Systematics and diversity of Australian pygopodoid geckos (Pygopodoidea, Gekkota, Squamata).

Paul M. Oliver

A thesis submitted in fulfilment of the

requirements for the degree of

Doctor of Philosophy

School of Earth and Environmental Sciences

The University of Adelaide

December, 2009

Table of Contents

Abstract

Lizards and snakes (squamates) are the most diverse endemic component of the Australian terrestrial vertebrate fauna; and three families of Pygopodoid gecko (Carphodactylidae, Diplodactylidae and Pygopodidae) together comprise the third most species rich squamate lineage within Australia. In this thesis I present the results of an analysis of the systematics and species diversity of components of the Australian pygopodoid gecko radation; specifically, I focus on establishing an overall systematic and temporal framework for the evolution of the entire clade, examining estimates of species diversity and interrelationships within three genera, and using the resultant phylogenetic framework to advance our understanding of how the onset and expansion of aridification across Australia may have affected evolution with this lineage.

In chapter two the phylogenetic relationships of all Australian pygopodoid genera (except *Orraya*) are examined, and temporal scale for their diversification is estimated based on Bayesian and Likelihood analyses of two nuclear genes. This work demonstrates that at least five extant lineages within this radiation diverged before the final separation of Australia from Antarctica, and that the clade has a long history within Australia equivalent to famous Gondwanan elements of the fauna, such as the Marsupials.

An analysis of systematic relationships within the genus *Diplodactylus* based on mitochondrial DNA and morphological data indicate that as recognised previously, it comprises two genetically distinct and morphologically diagnosable clades; we resurrect the name *Lucasium* for one of the these clades. Both genera appear to represent moderately diverse and broadly overlapping radiations of multiple taxa largely restricted

to arid and semi-arid Australia, but absent from relatively mesic coastal areas, especially along the east, suggesting semi-arid to arid habitats have a long history within Australia.

A multilocus (mitochondrial, alloyme and karyotypic) examination of species boundaries within the newly defined *Diplodactylus* increases estimates of species diversity from 13 to 29. A similar study of the single recognised species of *Crenadactylus*, reveals it to comprise a surprisingly ancient radiation of at least ten candidate species. The diversification of *Crenadactylus* species, some of the oldest cryptic vertebrate taxa yet identified, dates backs to the estimated onset of aridification and has important insights into this process. Together, these two studies demostrate that species diversity in many Australian vertebrates remains significantly underestimated, and that this inadequate taxonomy is masking important conservation and evolutionary information.

In chapter five I present a combined mitochondrial and nuclear phylogenetic analysis of the ecologically widespread genus *Nephrurus* (*sensu* Bauer 1990). Based on this phylogeny we propose a revised generic arrangment for this clade assigning the two most plesiomorphic and basal lineages to monotypic genera. Molecular dating reveals a strong correlation between the age of a specialised arid-zone clade and independent estimates for the major expansion of the arid zone.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Paul Oliver and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed above) resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

…….

Paul Oliver

February 2009

Acknowledgements

First and foremost I thank my official supervisors Mike Lee and Steve Cooper, and my unofficial supervisor Mark Hutchinson, for their advice, time, support and encouragement. I equally express my enormous gratitude to my labmates in the Lee lab - Andrew Hugall, Kate Sanders and Adam Skinner, without the enthusiasm, advice, support and not least of all patience of these people, it is certain that this thesis would be of lesser quality than it is. I also thank Mark Adams for his encouragement and producing great data that no-one else does anymore, and Steve Richards for the incredible career and life opportunities he has given me, just a small portion of which can be seen in the appendices of this paper. I would also like to take this opportunity to thank many co-authors on the papers that I present in this thesis (most of whom are listed above) who have all contributed enormously to the successful completion of this work.

I extend my further gratitude to a host of other students and staff at the Australian Centre for Evolutionary Biology and Biodiversity (ACEBB) that contributed time and advice towards this project, namely, but not limited to Kathy Saint, Terry Bertozzi, Andrew Breed, Takashi Kawikami, Duncan Taylor, Leanne Wheaton, Gaynor Dolman, Ralph Foster, Mark Sistrom, Racheal Dudaneic, Luke Price, Lizzie Perkins, Jaro Guzinski and many, many other people who made the long hours in the lab less onerous and more enjoyable.

I thank the staff from a number of Australian Museums for providing access to specimens and tissues in their care; namely Paul Doughty, Brad Maryan and Claire Stevenson (WAM), Paul Horner and Dane Trembath (NTM), Ross Sadlier and Glenn Shea (AM), Patrick Couper and Andrew Amey (QM), and of course Mark Hutchinson and Carolyn Kovach at SAM. I also thank Aaron Bauer and Todd Jackman for hosting me in the USA and providing some important advice and opportunities over the last three years.

Lastly, and on a more personal front, I would like to thank my wonderful family, and especially parents, for their unstinting support and encouragement over the last two and a half decades, this work is in many ways a culmination of that support. In a similar vein I extend my heartfelt thanks to a number my friends outside work who have been extremely supportive throughout this process; namely Sandy Foo, Praneet Keni, Navin Das, Beata Kucaba and Ian Wong.

This work was supported by grants from the Australia Pacific Science Foundation, Mark Mitchell Foundation and Australian Biological Resources Study. All SA Museum and University of Adelaide animal research is carried out under the supervision of the Wildlife and University of Adelaide (respectively) Animal Experimentation Ethics Committees.

