major sub-clades, with members of the subgenus *Axinurus* appearing basal. This molecular phylogeny was then used to examine hypotheses about the evolutionary relationships among species of *Naso*. This study demonstrates that distinct foraging modes and specialised body shapes arose independently at different times in the evolutionary history of the genus. Members of the subgenus *Axinurus*, characterised by a scombriform morphology, caudal fin structure and pelagic foraging mode, were consistently placed basal to the remaining *Naso* species, suggesting that pelagic foraging is plesiomorphic and benthic foraging derived in this genus.

The genus-level phylogeny (nuclear marker, ETS2) was used to obtain a range of age estimates for the most recent common ancestor of the genus *Naso*. These age estimates (range of 52MY to 43.3MY) were then used to estimate divergence times (by nonparametric rate smoothing method) of the nodes, giving rise to extant *Naso* species using the combined sequence data (from all loci). The reconstruction of the pattern of divergence of extant species indicates two sequences of events. The basal species characterised by pelagic foraging modes arose during the Eocene and Oligocene. Most of the remaining *Naso* species, including those characterised by benthic foraging, arose over a period of 20MY during the Miocene. Diversification during this period was associated with major plate tectonic and glaciation events, resulting in changes in sea level, ocean temperature and productivity regimes.

To examine further speciation events and the underlying processes, several comparative phylogeographic studies were undertaken. For this purpose, a *Naso*-specific fast

evolving mitochondrial marker was designed from the control region (d-loop). Firstly, a widely distributed species *N. vlamingii*, was selected to examine genetic connectivity and diversity throughout its distribution in the Indo-Pacific Ocean. Although very weak (overall $\square_{st}$ 0.005) genetic differentiation was obtained for this species over its sampled range, suggesting gene flow, there was some evidence of isolation by distance at the largest spatial scale (between Seychelles and the Philippines). This however, may be due to lack of samples from the Indian Ocean. There was no subdivision into distinct geographic regions, hence no evidence of cryptic speciation across the range sampled in this study, despite the presence of the central Indian Ocean barrier in its distribution range. Diversity indices (haplotype, h and nucleotide, $\square$) were extremely high ($h = 1.0$ and $\square = 15.1\%$), indicating that this species has had a long, but unstable evolutionary history, which has repeatedly allowed populations to diverge in isolation and to make secondary contact subsequently. A haplotype tree identified deep divergences for this species, suggesting that isolated populations diverged at times of low sea level. The lack of geographic partitioning in this species suggests that secondary contact was made between previously isolated and divergent populations at times of elevated sea level. The high dispersive capacity of the species is what allows secondary contact to occur between temporarily isolated populations.

Additionally, two sister species pairs were selected for comparative phylogeographic studies. One pair (*N. lituratus– N. elegans*) spanned the same distribution range as *N. vlamingii*, but the species pair was partitioned by ocean basins across this range (Indian-/Pacific Ocean). The second pair (*N. tuberosus – N. tonganus*) has an even more restricted distribution range (west Indian-/west Pacific Ocean) and was also partitioned by ocean basins. The two sister species diverged allopatrically, and species of both sister pairs (*N. lituratus– N. elegans* and *N. tuberosus – N. tonganus*) segregated into distinct clades. There was no distinct geographic subdivision throughout the sampling range for any of these species. Instead, low levels of genetic differentiation were recorded among populations of each species (overall $\square_{st}$ values ranged from 0.005 to -0.001) suggesting high levels of gene flow. Despite the presence of gene flow among populations there was some indication of reduced gene flow across the Indian Ocean (between Amirante of Seychelles and Cocos Keeling Island) for *N. elegans*.

There was no indication of gene flow between species in either of the sister pairs (*N. lituratus* – *N. elegans* F_{st}=0.75 and *N. tuberosus* – *N. tonganus* F_{st}=0.71), despite the fact that each pair was considered a single species as recently as 2001 and 2002 respectively. This indicates reproductive isolation, despite the potential for extensive dispersal in these species. The diversity indices were high for all of the species (*h* = 0.90 – 1.00, □ = 9.0 – 11.6%; *h* = 1.00, □ = 4.2 – 6.4% respectively), indicating deep divergences between haplotypes, as was the case for *N. vlamingii.* Several factors probably contribute to the population genetic structure of all five species: certain life history traits such as an extensive pelagic larval duration (up to 3 months) in combination with the ability of larvae to swim actively for extended periods, their long evolutionary history, the longevity (reach 30 – 40 years) and relatively short time to reach reproductive maturity coupled with overlapping generations (offspring) reproducing. The dispersive capacities of *N. vlamingii* are clearly the greatest of the five species studied, and may be attributed to the semi-pelagic adult lifestyle combined with the pelagic larval duration and a generalist dietary habit of this species.

This study has demonstrated that by applying a hierarchical approach (phylogenetic, temporal, and phylogeographic) in combination with biological, ecological and historical perspectives, it has been possible to elucidate the processes important in the diversification of the genus *Naso*.

Table of contents

List of Tables

List of Figures