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**Ecology of microhylid frogs in the
Australian Wet Tropics and implications for
their vulnerability to chytridiomycosis.**



Adult and juvenile *Copixalus ornatus*

Thesis submitted by
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in July 2010
for the degree of Doctor of Philosophy
in the School of Marine & Tropical Biology
James Cook University

STATEMENT OF SOURCES DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *Joint NHRMC/AVCC statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics: Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval numbers A931 and A960).

Research was carried out under Scientific Purposes permits issued by the Queensland Parks and Wildlife Service (WISP02464008 and WITK 02464108), and a Permit to Collect issued by the Queensland Environmental Protection Agency (ATH 08/005).

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STATEMENT OF CONTRIBUTION OF OTHERS

This thesis was co-supervised by Ross Alford, Lin Schwarzkopf and Stephen Williams. Professor Ross Alford provided input on experimental design, statistical assistance, and editorial advice on all chapters. Lin Schwarzkopf provided editorial assistance to several thesis chapters. Stephen Williams allowed access to transects that were established in the Atherton and Carbine uplands. Sara Bell offered advice on culturing *Batrachochytrium dendrobatidis* (*Bd*) and completing challenge assays, and provided editorial assistance on chapters three and four. PCR diagnostic tests for *Bd* were performed by Ruth Campbell, Stephen Garland and Andrea Philot at the school of Veterinary and Biomedical Sciences, JCU; and Alex Hyatt at the Australian Animal Health Laboratory at CSIRO.

Chapters two, four, five, and six are in the process of being prepared for submission to a variety of journals. Professor Ross Alford will appear as co-author on all publications from this thesis, Sara Bell will appear as a co-author on publications derived from chapters three and four, and Lin Schwarzkopf will appear as a co-author on the publication derived from chapter two. I was supported by an Australian Postgraduate Research Scholarship. Research was funded by the Australian Rainforest Co-operative Research Centre, and by tender 43/2004 from the Australian Department of Environment and Heritage (R. Alford, principal investigator).

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ACKNOWLEDGEMENTS

First and foremost I wish to thank the beautiful frogs for letting me prod, poke, swab, measure, inject and kidnap them. My work with microhylids has allowed me to see the infinite beauty in these humblest of creatures, and has taught me to be a more patient and gentle being. Rummaging through the rainforest floor in the pouring rain, with my ear to the ground, searching for microhylids are memories that I will always hold dear.

Thanks to my supervisor Ross Alford for putting up with my antics for another five years. It wasn't the smoothest ride, but your persistence and faith in me have always been appreciated, and I couldn't have made this journey without you. Thanks for all the enlightening comments and words of advice. Thanks also to Lin Schwarzkopf for giving me the greatest attention at the shortest notice.

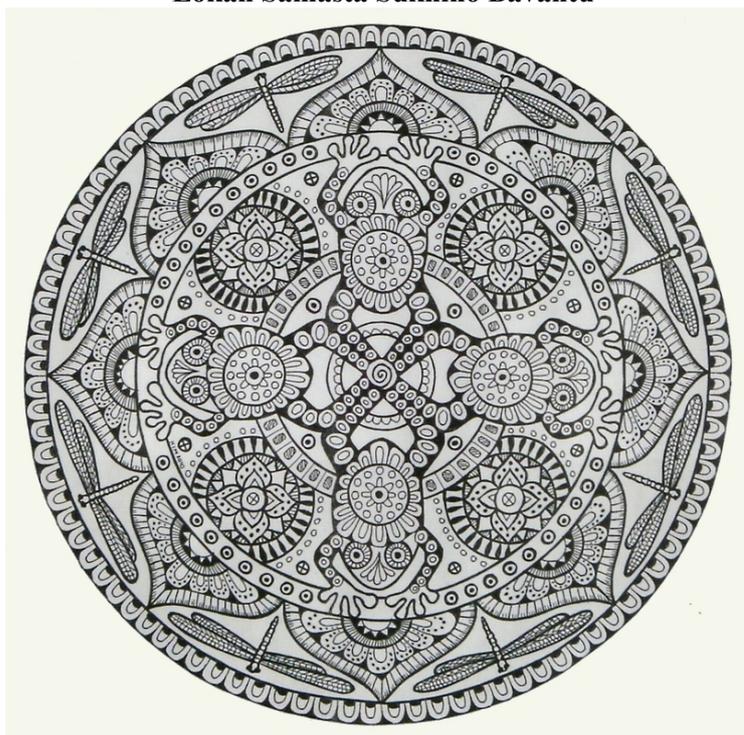
A special thanks to Sara Bell for teaching me the finer points of growing a pathogenic fungus. You made my experiences in the PC2 lab much more bearable, and I simply couldn't have completed my work on peptides or the experiment without you. Thanks also for going out of your way to answer all of my questions, and keep me up to date on all of the herpetological happenings at JCU when I wasn't in Townsville.