CHAPTER 1: GENERAL INTRODUCTION

1.1 The diverse Australian squamate fauna

Squamates (lizards and snakes) are the most diverse endemic component of the Australian vertebrate fauna (Pianka 1972, 1981). While snake diversity is relatively low by global standards, the lizard fauna is one of the most diverse in the world and includes over 600 recognised species (Wilson and Swan 2008). In contrast to this high species diversity, the squamate fauna is relatively poor at deeper phylogenetic levels, and is dominated by eight largely endemic and highly speciose radiations; the dragons (70+ species), the monitors (27+), venomous snakes (100+), the blindsnakes (40+), three major radiations of skinks (400+), and the pygopodoid geckos (including Pygopodidae (120+) (Greer 1989). The existence of multiple phylogenetically independent, but endemic, geographically bounded, and diverse Australian squamate radiations provides an excellent opportunity for comparative analysis of diversification processes on a continental scale. As a significant component of the terrestrial fauna, squamates are also likely to be a key group for understanding the timing and effects of major historical environmental changes within Australia (e.g Crisp *et al*. 2004). Unfortunately, the phylogenetic and systematic framework to undertake appropriate analyses is still lacking for many groups. The absence of answers to these basic systematic issues seriously impedes attempts to understand patterns of evolution within the diverse Australian squamate fauna.

1.2 Systematics of the Australian squamate fauna

Understanding of Australian squamate evolution has until recently been confounded by a lack of solid phylogenetic and especially temporal data (Greer 1989). Fortunately Australian squamate relationships and divergence dates have been the focus of a significant body of recent phylogenetic research, and at least preliminary molecular phylogenies have now been published for many major Australian groups (Donnellan *et al*. 1999; Melville *et al*. 2002; Reeder 2003; Jennings *et al.* 2003; Fitch *et al.* 2006;

Skinner 2007; Sanders *et al.* 2008; Hugall *et al.* 2008; Rawlings *et al*. 2008). This work has revealed that many previous phylogenetic and associated biogeographical hypotheses were compromised by both inadequate taxonomy and the absence of reliable timeframe for diversification (e.g compare generic limits and species estimates used by Pianka 1981 and Cogger and Heatwole 1981 with those in Wilson and Swan 2008). Nonetheless there remain major and significant gaps in our understanding, and comprehensive multilocus species level phylogenies based on a combination of nuclear and mitochondrial data have not yet been published for many of the more diverse groups.

Ongoing morphological and molecular work has also indicated that despite over a century of sustained taxonomic work, Australian squamate species diversity remains significantly underestimated. Indeed, if anything the rate of new species description has increased in the last two decades (Cogger 2000; Wilson and Swan 2008), spurred on significantly by the application of molecular techniques to identify morphologically similar but genetically distinct 'cryptic species' (Donnellan *et al.* 1993; Aplin and Adams 1998; Horner and Adams 2009). Nonetheless, while the problem of unrecognised cryptic Australian squamate species has been recognised for several decades (Donnellan *et al.* 1993) and has been the focus of a major research effort, there have been no systematic attempts to address the problem across all Australian squamates, and to estimate what percentage of the fauna remains unrecognised.

1.3 The "pygopodoid" geckos (Diplodactylidae, Carphodactylidae, and Pygopodidae).

Based on current estimates of species diversity, the third most diverse squamate lineage within Australia is an ecologically and morphologically diverse radiation of over 120 species of geckos in three families; the Pygopodidae, the Carphodactylidae and the Diplodactylidae (Han *et al*. 2004). These three families form a strongly supported clade (Donnellan *et al.* 1999; Gamble *et al*. 2008a), which was recently named the Pygopodoidea (Vidal and Hedges 2009). Pygopodoid geckos can be found across most of the Australian continent and have radiated into arboreal, terrestrial, saxicoline and even almost limbless fossorial forms (Greer 1989). Indeed, while molecular studies strongly

support their monophyly (Donnellan *et al.* 1999; Han *et al*. 2004; Gamble *et al.* 2008a), only a small number of morphological characters, most notably soft-shelled eggs and lidless eyes, and one synapomorphy, a complete external meatal closure muscle, characterise all the diverse array of taxa included within this clade (Kluge 1987; Greer 1989). In addition to a majority of species and genera in Australia, there are also at least 60 extralimital species in neighbouring landmasses, New Zealand and New Caledonia (Bauer and Sadlier 2000; Jewell 2008).

The Diplodactylidae is the most speciose family of pygopodoid geckos, and includes over sixty Australian species in six genera (but see Chapter 3). All extralimital pygopodoid geckos from New Zealand and New Caledonia are also currently placed within this family, although for a long time they were grouped with the padless Carphodactylids (Greer 1989; Bauer 1990; Han *et al*. 2004). Uniquely amongst the pygopodoids, all Diplodactylidae either possess toe pads, or show strong evidence of being secondarily padless (Kluge 1967; Greer 1989; Han *et al.* 2004). Within Australia the extant genera are relatively widespread and show considerable ecological diversity, but can be classified into predominately arboreal/saxicoline/scansorial genera (*Crenadactylus*, *Oedura*, *Pseudothecadactylus* and *Strophurus*) and predominantly terrestrial genera (*Diplodactylus, Lucasium* and *Rhynchoedura*). Although widespread in all but the most temperate south and mesic coastal regions, the highest diversity of species is found in semi-arid to arid habitats across the centre and west of the continent.

