

## Identification and characterisation of two haplosporidian parasites of oysters in north Western Australia.



Picture: An adult pearl oyster.

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## **Preface**

The work described in this thesis is that of the author alone unless otherwise stated in the text. None of the work has been submitted for any other qualification at this or any other university.

Douglas Bearham

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## Acknowledgments

While I'd like to claim all of this work as my own, it is not. I have benefitted from the ideas and advice of a large number of people and like all research it was built on the findings and discoveries of the researchers who came before. When it comes to naming names I would firstly, like to thank my supervisors Phil Nicholls and Shane Raidal for their hard work and invaluable advice and to Brian Jones for his knowledge and advice which was instrumental to the project. I'd also like to thank Zoe Spiers for her help throughout the course of the project. Zoe was involved in almost all aspects of the study in some way.

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- Bearham, D., Spiers, Z., Raidal, S., Jones, J.B., Burrenson, E.M., Nicholls, P.K (2008) Spore ornamentation of *Haplosporidium hinei* n.sp. (Haplosporidia) in pearl oysters *Pinctada maxima* (Jameson, 1901). *Parasitology*. **135**, (4) 521-527.
- Bearham, D., Spiers, Z., Raidal, S., Jones, J.B., Nicholls, P.K (2008) Spore ornamentation of *Minchinia occulta* n. sp. (Haplosporidia) in rock oysters *Saccostrea cucullata* (Born, 1778). *Parasitology*. **135**, (11) 1271-1280.
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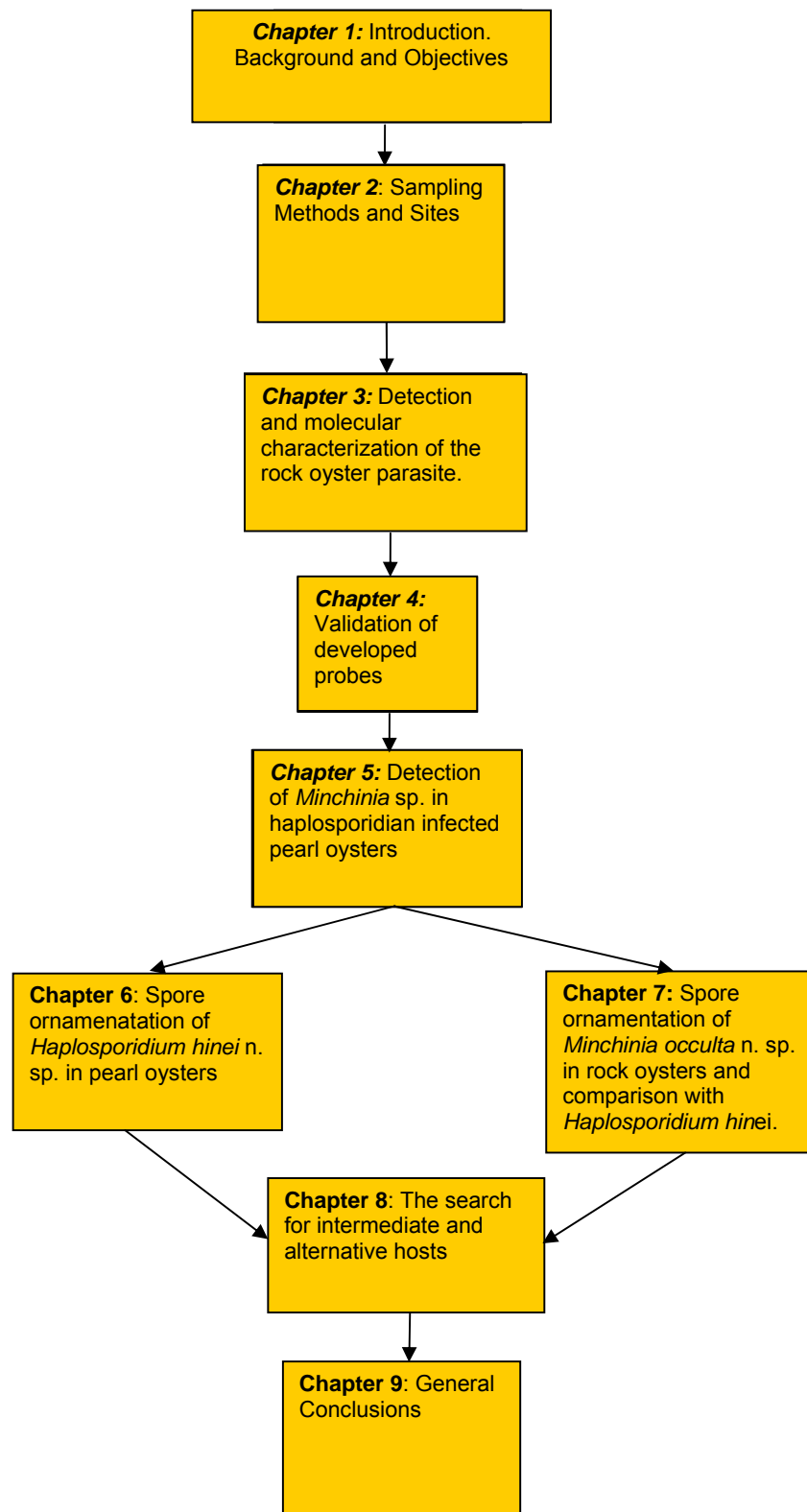
## List of Abbreviations, acronyms and definitions