Thanks to all of my beautiful friends and family for helping me throughout this process over the past few years, and for continually offering your love and support. There were times when I didn't think this thesis would ever come into fruition, but there were so many of you who had faith in me – cheers Nicole, Johnny, Jim, Kylie, Tim, Marty, Tereza, Rae, and Claire. A special thanks to Oma, Grandpa, Laura, PC, Pete, Rob, Jake, Nick and Luke who housed, fed and supported me through some of my most trying times. Thanks also to Kris and Jeff for your gracious hospitality, and the unceasing ability to make me smile.

Thanks to all the volunteers who helped me in the field, but especially to Raelene Hobbs, Claire Steel, and Tereza Tantar for helping with my research on so many occasions. You made field work even more wonderful, and you inspired me with your love and knowledge of all things froggy.

Finally, thank you to my wonderful parents Kurt and Irene who have been waiting for this for a long time. Thanks for always being there for me – both in the good and the bad times, and thanks for your patience, understanding and eternal wisdom. Big love to you.

Lokah Samasta Sukhino Bavantu



ABSTRACT

Chytridiomycosis is an emerging infectious disease of amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), and is responsible for causing mass mortality, population declines and extinctions of amphibian species in much of the world. The extent of pathological effects of chytridiomycosis varies amongst hosts, and terrestrial frogs and salamanders that have parental care of direct-developing eggs may tend to be less susceptible. The frog family Microhylidae is characterised by terrestrially-breeding species that reach their highest diversity in Australia in the Wet Tropics (WT) bioregion of northern Queensland. In this region, frogs of other families suffered severe declines and extinctions in association with outbreaks of chytridiomycosis. There is a lack of information on the responses of Australian microhylids to this emerging disease, and this project was carried out to gain explicit information on the interactions of microhylids with *Bd*.

The temporal calling patterns of two microhylid species (*Austrochaperina robusta* and *Cophixalus ornatus*) were examined at one site within the WT, using automated recordings every night for five entire wet seasons, over an 11-year period. Environmental variables were also recorded, to examine correlations between calling and weather conditions. The calling activity of *C. ornatus* and *A. robusta* fluctuated only slightly from year to year; the observed fluctuations were less than two-fold. There was no downward trend in average and maximum calls for either species over time, suggesting that population densities of these species have not suffered from major losses, and that variation in calling is unlikely to be due to climate change or an external factor such as *Bd*.

To determine if microhylids could be infected with *Bd* in the wild, I examined 595 samples from nine species, and found that none showed evidence of infection by *Bd*. When this data were regarded as a single sample representative of Australian microhylids, the upper 95% binomial confidence limit for presence of infection was 0.0062 (less than 1%). This suggests that microhylids have a very low prevalence of *Bd* in nature, and are either not susceptible, or are only slightly susceptible, to chytridiomycosis under natural conditions.