The family Carphodactylidae includes five genera (but see Chapter 6) of relatively large padless geckos. Based on comprehensive phylogenetic analyses, some clear morphological and ecological groupings are apparent within this family (Bauer 1990). The most speciose (16 species), but morphologically and ecologically relatively conservative group, are the arboreal leaf-tail geckos (genera *Orraya*, *Phyllurus* and *Saltuarius*) of mesic eastern Australia (Couper *et al.* 1993, 2008a; Hoskin *et al*. 2003). In contrast the terrestrial geckos of the genus *Nephrurus* (11 species) are widespread across Australia and show considerably more ecological and morphological diversity, including two species that are frequently placed into a separate genus, *Underwoodisaurus* (Bauer 1990; Wilson and Swan 2008). A final distinct lineage is the monotypic genus *Carphodactylus* from the Queensland wet tropics; amongst the many unique features of

this scansorial species is a tail that squeaks when shed (Bauer 1990; Wilson and Swan 2008). The Carphodactylidae range over most of Australia, and extend into some relatively temperate and mesic areas where the other two families are absent or depauperate.

The Pygopodidae, commonly termed "legless lizards", are the most morphologically aberrant living geckos (Greer 1989; Webb and Shine 1994). They have lost all functional limbs and diversified into spectacular array of highly specialised and divergent ecologies; they are widely regarded as the most adaptively diverse (though not most speciose) radiation of limb-reduced squamates apart from snakes (Patchell and Shine 1986; Shine 1986; Webb and Shine 1994). Particularly notable trends are a tendancy towards ecological specialisation and associated morphological adaptations in the genera *Aprasia*, *Lialis*, *Ophidiocephalus*, *Paradelma*, *Pletholax*, and *Pygopus* (Kluge 1976; Patchell and Shine 1986). The remaining genus *Delma* is relatively generalised, although it shows considerable variation in body size and proportions (Kluge 1974). Most genera are largely confined to Australia (although two species of *Lialis* occur in New Guinea) and at least one pygopod species can be found in most parts of Australia, with the exception of a small number of temperate coastal and southern areas (Kluge 1974).

While a number of recent papers have addressed systematic relationships within and between pygopodoid families and genera (Jennings *et al.* 2003; Hoskin *et al*. 2003; Melville *et al.* 2004; Pepper *et al.* 2006; Oliver *et al*. 2007), they still remain one of the more poorly understood radiations of Australian lizards. The phylogeny of the Pygopodidae is best understood due to a relatively recent phylogenetic study which included morphology and three genes (c-mos, ND2 and 16S), however even this work failed to strongly resolve most intergeneric relationships (Jennings *et al.* 2003). Intergeneric relationships in the Diplodactylidae and Carphodactylidae have not yet been examined in any detail, and a complete generic level phylogeny for the radiation based on slowly evolving nuclear genes has also not been published. Despite the widespread use of suitable molecular loci in other squamates, tissue collections and techniques, there are also no published species-level phylogenetic analyses for diverse genera that together

include nearly half of the recognised species diversity within Australia: most notably *Crenadactylus*, *Diplodactylus*, *Nephrurus* and *Oedura*.

Taxonomic investigations of several genera of pygopodoid geckos have also revealed numerous unrecognised cryptic species, and it seems likely that actual species diversity is far higher than currently recognised, both within Australia and extralimitally (Aplin and Adams 1998; Pepper *et al*. 2006; Bauer *et al*. 2006; Oliver *et al*. 2007; Couper *et al.* 2008a). The leaf-tail geckos of mesic eastern Australia provide the most spectacular example, in the last two decades 12 species and two new genera have been recognised (Couper *et al*. 1993, 1997, 2000, 2008a,b; Hoskin *et al.* 2003). Many of these species are extremely similar in external appearance and were only identified through the application of molecular techniques; indeed this radiation includes the first Australian reptile species diagnosed solely on molecular data (*Saltuarius wyberba*) (Couper *et al*. 1997). There seems no reason to assume that similar levels of diversity may not be contained within a number other widespread genera that have received little recent systematic attention, for instance *Crenadactylus*, *Diplodactylus* and *Oedura*.

1.4 Historical Biogeography of Australian squamates

Based on an extensive body of paleoclimatic, geological and phylogenetic data, it is widely accepted that Australian historical biogeography since the Oligo-Miocene has been dominated by two major processes 1) the ongoing and increasingly frequent invasion and subsequent radiation of novel lineages, particularly from the north as the Australian plate has migrated towards Asia (Cogger and Heatwole 1981; Keast 1981; Heatwole 1987; Hall 2001), and 2) the increasing extent and intensity of arid conditions (Bowler 1982; Martin 2006; Byrne *et al.* 2008). While the importance of these two processes on the biota has been accepted for many decades, the absence of a sound, dated phylogenetic framework has again impeded understanding of the tempo and pattern of evolutionary responses.

1.4.1 Geographic and temporal origins

Molecular dating has revolutionised our understanding of the relative ages and origins of some major Australian squamate radiations. Published data for the dragons (agamids) and venomous snakes (elapids) strongly support the contention that they are relatively recent Miocene radiations that colonised from the north after Australia had separated from Antarctica (Hugall and Lee 2004; Hugall *et al*. 2008; Sanders *et al*. 2008). While they have not been the foci of well-calibrated dating studies, current data also suggest that the *Sphenomorphus* group skinks, and varanids likewise colonised from the north some time during the Miocene (Reeder 2003; Hugall and Lee 2004; Skinner 2007). Unfortunately published data for the two remaining skink groups and the blindsnakes are few, and it is difficult to confidently assess both the timing of radiation and the origin of these groups, although work on each of these radiations is underway (A Skinner, S Donnellan pers. com.).

