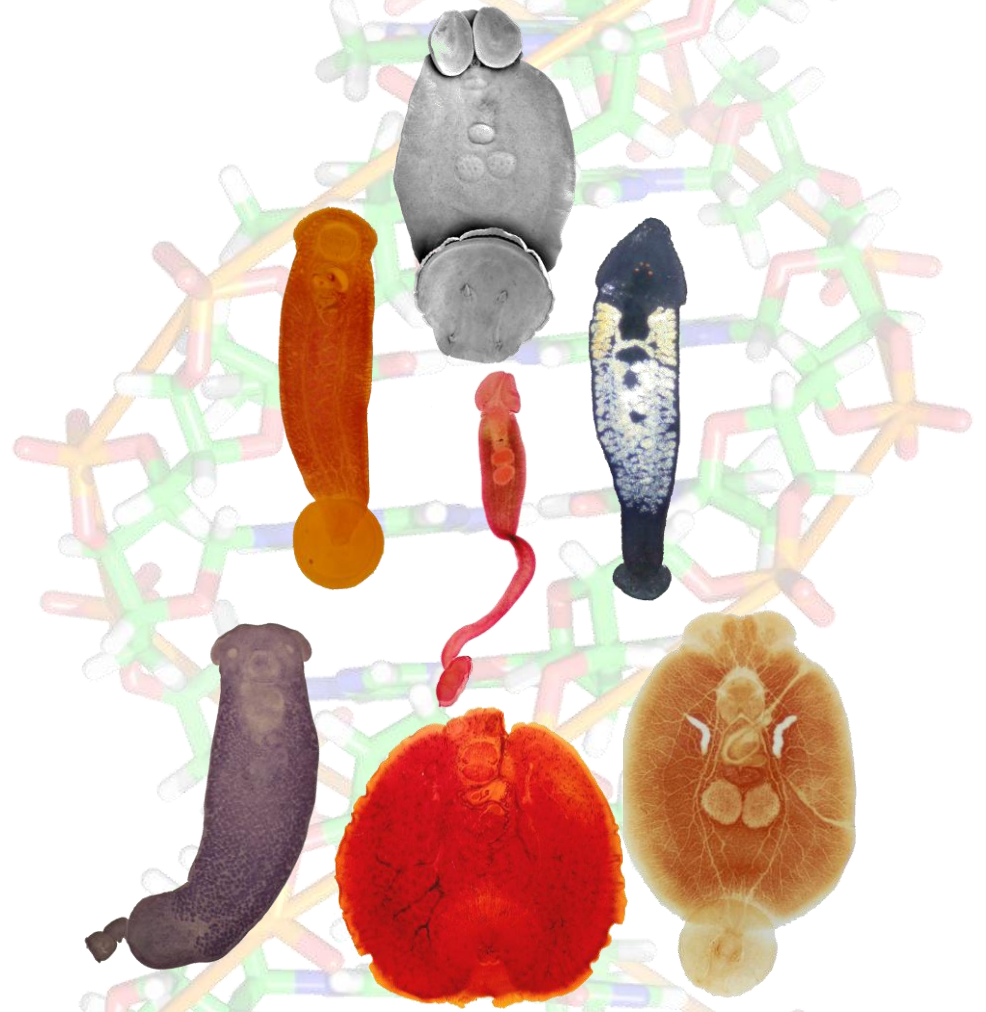


**FAMILY TIES: MOLECULAR  
PHYLOGENETICS, EVOLUTION  
AND RADIATION OF  
FLATWORM PARASITES  
(MONOGENEA: CAPSALIDAE)**



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Presented for the degree of Doctor of Philosophy

School of Earth and Environmental Sciences

The University of Adelaide, South Australia

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Elizabeth Perkins

February 24<sup>th</sup>, 2010

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Title page images: Top: *Benedenia seriolae* (I.D. Whittington & B. Cribb). Middle (left to right): *Nitzschia sturionis*; *Macrophyllida* sp.; *Dioncus remorae* (all images: I.D. Whittington). Bottom (left to right): *Encotyllabe* sp. (I.D. Whittington); *Capsala martinieri* (L.A. Chisholm); *Benedeniella posterocolpa* (I.D. Whittington).

## DEDICATION

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An unfortunate part of Marine Parasitology is the need to take the life of many spectacular, fascinating and beautiful fish. This PhD would not have been possible without them.

This thesis is dedicated to all the fish that were killed in the making of this PhD.