The susceptibility of the most common species of microhylid (*Cophixalus ornatus*) to *Bd* was tested in a series of laboratory experiments. Seven *C. ornatus* and five *Litoria wilcoxii* (susceptible controls) were exposed to increasing numbers of *Bd* zoospores and tested for infection using quantitative PCR assays. All *C. ornatus*, and four of the five *L. wilcoxii*, became infected by *Bd* at some point during the experiment. The mean intensity of infection, as measured by number of zoospore equivalents in infected individuals, was significantly higher in *L. wilcoxii* than in *C. ornatus*. All *C. ornatus* individuals eliminated their infections by the end of the experimental trials, whereas *L. wilcoxii* individuals still retained relatively intense infections.

Innate immune defenses in the form of antimicrobial peptides (AMPs) may be particularly important in providing resistance to chytridiomycosis, and highly effective AMPs could be responsible for the extremely low prevalences of *Bd* infection I found in nature. To test this hypothesis, the AMPs of 81 microhylids from six species were examined. Secretions containing skin peptides were collected by norepinephrine induction, and used in growth inhibition assays to measure their effectiveness against *Bd*. Sixty two percent of samples contained AMPs with at least some activity against *Bd*, and 17% showed 100% inhibition of *Bd* growth at the levels tested. Microhylid species produced peptides in quantities similar to two sympatric hylid frogs (*Litoria genimaculata* and *L. rheocola*). Mean protein secretion of microhylids did not differ significantly from these species, however, the overall protection of microhylids provided by AMPs was significantly lower than that of hylids. This suggests that AMPs are not likely to be responsible for the low prevalence of infection by *Bd* in Australian microhylids.

Thermal and hydric environmental variables are known to affect the prevalence of *Bd* infections and the occurrence of epidemic outbreaks of chytridiomycosis, as *Bd* requires water to reproduce, and has a thermal optimum of 17-25°C. Measurements of the body temperatures of frogs were combined with longer-term temperature and moisture data collected from permeable and impermeable agar models. These models were placed in frog retreat sites to document the thermal and hydric environments that microhylids experience in the field. Models never lost more than 20% of weight after periods exceeding 96hrs, suggesting that retreat sites provide insulation from dehydration. Models produced an accurate outline of the thermal envelope of microhylids as 90% of frogs had surface temperatures that were inside the range of the models. Surface temperature readings of 197 frogs were within the growth range for *Bd*, and model thermal data suggested that at two of the four transects (Carbine uplands and Paluma), temperature levels during the period of data collection were entirely within the optimal range for *Bd* growth. However, at Bellenden Ker, models were below the optimal range for *Bd* growth 70 percent of the time, and at the Atherton uplands, model temperatures reached levels that were above the range for optimal growth of *Bd* 24 percent of the time. Model temperatures also reached levels above 28°C, which may cause the fungus to stop growing. Given that data were collected over a narrow time window, and that *Bd* has a narrow range of tolerance for temperature and moisture conditions, the data indicate that microenvironments used by microhylids are likely to affect the overall dynamics of the host-pathogen system. Some individuals and species may be less susceptible to *Bd* infection due to the environments they use.

In summary, the results of this study provide new levels of understanding of the interactions between Australian frogs of the family Microhylidae and the amphibian chytrid fungus. Despite inhabiting environments where *Bd* has caused declines in other frog species, they have not suffered population losses associated with *Bd*, and *Bd* infections are either at very

low prevalence or are entirely absent in nature. This apparent resistance to infection does not appear to be solely due to AMPs, as samples collected from microhylids in the field were no more effective against *Bd* in microhylids than are those of hylid species. Microhylids are not constitutively immune to infection by *Bd* from some other, unknown mechanism intrinsic to themselves, since *C. ornatus* readily became infected in laboratory experiments. Field data showed that the thermal environments experienced by microhylids may contribute to their near-immunity to *Bd* infection in nature, but are unlikely to explain it entirely. One potentially important source of resistance to *Bd* infection that was not evaluated in the present study is the contribution of symbiotic skin microbes, and future work should evaluate this, as it is possible that the Australian microhylids have a skin microbiota that is particularly effective at combating *Bd* infection in the field.

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