In striking contrast, both the distribution of lineages and a number of preliminary phylogenetic dating studies strongly suggest that the pygopodoids are a relatively ancient component of the Australasian fauna that has persisted in the region since well before the separation of Australia and Antarctica (Cogger and Heatwole 1981; King 1987; Gamble *et al.* 2008a). A number of recent phylogenetic studies have also estimated divergence dates for clades within this group that extend to well before the Miocene (Jennings *et al.* 2003; Pepper *et al*. 2006; Oliver *et al.* 2007). These data suggest that deeper nodes within the pygopodoids might significantly pre-date most other Australian lineages of squamates, and may be of equivalent antiquity to famously endemic vertebrate groups such as the marsupials, Australasian passeriform birds and myobatrachid frogs (Barker *et al*. 2004; Roelants *et al.* 2007; Beck 2008). However, no comprehensive modern molecular attempt has been made to estimate the number and age of deeply divergent lineages within the Pygopodoidea.

1.4.2 Aridification

Since its final separation from Antarctica in the late the Oligocene, the Australian continent has also undergone a profound climatic change; from predominantly mesic to predominantly arid (Bowler 1982; White 1994; Byrne *et al*. 2008). Based a suite of

different data a broad timeline for the onset and spread of aridification within Australia has been proposed (Martin 2006; Byrne *et al.* 2008). It is hypothesised that arid conditions, and at least some arid lineages, date back to at least the mid Miocene and potentially much earlier, and that the late Miocene (10-6) Myr was a time of significant diversification amongst many lineages which now populate the arid zone. It is also predicted that as the arid zone is a younger habitat, much of its diversity will be derived from ancestors in more mesic biomes.

Squamates (and especially lizards) are the dominant terrestrial vertebrates in the Australian arid zone, and are a key group for understanding the history of the Australian arid biome and its biota. A significant component of diversity in all Australian squamate families is currently found in arid and semi-arid climates. At least one study has also found evidence for a significant upturn in rates of diversification within one Australian lizard clade that may be associated with successful adaptation to expanding arid conditions (Rabosky *et al.* 2007). However as many Australian squamate groups apparently colonised the continent during the Miocene, it may be difficult to separate the effects of increasing aridity on diversification, from elevated rates of speciation and evolutionary change (Schulter 2000) immediately following colonisation of Australia as a whole. This caveat is especially relevant to the potential timing of major aridification in the early to mid Miocene, which overlaps with the putative timing of arrival for many immigrant groups.

The likely ancient, Gondwanan ancestry of the Pygopodoid geckos suggests they offer a valuable phylogenetic contrast to many other major extant groups of Australian squamates (which have recent, northern origins). At least some lineages in all three families occur in the arid zone and have adapted successfully to this new and challenging biome. If these lineages have been present within Australia since before the break-up of east Gondwana, patterns of diversification in the Miocene are unlikely to be confounded by this colonisation effect, and are more likely to be attributable to extrinsic abiotic factors associated with environmental change. The existence of at least three evolutionarily divergent and putatively relatively ancient lineages within the pygopodoids (the three recognised families) also provides a unique opportunity to compare patterns of diversification across ecologically diverse lineages with ancient Gondwanan origins.

1.5 The aims of the thesis

The overall objective of the work in this thesis was to examine the systematics, diversity and evolutionary history of Australian pygopodoids at various hierarchical levels, with specific reference to historical patterns of diversification, and the effects of aridification since the late Oligocene/Miocene. Within this broader framework, the research consisted of a series of smaller aims.

Aim 1. Determine the phylogenetic relationships, pattern and timing of diversification between and within the three families of Pygopodoidea using slowly evolving nuclear loci and recently developed techniques for Bayesian estimation of divergence dates.

Aim 2. Use a combination of genetic loci and other techniques, including anatomy, to examine interspecific and generic relationships in the historically problematic and potentially non-monophyletic genera *Diplodactylus* and *Nephrurus*.

Aim 3. Complete a comprehensive assessment of levels of cryptic species diversity within the genera *Crenadactylus* and *Diplodactylus*, using a combination of complementary molecular techniques to identify historically divergent lineages (DNA sequencing) and genetically cohesive (allozymes) populations (i.e species).

Aim 4. Use the data gathered towards aims 1-3 to examine for both concerted and/or idiosyncratic patterns of diversification or evolutionary change within the pygopodoid geckos, and whether these patterns correlate with major changes in the Australian environment since the late Oligocene/Miocene, especially aridification.

1.6 Thesis structure

The main body of this thesis comprises five papers that have either been published or have been submitted for publication. They are presented in the format of the relevant journal preceded by a title page and statements of authorship. Supplementary information

is provided at the end of each chapter. A final chapter presents a synthesis of my work, highlighting both significant advances in our knowledge and obvious areas for further research.

The appendices comprise five published papers resulting from work done concomitantly with the research presented herein. I was senior author on three of these, and contributed significantly to the remaining two. All pertain to the systematics of Australasian geckos. Appendices 1-3 are descriptions of new or poorly known Melanesian geckos in the genera *Cyrtodactylus* and *Gehyra*. Both genera are also important components of the Australian fauna, and improved resolution of species diversity is important to understanding their historical biogeography.

Appendix 4 is the description of the first of many new Australia geckos in the genus *Diplodactylus* identified and characterised as part of this work. This paper demonstrates how independent data sources (allozymes, mitochondrial DNA and morphology) may be employed to delineate species boundaries in problematic groups.

