'Through the looking glass': Diversity and its functional significance in marine benthic microbial eukaryotes

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This research is supported by an Australian Government Research Training Program Scholarship.

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The woods are lovely, dark and deep,

But I have promises to keep,

And miles to go before I sleep,

And miles to go before I sleep.

-*Stopping by Woods on a Snowy Evening*, Robert Frost

I shall be telling this with a sigh

Somewhere ages and ages hence:

Two roads diverged in a wood, and I—

I took the one less travelled by,

And that has made all the difference.

-*The Road Not Taken*, Robert Frost

ORIGINAL PUBLICATIONS

Publications included in this thesis:

1. **Verma, A.**, Hoppenrath, M., Harwood, T., Brett, S., Rhodes, L., Murray, S., 2016, Molecular phylogeny, morphology and toxigenicity of *Ostreopsis* cf. *siamensis* (Dinophyceae) from temperate south-east Australia, Phycological Research 64(3), 146-59

2. **Verma, A.**, Hoppenrath, M., Dorantes-Aranda, J.J., Harwood, D.T. and Murray, S.A., 2016. Molecular and phylogenetic characterization of *Ostreopsis* (Dinophyceae) and the description of a new species, *Ostreopsis rhodesae* sp. nov., from a subtropical Australian lagoon. Harmful Algae, 60, 116-130.

3. **Verma, A.**, Kohli, G. S., Hoppenrath, M., Harwood, T., Kuzhiumparambil, U., Ralph, P. J., Murray, S. A., 2017 Systematics and diversity of the genus *Ostreopsis* in the East Australian Current region. In Proceedings of the 17th International Conference on Harmful Algae. International Society for the Study of Harmful Algae 2017.

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PREFACE

Lewis Carroll's 'Alice's Adventures in Wonderland' (1865) and 'Through the Looking-Glass and What Alice Found There' (1871) have fascinated readers for generations and have had a considerable impact on popular culture. Characters and references from these books have been used by scientists to explain the intricate phenomenon in microbial ecology, and particularly in marine microbial eukaryotes. Van Valen's 'Red Queen' hypothesis as a metaphor for an evolutionary 'arms race' and the 'Cheshire Cat' as a symbol of the complex phenomenon of sexual reproduction in *Emiliania huxleyi* are a few popular examples.

'*Through the looking glass*' depicts mirrors as a gateway to the wonderland and reflective of how mirrors are often illusionary. The book's finale centred around a game of chess where Alice finds herself as a pawn in the bigger game, highlights the importance of strategy to survive. The 'mirrors' and 'chess' from the storyline are symbolic of cryptic diversity and functional traits that exist in marine microbial eukaryotes, at a species, population, genetic and metabolic levels, enabling them to survive in the changing oceanic conditions. Cryptic diversity and strategic functional traits in *Ostreopsis* species are the fundamental questions that I have pursued in my dissertation and hence used the book title as a reference to symbolise the details of my aims and findings. The reference also highlights the enigmatic 'wonderland' of marine microbial eukaryotes that we witness through the lenses of a microscope (looking glass).

"*There is a place like no other on earth. A land full of wonder, mystery and danger! Some say, to survive it, you need to be as mad as a hatter."*

-Lewis Carroll, *Alice's Adventures in Wonderland*

THESIS ABSTRACT

Marine microbial eukaryotes are of immense ecological and evolutionary significance in marine ecosystems. Understanding their biodiversity and functional evolutionary traits are key to improving our understanding of marine ecosystem functioning. The East Australian Current (EAC) is a global climate change hotspot, and yet we lack in our understanding of its impact on phytoplankton distribution and dynamics. *Ostreopsis* species have been reported to cause severe blooms and produce palytoxin (PLTX) - like compounds all around the globe but we do not have basic information on the distribution and dynamics of *Ostreopsis* species in Australia.

In this dissertation, I established the first comprehensive report of *Ostreopsis* species from Australian waters and explored cryptic diversity and functional traits in this genus. Extensive sampling along a north-south gradient of 1800 km from sub-tropical to temperate waters yielded the identification of three species, including a novel pseudo-cryptic *Ostreopsis rhodesae* from the Great Barrier Reef, along with *Ostreopsis* cf. *ovata*. *Ostreopsis* cf. *siamensis* was identified at all locations and its eco-physiological traits and genetic population structure were investigated. The genetic diversity in the northern sub-tropical locations was greater compared to the more southern locations, reflecting a long-standing divergence and local radiations originating from the ancestral population and a potential southward range expansion, which may be related to the intensification of the EAC over the past century.

Intra- and inter-population variations in physiological traits were investigated to understand its range expansion and functional trade-offs. This is the first study to our knowledge to report growth rates, cell size, cellular toxic concentrations and photobiological parameters on fifty-three clones of a marine protist, in order to investigate intra-specific diversity in key functional traits. The toxin biosynthesis pathway in the three species was investigated using *de novo* transcriptomics and compared to *Coolia malayensis*. All essential domains needed to synthesize a PLTX-like carbon backbone were identified in the three *Ostreopsis* species and were also found in the non-PLTX producing *C. malayensis*. Putative molecules with potential polyketide-like backbone structures were reported in this investigation using non-targeted metabolomics,

suggesting a greater diversity of polyketide compounds amongst these species than previously anticipated.

Results from this dissertation add to the knowledge of species biodiversity, population structures, eco-physiological traits and toxin biosynthesis mechanisms in marine microbial eukaryotes, and *Ostreopsis* species in particular.