## PUBLICATIONS ARISING FROM PhD CANDIDATURE

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Glennon V, **Perkins EM**, Chisholm LA, Whittington ID (2008) Comparative phylogeography reveals host generalists, specialists and cryptic diversity: Hexabothriid, microbothriid and monocotylid monogeneans from rhinobatid rays in southern Australia. *International Journal for Parasitology* **38**, 1599–1612. (doi:10.1016/j.ijpara.2008.05.017)

**Perkins EM**, Donnellan SC, Bertozzi T, Chisholm LA, Whittington ID (2009) Looks can deceive: Molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification. *Molecular Phylogenetics and Evolution* **52**, 705–714. (doi:10.1016/j.ympev.2009.05.008)

**Perkins EM**, Donnellan SC, Bertozzi T, Whittington ID (2010) Closing the mitochondrial circle on paraphyly of the Monogenea (Platyhelminthes) infers evolution of diet in parasitic flatworms. *International Journal for Parasitology* **In Press**.

## TABLE OF CONTENTS

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ABSTRACT.....	vii
ACKNOWLEDGEMENTS.....	ix
CHAPTER I	
General Introduction.....	2
CHAPTER II	
Looks can deceive: Molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification.....	17
CHAPTER III	
Closing the mitochondrial circle on paraphyly of the Monogenea (Platyhelminthes) infers evolution of diet in parasitic flatworms .....	44
CHAPTER IV	
When the worms turned: molecular dating for diversification of the parasitic Platyhelminthes, Monogenea and Capsalidae (Monopisthocotylea)...	67
CHAPTER V	
Collecting souvenirs: Family of ectoparasitic Platyhelminthes (Monogenea: Monopisthocotylea) displays extensive host switching across marine fishes .....	80
CHAPTER VI	
General Discussion.....	107
REFERENCES.....	116
APPENDICES	
APPENDIX I: Reprint of published paper.....	140
APPENDIX II: Copyright transfer statement for Chapter II.....	141
APPENDIX III: Parasite taxa used in analyses in Chapter II.....	144
APPENDIX IV: The maximum likelihood tree derived from analyses of the combined nuclear sequence data for the Capsalidae in Chapter II.....	155
APPENDIX V: Parasite taxa used in analyses in Chapter IV.....	157
APPENDIX VI: Additional Materials and Methods, Results and Discussion for analyses in Chapter IV.....	174

APPENDIX VII: Additional taxa used in molecular dating analyses in Chapter IV.....	180
APPENDIX VIII: A 50% majority rule consensus tree produced from Bayesian inference analyses of the combined nuclear and mitochondrial sequence data for 78 capsalid taxa and 29 outgroup taxa in Chapter IV.....	182
APPENDIX IX: A 50% majority rule consensus tree produced from Bayesian inference analyses of the combined nuclear sequence data for 77 capsalid taxa and 30 outgroup in Chapter IV.....	184
APPENDIX X: The maximum likelihood tree derived from analysis of the combined nuclear and mitochondrial sequence data for the Capsalidae in Chapter IV .....	186
APPENDIX XI: GenBank numbers for sequences of fish taxa used in analyses in Chapter V.....	188
Appendix XII: Table of fossil calibrations, cladogram of phylogenetic relationships presented in Nelson (2006) and justification for constraints used in molecular phylogenetic analyses of fish data in Chapter V.....	195

## ABSTRACT

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The Capsalidae is a diverse family of ectoparasites of marine fish (Platyhelminthes: Monogenea). It is a large family with approximately 180 described species and many more yet to be discovered. Capsalids have a global distribution and parasitise a diversity of hosts from the Chondrichthyes to bony fishes. A morphological classification exists for the family based on a few key characters such as testes number, their arrangement and morphology of the posterior attachment organ (haptor). Phylogenetic relationships within the family and its position within the Monogenea are largely unexplored. I have used various molecular phylogenetic techniques to resolve relationships and explore the evolution and radiation of this family. Specimens from the Capsalidae and other monogenean families (outgroups) were obtained through fresh collections and generous donations by other parasitologists. Specimens were stored in 95% undenatured ethanol. Three unlinked nuclear genes (*28S ribosomal RNA*, *Histone 3* and *Elongation Factor 1  $\alpha$* ) and two mitochondrial genes (*Cytochrome Oxidase 3* and *Cytochrome B*) were amplified for 78 capsalid taxa in 28 genera representing all nine subfamilies and 30 outgroup taxa (eight Polyopisthocotylea and 22 Monopisthocotylea). Analyses showed the Capsalidae is monophyletic, with the sister group remaining unresolved. Some analyses supported Gyrodactylidae and Udonellidae as the sister group but in other analyses, it was unresolved with the Monocotylidae and Microbothriidae also possible sister groups. The Capsalinae, Encotyllabinae and Nitzschiinae are monophyletic, but analyses did not support monophyly for the Benedeniinae, Entobdellinae and Trochopodinae. Monophyly was supported for *Capsala*, *Capsaloides*, *Encotyllabe*, *Entobdella*, *Listrocephalos*, *Neobenedenia*, *Nitzschia* and *Tristoma*, but *Metabenedeniella* is paraphyletic and *Benedenia* and *Neoentobdella* are polyphyletic. Comparisons of the distribution of character states for the small number of morphological characters on a molecular phylogeny show a high frequency of apparent homoplasy. Consequently the current morphological classification for the Capsalidae shows little correspondence with the phylogenetic hypotheses I present.