Appendix 5 presents a combined morphological and genetic analysis of the relationships of a problematic, but important pygopodid fossil '*Pygopus' hortulanus* (Hutchinson 1997). While clearly a pygopodid, the relationships of this fossil to extant pygopodids are found to be difficult to resolve; indicating that the error for age estimates associated with this fossil is far higher than has been widely recognised. This is likely to be a problem for many dating analyses, which uncritically and without explicit analysis use fossils to constrain the age of nodes in phylogenetic trees.

A comment on terminology

The name Pygopodoidea, for the clade containing all three families of gecko under study here, was proposed only recently (Vidal and Hedges 2009). Reflecting this, in some chapters of this thesis that were written prior to this publication, I used the term diplodactyloids to informally refer to this clade. In all work done subsequent to 2008 (i.e. Chapters 1 and 5-7) I refer to this clade as the pygopodoids or Pygopodoidea.

CHAPTER 2

Molecular evidence for Gondwanan origins of multiple lineages within a diverse Australasian gecko radiation.

Oliver, P.M[1,2], Sanders KL[1]

1. Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide.

2. Vertebrates, South Australian Museum, North Terrace, Adelaide, SA Australia.

Journal of Biogeography (2009), 36: 2044-2055.

NOTE:
Statements of authorship appear in the print copy of the thesis held in the University of Adelaide Library.

Oliver, P.M. & Sanders, K.L. (2009) Molecular evidence for Gondwanan origins of multiple lineages within a diverse Australasian gecko radiation
Journal of Biogeography, v. 36(11), pp. 2044-2055

CHAPTER 3

Phylogenetic relationships in the lizard genus *Diplodactylus* Gray, 1832, and resurrection of *Lucasium* Wermuth, 1965 (Gekkota, Diplodactylidae).

P.M. Oliver[1,2], M.N. Hutchinson[1,2], S.J.B. Cooper[1,2]

1. Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide.

2. Vertebrates, South Australian Museum, North Terrace, Adelaide, SA Australia.

Australian Journal of Zoology (2007), 55: 197-210.

Oliver, P.M., Hutchinson, M.N. & Cooper, S.J.B. (2007) Phylogenetic relationships in the lizard genus *Diplodactylus* Gray and resurrection of *Lucasium* Wermuth (Gekkota, Diplodactylidae).
Australian Journal of Zoology, v. 55(3), pp. 197-210

CHAPTER 4

The taxonomic impediment in vertebrates: DNA sequence, allozyme and chromosomal data double estimates of species diversity in a lineage of Australian lizards (*Diplodactylus*, Gekkota).

P.M. Oliver[1,2], M. Adams [2], M.S.Y. Lee[1,2], M.N. Hutchinson[1,2], P. Doughty[3]

1. Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide.

2. Vertebrates, South Australian Museum, North Terrace, Adelaide, SA Australia.

3. Herpetology, Western Australian Museum, Perth, Western Australia 6000, Australia.

Proceedings of the Royal Society London: Biological Sciences (2009) 276: 2001-2007.

Oliver, P.M., Adams, M., Lee, M.S.Y., Hutchinson, M.N. & Doughty, P. (2009) The taxonomic impediment in vertebrates: DNA sequence, allozyme and chromosomal data double estimates of species diversity in a lineage of Australian Lizards (Diplodactylus, Gekkota).
Proceedings of the Royal Society London: Biological Sciences, v. 276(1664), pp. 2001-2007

Molecular evidence for ten species and Oligo-Miocene vicariance within a nominal Australian gecko species (*Crenadactylus ocellatus*, Diplodactylidae)

Paul M. Oliver [1,2*], Mark Adams [3], Paul Doughty [4]

[1] Australian Centre for Evolutionary Biology and Biodiversity, The University of Adelaide, Darling Building, Adelaide SA 5005, Australia and [2] South Australian Museum, Adelaide, SA 5000, Australia

[3] Evolutionary Biology, South Australian Museum, Adelaide, SA 5000, Australia

[4] Terrestrial Zoology, Western Australian Museum, 49 Kew St, Welshpool WA 6106, Australia

[*]Corresponding author

Email address

Paul Oliver - [*]paul.oliver@adelaide.edu.au

Mark Adams - Mark.Adams@samuseum.sa.gov.au

Paul Doughty - Paul.Doughty@museum.wa.gov.au

Abstract

Background

Molecular studies have revealed that many putative ‘species’ are actually complexes of multiple morphologically conservative, but genetically divergent 'cryptic species'. In extreme cases processes such as non-adaptive diversification (speciation without divergent selection) could mask the existence of ancient lineages as divergent as ecologically and morphologically diverse radiations recognised as genera or even families in related groups. The identification of such ancient, but cryptic, lineages has potentially important ramifications for conservation, biogeography and evolutionary biology. Herein, we use an integrated multilocus genetic dataset (allozymes, mtDNA and nuclear DNA) to test whether disjunct populations of the widespread nominal Australian gecko species *Crenadactylus ocellatus* include distinct evolutionary lineages (species), and to examine the timing of diversification amongst these populations.

Results

We identify at least 10 deeply divergent lineages within the single recognised species *Crenadactylus ocellatus*, including a radiation of five endemic to the Kimberley region of north-west Australia, and at least four known from areas of less than 100 square kilometres. Lineages restricted to geographically isolated ranges and semi-arid areas across central and western Australia are estimated to have began to diversify in the late Oligocene/early Miocence (~20–30 Mya), concurrent with, or even pre-dating, radiations of many iconic, broadly sympatric and much more species-rich Australian vertebrate families (e.g. venomous snakes, dragon lizards and kangaroos).

