Systematics, Phylogeny, Phylogeography and Reproduction of *Neotrigonia* (Bivalvia: Palaeoheterodonta)

Ana Glavinic, BSc (Hon.)

Thesis submitted in fulfilment of the requirements for the
Degree of Doctor of Philosophy

School of Biology, Faculty of Science and Engineering
Flinders University
Adelaide, Australia

June 2010

Table of contents

List of tables

List of figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Appendix 4

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

~ Ana Glavinic

June, 2010

Acknowledgments

Foremost I would like to thank my supervisors Prof. Greg Rouse, Dr Kirsten Benkendorff, and Prof. Peter Fairweather, for exceptional academic mentoring, guidance and enthusiasm. Thank you Kirsten, for quick turnaround time with drafts, scientific freedom, laboratory space and tremendous amount of support. I am extremely grateful to Greg and Nerida, to sum it up simply, without your support this thesis would have never taken shape. Peter thank you for jumping in at the deep end, your support and encouragement have helped me cross the finish line.

Over the years many people have assisted me either in the field or the laboratory. I would like to formally express my gratitude to Lauren Helgen, Patrick Laffy, Lyndlee Easton, Elena Kupriyanova, Chantell Westly, Joy Frances-Hayes, Peter Costello, and Joseph Bain. I am very thankful to Nerida Wilson and Elena Kupriyanova who unselfishly gave constructive advice for Chapter 3 and 4, and Patrick and Chantell for their feedback for Chapter 5. Thank you Darko Bogdanovic for teaching me tricks of Adobe Illustrator. Thanks to the helpful staff at South Australian Museum, Australian Centre for Evolutionary Biology and Biodiversity and Adelaide Microscopy Centre. Thanks to the Ngerin crew.

I had a lot of emotional support from my friends and without their friendship the PhD duration would have been unbearable; thanks to Aida and Neda for being the best friends one could have. Also thank you to all my friends and family overseas, who repeatedly expressed interest in what I was doing and through that showed support and ensured my sense of belonging. I am indebted to: Melanie Sulda, Melissa Gregory, Patrick Laffy, Chevaun Smith, Alexis Young and all others from 3rd floor for listening to my lunch time moans. Collectively to room 039, thank you for putting up with me through my grumpiest states.

My patient and supportive family deserves a big thank you, especially to my parents Filip and Ljiljana and my grandparents Katica and Damjan who with their combined effort and love have made me in to the person I am today. Thank you to my lovely Jo, encouraging Ivana and Brendan and my little rays of sunshine Mia and Oliver. Jo-thank you for everything that makes our life together the way it is. Ivana-most of all thank you for never backing off and always cheering me on to achieve what I never conceived possible.

This research would not have been feasible without generous founding from: Australian Biological Resources Study **(**ABRS**),** Mark Mitchell Foundation, Nature SA Foundation, Linnean Society, Waterhouse Club, PADI and Flinders University.

Preface

This is a resubmission of a thesis, which was examined by two anonymous examiners and subsequently read by two advisors chosen by the Faculty. The Faculty permitted limited time for this revision. I have now addressed all of the comments made by examiners, and also acknowledge the helpful advice and suggestions made by advisors. This version of a thesis is a significant improvement upon the original.

This thesis comprises a series of manuscripts dealing with separate parts of the research project. This format is not typical of a traditional thesis, so there are some differences that need to be recognised beforehand. Firstly the tables and figures are not embedded within the text but are found at the end of each manuscript. Secondly, separate reference lists are provided at the end of each chapter. Finally some sections of the thesis can be repetitive at times because each chapter is meant to stand alone and thus can be read independently of the rest of the thesis. I apologise in advance for the repetition.

I wrote all of the chapters, however, other authors have been included for the purpose of publication and to acknowledge here their contribution to each of the separate pieces of work. The following table indicates the contribution of these co-authors to the piece of work:

Contribution of authors to manuscripts in the thesis:

Chapter	Concept	Method	Data analysis	Manuscript preparation
I	AG	AG	AG	AG/KB/PF
II	AG	AG/GR	AG	AG/GR/KB
III	AG	AG/GR	AG/GR	AG/NW/KB
IV	AG	AG/NW/GR	AG/NW/GR	AG/NW/GR
V	AG	AG/GR/ KB	AG/GR/ KB	AG/GR/ KB
VI	AG	AG	AG	AG/KB/PF

Where AG=Ana Glavinic, KB= Kirsten Benkendorff*, GR= Greg Rouse*, NW= Nerida Wilson[@] and PF= Peter Fairweather*. * = co-supervisors, @ = a collaborator of Prof. Rouse

Glossary

This glossary contains less common biological terms, but also common terms, which are used throughout the thesis to interpret a particular function or a morphological character.

acinus – singular Acini- a small saclike dilatation in ovaries containing eggs

acrosome - A caplike structure at the anterior end of a spermatozoon that produces enzymes aiding in egg penetration.

ciliary tracts- the respiratory tract that sweep in unison and help to sweep away fluids and particles.

ctenidia - A gill like structure, a respiratory apparatus of a mollusc.

denticulated- Finely toothed or notched, its use in the thesis is to describe the shell margin.

dissoconch – juvenile bivalve shell

eucheton- a small area on the shell near the umbo in the shape of the shield or a key hole like eucheton.

median carinae - Median carinae is a prominent feature on *Trigonia* and *Eotrigonia* specimens and it separates flank with radial ribs away from an area with parallel costae. In case of *Neotrigonia* this line is not as obvious, but it is present separating area from the flank.

metamorphic line- a shell feature delimitating prodissoconch from dissoconch

micropyle - a very small opening in the vitelline layer of an oocyte

oogonia - A cell that arises from a primordial germ cell (protogonia) and differentiates into an oocyte in the ovary.

palps- an elongated, often segmented appendage usually found near the mouth in invertebrate organisms such as molluscs.

prodissoconch – prejuvenile bivalve shell.

protogonia- a primordial germ cell of an oocyte.

spinous- pertaining to or like a spine, in the thesis it is used to describe shell rib ornamentation.

synonymisation- the act of identifying two known species to be identical and therefore synonyms.

vitelogenesis- process of yolk formation in an developing egg.