bp	Base pair(s)
ddH <sub>2</sub> O	Double distilled water
dNTP	dATP, dCTP, dGTP or dTTP
DIG	Digoxigenin
ECE	Epispore Cytoplasmic Extensions
EDTA	Ethylene diamine tetraacetic acid
H&E	Hematoxylin and Eosin
Inflammation	Infiltration of haemocytes in oyster tissues
ISH	In situ hybridization
Longline	Rope with anchors and bouys attached. Common method used to maintain pearl oysters in the water column.
MDS	Multidimensional scaling
min	Minutes
MSN	Minimum Spanning Network
NPV	Negative predictive value (or number of negative test results for truly disease-free animals divided by the total number of negative test results.
OIE	Office Internationale d'Epizootie or World Organisation for Animal Health.
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
Pearl Oyster	Pinctada maxima
Rock Oyster	Saccostrea cucullata
s	Seconds
SEM	Scanning Electron Microscopy
Sensitivity	The number of disease-free animals that test negative divided by the number of truly disease-free animals.
SSC	Standard Saline Citrate
SSU	Small Subunit region of the rRNA gene
Spat	Juvenile pearl oysters
Sydney rock oyster	Saccostrea glomerata
TBSBT Triton X-100	Tris-buffered saline containing 3% bovine serum albumin and 0.1% Triton X-100
TE	Buffer containing 10 nM Tris – HCl (pH 8.0), 1 nM EDTA
TEM	Transmission Electron Microscopy
Tris	Trishydroxymethylaminomethane
Tropical oyster	Saccostrea echinata

## Summary

A cryptic haplosporidian parasite was detected infecting rock oysters from the Montebello Islands in north-western Australia using a PCR targeting the parasite's small ribosomal subunit gene. The PCR products were cloned and sequenced along with the remaining sections of the parasite's SSU rRNA gene. Phylogenetic analysis of the sequence generated indicated a *Minchinia* species (Haplosporidia). The SSU sequence generated was used to develop two *in situ* hybridisation assays to visualise the parasite in H/E sections as well as a PCR assay to detect the parasite. The molecular assays were assessed for specificity and sensitivity and were then used to compare the parasite to previous haplosporidian parasite infections of pearl oysters. Both assays produced positive results from the infected pearl oysters but not from other closely related haplosporidian species. An SEM and TEM electron microscopy analysis was performed on spores from both parasite species. The spores of the pearl oyster parasite had two spore wall filaments wound around the spore originating for a posterior thickening while the spores of the rock oyster parasite were covered in microtubule-like structures. These data suggests pearl oysters were co-infected with both the *Haplosporidium* sp. and the *Minchinia* sp. detected in rock oysters. No evidence of a posterior thickening could be found on the spores of the rock oyster parasite. Attempts to detect the parasite at the previous geographic sites of its detection in pearl oysters resulted in detection of the *Minchinia* species in tropical oysters in the Kimberley region of Western Australia by *in-situ* hybridisation.

## Thesis Flow Diagram





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