Conclusions

Instead of a single species, *Crenadactylus* is a surprisingly speciose and ancient vertebrate radiation. Based on their deep divergence and no evidence of recent gene flow we recognise each of the ten main lineages as candidate species. Molecular dating indicates that the genus includes some of the oldest vertebrate lineages confounded within a single species yet identified by molecular assessments of diversity. Highly divergent allopatric lineages are restricted to putative refugia across arid and semi-arid Australia, and provide important evidence towards understanding the history and spread of the Australian arid zone, suggesting at a minimum that semi-arid conditions were present by the early Miocene, and that severe aridity was widespread by the mid to late Miocene. In addition to documenting a remarkable instance of underestimation of vertebrate species diversity in a developed country, these results suggest that increasing integration of molecular dating techniques into cryptic species delimitation will reveal further instances where the taxonomic impediment has led to profound underestimation of not only species numbers, but also highly significant phylogenetic diversity and evolutionary history.

Background

Whereas traditional field and morphological studies continue to discover new species [1], complexes of phenotypically similar unrecognised taxa are now increasingly identified through molecular systematic examination of 'known' taxa [2, 3, 4]. Documenting this wealth of ‘cryptic species’ (two or more morphologically similar, but not necessarily identical, species confounded within one) is a priority of modern systematic research [5]. All species, however, are not equal: their phylogenetic distinctiveness (i.e. evolutionary distance from nearest living relatives) can vary enormously [6, 7, 8]. Many clades are characterised by relative morphological stasis over very long time periods [9]; within such groups, 'cryptic species' might be divergent lineages as ancient as ecologically diverse nominal "genera" or even "families" of more morphologically variable clades [9, 10]. Identifying such ancient cryptic diversity is likely to provide important insights into biogeographic history and processes of morphological stasis, and is essential for the effective allocation of conservation resources to preserve the maximal breadth of evolutionary diversity [5]. Nonetheless, even though the techniques are readily available, cryptic species assessments have not systematically integrated techniques such as internally calibrated molecular dating to assess the phylogenetic diversity [6, 7] of newly identified taxa.

Pygopodoid (formerly diplodactyloid or diplodactylid) geckos are a Gondwanan radiation of lizards restricted to Australia and surrounding islands [11, 12]. A recent molecular phylogenetic study of the pygopodoids, found the monotypic genus *Crenadactylus* to be among the most divergent extant lineages [12]. The single nominal species in the genus, *Crenadactylus ocellatus* is a secretive scansorial lizard, Australia's

smallest gecko species (< 59 mm snout-vent length), and broadly distributed across isolated patches in the west, centre and north of Australia [13]. Two papers have examined the taxonomy of this species over the last three decades and four subspecies are now recognised [14, 15]. A more recent molecular study revealed very deep genetic divergences between these nominal subspecies [12]; and at least one recognised subspecies (*C. o. horni*) also spans multiple deeply isolated and disjunct biogeographic regions [13], suggesting the genus may harbour additional species level diversity.

Crenadactylus are rarely collected over much of their range, many northern populations are known from very few sites and poorly represented in museum collections, and it is only through recent extensive fieldwork that sufficient samples have become available for a comprehensive genetic analysis. In this study we used independent mitochondrial (*ND2*) and nuclear (*RAG1*, *C-mos*, allozymes) loci to estimate specific and phylogenetic diversity within the nominal species '*Crenadactylus ocellatus*' from localities spanning its wide range across arid and semi-arid Australia. Populations for which there was congruent evidence of lack of gene flow and historical independence (fixed allozyme differences and relatively high mtDNA divergence and monophyly) were regarded to represent candidate species (see methodology outlined in detail elsewhere [4]). This new sampling and data revealed a striking instance of severe underestimation of phylogenetic diversity, with important ramifications for both conservation, and understanding the environmental history of Australia.

Results

Species diversity and distributions

An initial Principal Co-ordinates Analysis (PCO) of allozyme data for all 94 individuals (Figure 1A) revealed the presence of six primary clusters, one for each of six different geographic regions: South West, Carnarvon Basin, Cape Range, Pilbara, Kimberley, and Central Ranges. Each cluster was diagnosable from all others by 6–19 fixed differences, supporting their status as distinct taxonomic entities. Follow-up PCOs on each cluster found only modest within-group heterogeneity (i.e. no obvious subgroups, or subgroups differing by less than three fixed differences) in all but one regional cluster, namely that representing the Kimberley specimens. Here, PCO identified five genetically distinctive subgroups (Kimberley A-E; Figure 1B), each differing from one another by 4–14 fixed differences, and all characterized by "private" alleles at one or more of the loci (displaying fixed differences (range = 1–4 loci; Table S4). A final round of PCOs on subgroups Kimberley B and Kimberley E (the only two Kimberley lineages represented by more than one specimen) did not reveal any obvious genetic subdivision (Additional file 1, Tables S1 and S2**)**.

Bayesian and maximum likelihood phylogenetic analyses of nuclear and mitochondrial data identified these same ten groups as both deeply divergent lineages (Additional file 1, Table S1) and reciprocally monophyletic where multiple samples were available (Figure 2a,b). Minimum corrected and uncorrected pairwise (mitochondrial) genetic divergences between candidate species (> 22.1/15.3%) were much higher than maximum distances within candidate species (< 11.6/9.7%) (see Additional file 1, Tables S3 and S4, respectively), further emphasising their long periods of historical isolation.