I also sequenced the first complete mitochondrial (mt) genome for a capsalid species, *Benedenia seriolae*. The mt genome of *B. seriolae* shows some tRNA

rearrangements in comparison to three *Gyrodactylus* spp. (Gyrodactylidae), the only other complete monopisthocotylean mt genomes sequenced. It also lacks the duplicated, conserved non-coding regions present in *Gyrodactylus* spp. making the genome smaller in size. I combined this genome with other available platyhelminth mt genomes to investigate the monophyly of Monogenea and the evolution of diet across the Neodermata. Results confirm paraphyly for the Monogenea and also suggest paraphyly for the epidermal feeding Monopisthocotylea. I hypothesise that the Monopisthocotylea represent the first shift to parasitism in the Neodermata from a free living ancestor and following this, there was a dietary shift to blood feeding (Polyopisthocotylea). The Digenea and Cestoda independently evolved dietary specialisations to suit their diverse microhabitats and broad range of vertebrate final hosts.

Using *Elongation Factor 1  $\alpha$*  I have estimated molecular divergence dates for the Neodermata, Monogenea and Capsalidae. Molecular clock analyses estimate the Neodermata diverged from the free living platyhelminths 513 million years ago (mya) (95% HPD [highest posterior density]: 473–605) before the appearance of vertebrates in the fossil record. The Monogenea diverged from the other neodermatan classes 441 mya (95% HPD: 420–547) coinciding with the appearance of fish in the fossil record. The most recent common ancestor of the Capsalidae arose approximately 235 million years ago (95% HPD: 200–274) following the Permian/Triassic extinction event and coinciding with the diversification of bony fishes in the marine environment.

I compared the capsalid phylogeny to a phylogeny of fishes that I generated using eight nuclear and three mt genes to explore the evolution and the radiation of the Capsalidae across fishes. There is no significant global signal of coevolution with only five parasite-host associations that may be a result of coevolution. Estimated molecular divergence dates are also compared and do not reveal temporal congruence. Radiation of the family may have been driven by host switching constrained by shared host ecology, biology, behaviour and plasticity in morphological adaptations by the parasites.

This study advances our understanding of the evolution of this monogenean family and provides insights into the evolution of the Neodermata and the complicated realities of reconstructing the evolutionary history of parasitic groups.



## ACKNOWLEDGEMENTS

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*To strive, to seek, to find, and not to yield*

*Ulysses*

Alfred Lord Tennyson

This PhD has been an incredible journey, full of highs, lows and everything in between. On more than one occasion I had to wonder to what I'd got myself into! I have been exceptionally fortunate to have this opportunity and recognise just how far I have come in these past few years. This journey would not have been possible without the support of many people. I must first acknowledge the endless support of my supervisors, Associate Professor Ian Whittington and Professor Steve Donnellan. Their commitment of time, energy and brain power has made this project possible and furthered my learning beyond what I could have imagined. The extensive knowledge you have both brought to my project has been invaluable.

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Last, but absolutely not least, I thank my family. You are the ones who have made me who I am and your support, interest and love have carried me through both the good and hard times during this PhD. You have been my refuge and my peace of mind. I am forever in your debt.

## NOTES ON CHAPTER STYLES

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Chapter II is published in *Molecular Phylogenetics and Evolution* and therefore follows the journal style precisely. A statement of authorship that details publication information and declares co-author contributions precedes Chapter II.

I will submit my other data chapters for publication and each is written in a style suitable for a specific journal as indicated on each title page. As such, the text also reflects multiple authors who contributed similarly as declared for Chapter II (e.g. secured funds, project supervision, guidance on laboratory protocols, field assistance and comments on chapter drafts during thesis preparation).

A reprint of the published chapter is in Appendix I. The format of my thesis complies with that outlined under “Specifications for Thesis 2009” provided by the Adelaide Graduate Centre at The University of Adelaide (see:

[www.adelaide.edu.au/graduatecentre/pdf/specifications\\_thesis\\_2009.pdf](http://www.adelaide.edu.au/graduatecentre/pdf/specifications_thesis_2009.pdf))