Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
Evolution of diversity: analysis of species and speciation in *Hemiandrus* ground wētā

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy
in
Zoology

Massey University
New Zealand

Briar Leigh Taylor Smith
2015
In loving memory of my grandad,

Bruce Smith

“The important thing is not to stop questioning. Curiosity has its own reason for existing. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvellous structure of reality. It is enough if one tries merely to comprehend a little of this mystery every day. Never lose a holy curiosity.”

- Albert Einstein
Abstract

Patterns of biodiversity and endemism in New Zealand are explored, with a focus on the ground wētā genus *Hemiandrus*. I first investigated factors that determined regional levels of endemism using a generalised linear model based on analysis of 2322 species of endemic New Zealand invertebrates. I found that widespread species are uncommon in New Zealand and most invertebrates occupied few regions. Number of endemic species per region was positively correlated with total number of species and size of the region 3 million years ago. Within one clade of *Hemiandrus* I found that North and South Islands differed in how they were occupied: South Island had many species with small non-overlapping ranges, whereas North Island was largely dominated by a single species. This is likely due to differences in age of different parts of New Zealand, yet this pattern was absent in another clade of ground wētā species, showing that properties of species themselves also have a large impact on species ranges and speciation.

I applied several strategies to the *Hemiandrus maculifrons* species complex to test putative species boundaries (chapter 3). I compared morphological methods (Gaps in Continuous Characters across Geography (GCCG)) and genetic methods (Bayesian Species Delimitation, Rosenberg's P(AB), P(Randomly Distinct), P ID(Liberal)). Some of these strategies indicated that all or nearly all mtDNA clades tested represented separate species, while others indicated that no clades were likely to be distinct species. I concluded that *H. maculifrons* comprises three species (plus an under-sampled microendemic species, chapter 4); a conclusion that is discordant with the results of the “species delimitation” methods but consistent with other genetic, morphological and distributional data.

Since the genus *Hemiandrus* was thought to comprise only nine named species but dozens of alleged species, I tested whether the purported diversity accurately reflected biological diversity in the genus or whether it was exaggerated due to speculative classification (chapter 5). To do this, I applied traditional techniques to search for qualitative or quantitative differences between individuals using a model where species are separately evolving lineages that form separate genotypic clusters with no or few intermediates when in contact (Mallet 1995). Most proposed operational taxonomic units were supported, but some names appear to be synonyms while others appear to encompass more diversity than previously recognised. I concluded that *Hemiandrus* comprises at least 25 species, but as specimens representing all tag-names1

---

1 A tag-name is an informal name that indicates an entity that may be a separate species, monophyletic group or separate interbreeding population of uncertain taxonomic rank (Leschen *et al.* 2009).
were not available, additional diversity may exist within *Hemiandrus* than recognised here.

Phylogenetic analysis of mtDNA sequences identified two major clades within New Zealand *Hemiandrus*. Using nuclear markers and morphological traits I found strong support for these two clades. Derived shared traits were identified that can determine to which clade each species belongs. Concordance between genetic markers (four loci) and morphology resolved evolutionary relationships from which I propose dividing the group into two separate genera.
Preface

The overall aim of this research project, “Evolution of diversity: analysis of species and speciation in Hemiandrus ground wētā”, was to examine patterns of biodiversity and endemism in New Zealand, focusing particularly on the genus Hemiandrus (ground wētā). This genus has been suggested to comprise high species diversity and many taxa with restricted ranges, but diversity within this genus is largely unquantified.

In chapter one I explore the patterns and drivers of microendemism in New Zealand invertebrates. The finding that widespread species are uncommon in New Zealand and most invertebrates occupied few regions led me to question why a single ground wētā species, H. maculifrons, is found so extensively throughout both main islands of New Zealand (chapter 3). I explored this using multiple lines of evidence, including nuclear markers developed in chapter 2 with the help of Eddie Dowle, who sequenced multiple anostostomatid mtDNA genomes for her PhD thesis (2013). My results from chapter 3 suggest that H. maculifrons comprises multiple species and these are described in chapter 4 along with two morphologically similar species.

In chapter 5 I assess the overall purported species diversity within Hemiandrus. I first examined specimens collected during extensive field work and from assorted collections, in order to gain an understanding of morphological variation within the genus. Based on this background knowledge I was then able to match specimens to tag-names. Most tag-names do reflect biological diversity, although some appear to be synonymies, while others may comprise several species. Two of these tag-named species are described in chapter 6: Taylor Smith, B. L., Morgan-Richards, M., & Trewick, S. A. (2013). New Zealand ground wētā (Anostostomatidae: Hemiandrus): descriptions of two species with notes on their biology. New Zealand Journal of Zoology, 40(4), 314-329.