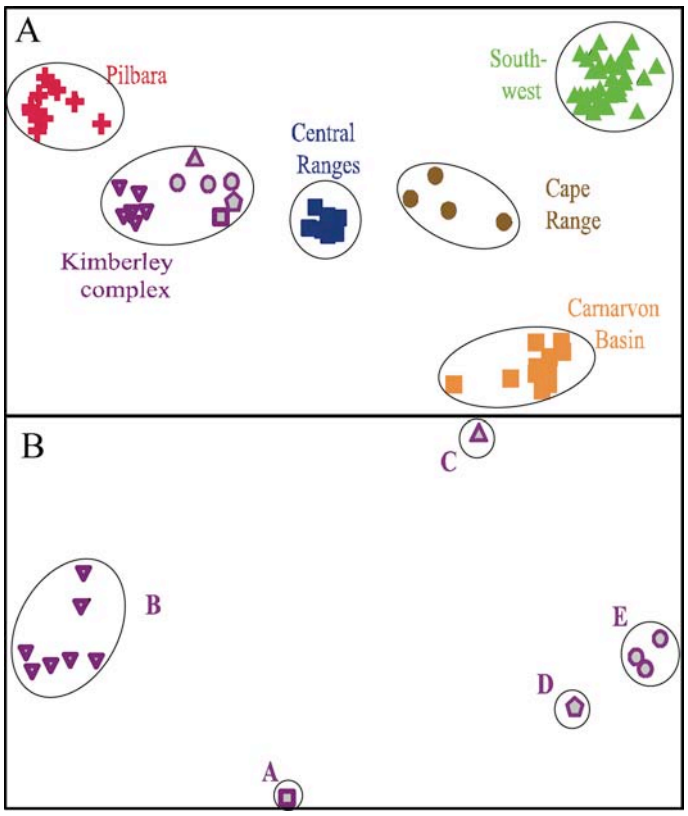

Figure 1. Allozyme data for *Crenadactylus*

Selected Principal Co-ordinates Analyses, based on the allozyme data. The relative PCO scores have been plotted for the first (X-axis) and second (Y-axis) dimensions. (*A*) PCO of all 94 *Crenadactylus*. The first and second PCO dimensions individually explained 30% and 16% respectively of the total multivariate variation. (*B*) PCO of the 13 Kimberley *Crenadactylus*. The first and second PCO dimensions individually explained 51% and 11% respectively of the total multivariate variation.

Based on both independent and combined analysis of mitochondrial and nuclear sequence data (Figure 2, Additional file 2) the basal dichotomy within *Crenadactylus* was between a south/western clade (three major lineages) and a north/central clade (seven major lineages). The south/western clade included three parapatric lineages, two endemic to the Cape Range area and Carnarvon coast respectively, and a more deeply divergent lineage widespread throughout the southwest of Western Australia. The north/central clade comprised an endemic radiation of five allopatric lineages from the Kimberley

(northern Western Australia), and a pair of sister taxa from the Pilbara region and the Central Ranges (Figures 2b,d). Allopatric populations within the north/central clade are largely restricted to rocky ranges and showed high levels of geographically structured mtDNA diversity, while the two widespread taxa in the south/western clade were not restricted to ranges, and were characterised by very low levels of mtDNA divergence across their distribution, suggestive of significant recent gene flow or range expansion (Additional file 1, Table 4).

Divergence dating and age of cryptic radiation

Topology and node support for the pygopodoid phylogeny recovered by the dating analyses was consistent across nuclear and combined datasets, and with similar datasets presented elsewhere [12]. The 95% height intervals for all age estimates were relatively wide (Table 1), due to our explicit incorporation of calibration error. Using the estimated age of *Crenadactylus* from the nuclear and combined analysis as secondary prior, the 95% CI for the estimated mean rate of mitochondrial sequence evolution per lineage per million years within *Crenadactylus* was between 0.96–2.24% (nuclear calibrations) to 0.72–1.76% (combined calibrations), broadly consistent with published estimates of rates from other squamate groups (0.47–1.32% per lineage per million years) [16].

Actual and relative age estimates for the four major clades of pygopodoids (C, D, E, F (see methods)) were broadly similar (Figure 1, Table 1). However, the estimated age of crown *Crenadactylus*, and the relative age of this radiation against the other three major related Australian pygopodoid gecko radiations were significantly older when using combined data as opposed the nuclear data alone (Table 1). Saturation of the