Abstract

This research investigates the evolution of *Neotrigonia* species (Bivalvia: Palaeoheterodonta), the remaining extant genus of the Trigonioida, a group of bivalves endemic to Australian waters. The intent of this research was to review the current systematics, investigate phylogeny and phylogeography of the genus, and advance scientific knowledge in regard to the presence of doubly uniparental inheritance in *Neotrigonia*, as well as to address some aspects of reproductive strategy and outline the process of oogenesis. The research has resulted in a thesis in manuscript format, where Chapter 1 is a general introduction to the thesis as a whole, Chapters 2-5 inclusive are research manuscripts, and Chapter 6 is a general discussion of the completed research.

In chapter 2, the type material of all of the seven extant, nominal species of *Neotrigonia* Cossman 1912 are reviewed and illustrated, based on available museum specimens and fresh collections. The type localities and currently-known distributions for each extant species are included. A cladistic analysis was performed using morphological characters of *Neotrigonia* species living and fossil, using *Eotrigonia subundulata* and *Trigonia miriana* as an outgroup. Results from parsimony analysis show that all *Neotrigonia* form a monophyletic clade, in which living and fossil *Neotrigonia* form reciprocally monophyletic sub-groups. The species status of *Neotrigonia bednalli*, Verco 1907, is revised based on examination of all available types, museum specimens and a relatively large number of newly-collected specimens from southern Australian waters. This assessment suggests that *N. bednalli* is a junior synonym of *N. margaritacea*. Species status is accepted for *N. gemma*, *N. lamarckii*, *N. uniophora*, *N. strangei* and *N. kaiyomaruae*. However, reclassification of *N. strangei* specimens from Western Australia to *N. margaritacea* would revise the previously disjunct distribution of this species to a narrower range in NSW. This chapter demonstrates the limitations in relying on shell morphology only for species classification in the *Neotrigonia*.

The contemporary knowledge of ocean currents, temperatures, and geological and climatic history across southern Australian waters represents a useful framework for phylogeographical analyses. There are already a number of studies that show coincident distribution patterns within some marine invertebrate groups across the Maugean, Flindersian and Peronian marine provinces. In Chapter 3, I examine the genetic structure of *Neotrigonia margaritacea* and *Neotrigonia lamarckii*.

Phylogenetic analyses based on COI and ITS gene sequence data reveals a split between southern Australian *Neotrigonia margaritacea* and eastern Queensland *Neotrigonia lamarckii*. The molecular analyses confirmed my synonymisation of *N. bednalli* to *N. margaritacea*. Population genetic analyses of the *Neotrigonia margaritacea* COI gene, in four different populations located hundreds of kilometres apart, revealed insight into genealogical pathways amongst haplotypes. These networks showed that there was no shared haplotypes among populations and most populations were significantly far from panmixia. The highest haplotype diversity was recorded from the Port Lincoln (South Australia) population. Haplotype variations across the range are discussed in terms of estimated population sizes and geographical barriers.

Several species of bivalves have been reported to have two mitochondrial DNA types, maternal and paternal. This system of mtDNA inheritance is known as doubly uniparental inheritance (DUI). In Chapter 4, the presence of the DUI phenomenon in *Neotrigonia margaritacea* is investigated within a phylogenetic framework for Paleoheterodonta, using COI and 16S rDNA molecular data. Results indicate the presence of DUI in *Neotrigonia margaritacea* and provide evidence for a masculinization event within this taxon. This phenomenon has so far been identified in six superfamilies of bivalves, so the new record of DUI in *N. margaritacea* was incorporated into a phylogenetic tree addressing the question of a single or multiple origins of DUI in Bivalvia. Parsimony transformations indicate that DUI is likely to be the ancestral state for all Bivalvia.

In Chapter 5, the ultrastructural stages of female gametogenesis are described for *Neotrigonia margaritacea*. The morphology of oocytes and gonad tissue are described for the first time using electron microscopy and histology techniques. Throughout the summer period, the ovary contains oocytes in various developmental stages. Oocytes develop from oogonia derived from protogonia and then undergo three distinct stages of oogenesis: previtellogenesis; vitellogenesis; and postvitellogenesis (or presence of mature oocytes). Based on gonad tissue and oocyte morphology, and as well as laboratory observations, it is inferred that *Neotrigonia margaritacea* is sequentially tachitictic, thus a trickle (continuous) spawner over an extended summer season.

In conclusion the museum collections of *Neotrigonia* and current systematics have provided valuable information on classification and distribution of this relic

bivalve genus. Morphological analysis has enabled preliminary synonymisation of species to establish species distributions. The results from molecular data confirmed aspects of phylogeny and revealed phylogeographic structure of *Neotrigonia margaritacea* in Southern Australian waters. The new molecular information regarding the presence of DUI and novel insight into reproductive strategies further our understanding of the evolutionary affinities of *Neotrigonia*. Based on the integration of these multidisciplinary results conservation assessment is suggested for *Neotrigonia margaritacea*.