In chapter 7 I show that the high species diversity within Hemiandrus compared to other New Zealand anostostomatid genera may be partially explained by ground wētā comprising multiple morphologically and genetically distinct genera.

These chapters are intended for publication and so references are presented at the end of each chapter.
Steve and Mary, you are incredible friends and supervisors. I am going to miss working with you. You create an amazing environment for students and I feel privileged to have been part of it. Thanks to my friends in the Phoenix group, especially my number one Phoenix, Elizabeth Daly. Thanks to the Trewicks and Dalys for welcoming me into your families when I was in Palmerston North.

Thank you to Grace Hall and Birgit Rhode for assistance with the NZAC collection and microscopy help; to John Early and Dhahara Ranatunga for assistance with the Auckland Museum collection; to Eric Edwards for advice and providing me with specimens from Fiordland; to our secretary Sharon Wright and our technicians Tracy Harris, Paul Barret, Cleland Wallace and Shaun Nielsen; to Edwina Dowle, Gillian Gibb and Trish McLenachan, for always being there to answer my questions and also to Eddie for grasshoppers, helping me with genome assembly and for getting things down from high shelves; to Niki Murray at Manawatu Microscopy and Imaging Centre for egg chorion photographs and my golden eggs; to Ian Stringer and Mike Wakelin for assistance with LENVZ data; to Lesley van Essen for mining data from the FNZ series; to Matt Irwin for GIS help and Jean Sanderson for assistance with statistics; to Adele Reweti and Aaron Gillespie for letting me put out traps on your property; to Cilla for being an awesome mentor in the early stages of my research; to Esta Chappell, Peter Johns and Darryl Gwynne for your correspondence regarding ground wētā; also to Darryl and Sarah Gwynne for the refreshing few days collecting at Tekapo; to the Entomological Society of New Zealand, the Brian Mason Scientific & Technical Trust, Massey University Doctoral Scholarship fund, Department of Conservation data deficient fund and Massey University Institute for Agriculture and Environment Doctoral Bridging fund for financial support.

Thank you to all those who have collected alongside me: Knoll Smith (my number one wētā hunter), Kane O’Keeffe, Eddie Dowle, Marianna Bulgarella, Melissa Griffin, Shelley Myers, Amelia Corin, Steve Corin, Bridget Reweti, Rob Wilson, Angus Wilson, George Wilson, Regan Smith, Mary Morgan-Richards, Edward Trewick, Bianca Trewick, Niki Minards, Christina Rowe, Stefanie König. A big thank you to those who provided me with an abundance of ground wētā: Mike Lusk, Jess Costal, Jo Fitness, Andrew Blayney, Ian Millar and Tony Jewell. Collecting was also aided and abetted by: Marty Haigh, Rod Hitchmough, Julia Goldberg, Cindy Coreman, R. Goudsward, Renae Pratt, Mike Wakelin, Christina Painting, Gareth Boyt, Pete Shaw, Jay McCartney, Lorraine Cook, Ollie Ball, P. Van Veen, Dave Seldon, Chris

Thank you to Kane and my family for the support, encouragement and understanding.
## Contents

Abstract .......................................................................................................................................... i  
Preface .......................................................................................................................................... iii  
Acknowledgements ...................................................................................................................... iv  
Chapter 1. Microendemism in New Zealand ............................................................................... 13  
   Introduction ............................................................................................................................. 13  
   Methods ................................................................................................................................... 21  
   Results ..................................................................................................................................... 26  
   Discussion ............................................................................................................................... 33  
   Conclusions ............................................................................................................................. 36  
   Appendix ................................................................................................................................. 37  
   References ............................................................................................................................... 38  
Chapter 2. Nuclear marker development ..................................................................................... 41  
   Introduction ............................................................................................................................. 41  
   Methods ................................................................................................................................... 43  
   Results ..................................................................................................................................... 48  
   Discussion ............................................................................................................................... 53  
   Conclusion ............................................................................................................................... 54  
   Appendix ................................................................................................................................. 55  
   References ............................................................................................................................... 56  
Chapter 3. Marvellous *maculifrons*: discovering and interpreting genotypic clusters in a widespread ground wētā (Anostostomatidae: *Hemiandrus*) ....................................................... 58  
   Introduction ............................................................................................................................. 58  
   Methods ................................................................................................................................... 62  
   Results ..................................................................................................................................... 68  
   Discussion ............................................................................................................................... 89  
   Conclusions ............................................................................................................................. 92  
   Appendix ................................................................................................................................. 93
List of tables and figures

Chapter 1:

<table>
<thead>
<tr>
<th>Figure 1.1</th>
<th>Microendemism in Madagascar</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.2</td>
<td>Endemism in Europe and Israel</td>
<td>16</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Regional endemism in New Zealand</td>
<td>17</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>New Zealand entomological regions and land area 3Ma</td>
<td>19</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Range restriction hypothesis graph</td>
<td>19</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Maps showing microendemism and diversity in New Zealand entomological regions</td>
<td>27</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>Relationship of microendemism to diversity and to well-sampled regional endemics</td>
<td>28</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Number of regions each of the 2322 invertebrate species was collected from</td>
<td>29</td>
</tr>
<tr>
<td>Figure 1.9</td>
<td>Lycosidae and Simuliidae sampling frequency distribution</td>
<td>29</td>
</tr>
<tr>
<td>Figure 1.10</td>
<td>The distribution of Simuliidae sampling effort</td>
<td>30</td>
</tr>
<tr>
<td>Figure 1.11</td>
<td>Correlation between endemism and land area 3Ma</td>
<td>31</td>
</tr>
<tr>
<td>Table 1.1</td>
<td>Invertebrate species diversity and endemism</td>
<td>20</td>
</tr>
<tr>
<td>Table 1.2</td>
<td>Correlation of environmental variables</td>
<td>25</td>
</tr>
<tr>
<td>Table 1.3</td>
<td>Generalised linear model results</td>
<td>32</td>
</tr>
</tbody>
</table>

Chapter 2:

| Figure 2.1 | Primers were designed based on the alignment of Hemiandrus pallitarsis and H. maculifrons contigs | 43   |
| Figure 2.2 | Chromatograms with double peaks | 45   |
| Figure 2.3 | NU2 in Deinacrida connectens | 48   |
| Figure 2.4 | Bayesian mitochondrial DNA (COI) phylogeny | 49   |
| Figure 2.5 | Unrooted phylogenies of NU2 and NU3 alleles | 50   |
| Figure 2.6 | Phylogeny of NU13 alleles rooted with tree and giant wētā | 51   |
| Figure 2.7 | Relationship between nuclear and mtDNA genetic distances | 51   |
| Figure 2.8 | Median joining networks of nuclear haplotypes | 52   |
| Table 2.1  | Number of individuals, variable sites and haplotypes for each Hemiandrus species sequenced | 45   |
| Table 2.2  | Nuclear loci details | 47   |

Chapter 3:

| Figure 3.1 | The distribution of New Zealand ground wētā | 60   |
| Figure 3.2 | Morphometric characters measured for Hemiandrus maculifrons specimens | 62   |
Figure 3.3  Section of COI mtDNA chromatogram shows a probable numt…….  64
Figure 3.4  Bayesian mtDNA COI phylogeny of H. maculifrons……………….  70
Figure 3.5  Haplotype networks and sampling locations…………………….  71
Figure 3.6  Nuclear allele networks…………………………………………….  75
Figure 3.7  Variation in H. maculifrons male subgenital plate shape and pronotal patterning……………………………………………………  77
Figure 3.8  Morphometric traits that differed significantly among male H. maculifrons in different mtDNA lineages and clades………………….  79
Figure 3.9  Morphometric traits that differed significantly among female H. maculifrons in different mtDNA lineages and clades……………….  80
Figure 3.10  Principal component analysis of H. maculifrons body size measurements…………………………………………………………  82
Figure 3.11  Variation in spine and peg number among Hemiandrus maculifrons mtDNA clades……………………………………………….  85
Figure 3.12  Results of principal component analysis of female ovipositor shape…………………  86
Figure 3.13  Results of principal component analysis of mid tibial spine position…………………  87
Table 3.1  Sample sizes and species delimitation statistics for clades within H. maculifrons………………………………………………………….  73
Table 3.2  Nuclear alleles within three clades at Lewis Pass…………………...  76
Table 3.3  Variation in qualitative and discontinuous quantitative traits within H. maculifrons and ANOVA results……………………………..  78
Table 3.4  Variation in continuous quantitative traits within H. maculifrons and ANOVA results………………………………………………….  81
Table 3.5  Correlation between the pooled body sizes of North A and North C females and longitude………………………………………..  82
Table 3.6  Male H. maculifrons discriminant function analysis results………………...  83
Table 3.7  Female H. maculifrons discriminant function analysis results………………...  84
Table 3.8  Summary of genetic and morphometric evidence for subgroups within H. maculifrons………………………………………………  88

Chapter 4:

Figure 4.1  Bayesian mtDNA COI phylogeny showing the relationships among species of the Hemiandrus maculifrons cryptic species complex, and their minimum distribution in New Zealand………………….  105
Figure 4.2  Representative female individuals of each of the five ground wētā species described in this chapter……………………………………”