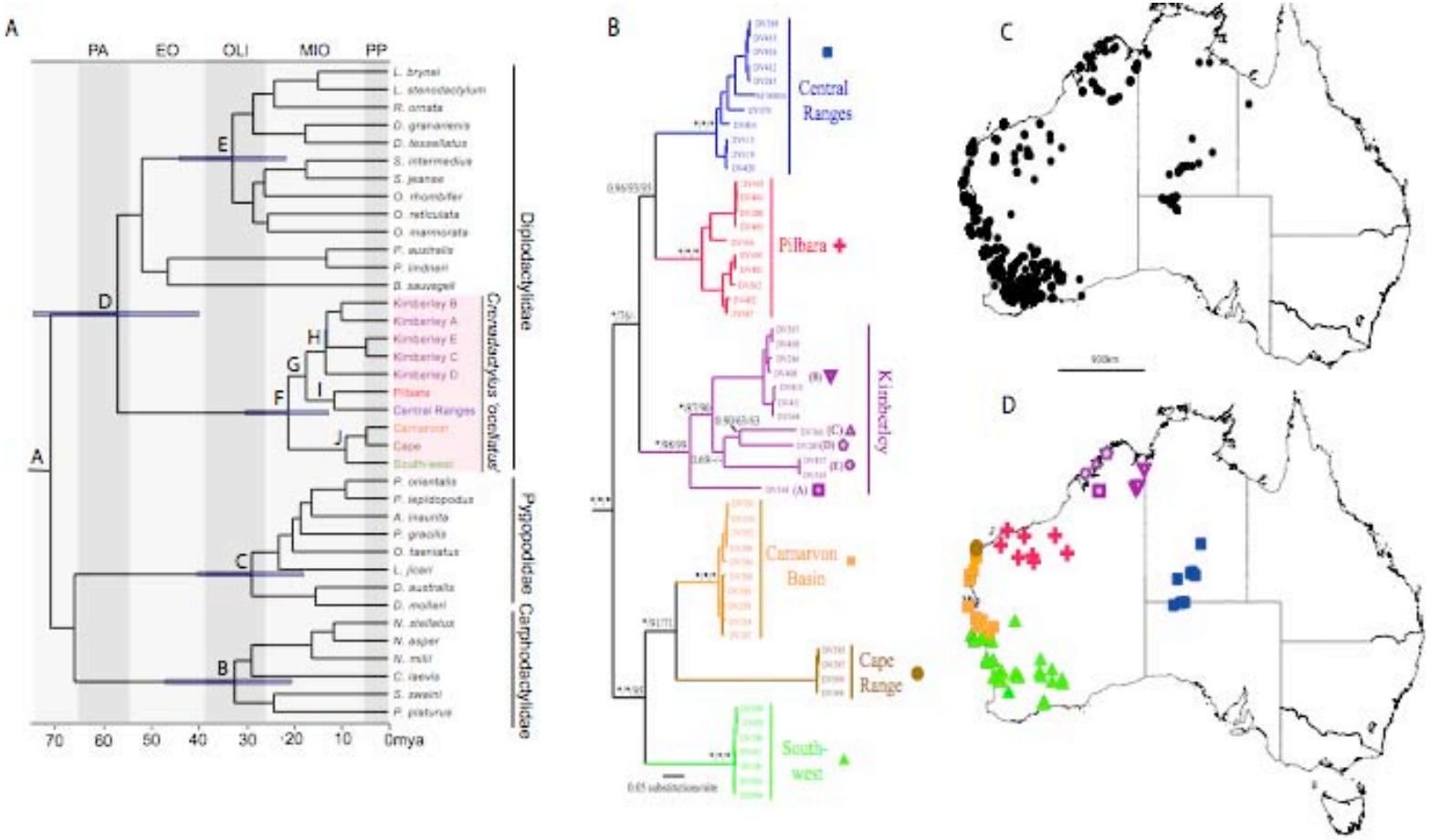

Figure 2. Phylogeny and distribution of *Crenadactylus*

(*A*) Bayesian chronogram showing estimated age of ten candidate species of *Crenadactylus* and exemplars of major lineages of pygopoids based on concatenated nuclear dataset. Letters at major nodes correspond with those in Table 1. (*B*) Bayesian consensus tree from ND2 data showing structure and relationships between ten candidate taxa of *Crenadactylus* with Bayesian, ML and MP support values for key nodes. (*C*) Known localities of *Crenadactylus* based on Australian Museum voucher specimens. (*D*) Localities and nominal taxonomic designation for each genetically typed specimen included in our analyses.

mitochondrial component of the combined data, and/or stochastic error given the relatively few substitutions in the nuclear dataset may explain this discrepancy. The older dates from combined datasets are viewed as a potential maximum while the younger dates from the nuclear data are viewed as a conservative minimum. Nuclear data suggest that the initial diversification of crown *Crenadactylus* occurred in the late Oligocene to early Miocene (10–30 million years ago (mya)), and that it is probably slightly younger, but nonetheless broadly concurrent with diversification in the other three major Australian clades of Pygopodoidea (Table 1). If the combined analysis is more correct than the nuclear only analysis it would indicate that crown *Crenadactylus* is significantly older (i.e. late Oligocene 20–40 mya). Both datasets indicate that the four major geographic isolates of *Crenadactylus* (Western/South-west, Central Ranges, Pilbara and Kimberley) had all diverged by the late Miocene, approximately 10 mya.

Table 1: Bayesian age estimates.

Comparison of mean and range (95% posterior density distribution) of divergence time estimates for selected outgroup and *Crenadactylus* nodes based on Bayesian dating analyses (BEAST) of three different sets of alignment data. Age estimates are in millions of years and letters alongside major splits correspond with labels in Fig. 2a.

	nuclear	Combined	combined no 3rds
Posteriors			
Outgroups			
Root	113.9 (82.7-145.2)	113.3 (81.5-142.8)	114.5 (84.3-145.7)
(A) Pygopoidea	69.3 (51.0-89.4)	65.4 (47.0-83.6)	67 (48.0-85.1)
(B) Carphodactylidae	31.5 (19.9-36.7)	39.7 (27.2-54.5)	36.7 (23.9-50.3)
(C) Pygopodidae	28 (17.5-39.2)	28.2 (19.2-38.2)	26.2 (17.2-35.6)
(D) Diplodactylidae	55.6 (38.9-72.9)	56.2 (40.8-73.3)	56.4 (39.2-72.8)
(E) Core Diplodactylidae	32 (21.0-42.9)	37.1 (26.5-49.4)	34.8 (23.2-46.4)
Crenadactylus			
(F) Crown	20.5 (12.3-29.3)	31.5 (21.7-41.9)	30.7 (20.6-41.4)
(G) Northern	16.9 (9.9-24.0)	27 (18.5-36.4)	25.9 (17.2-35.6)
(H) Kimberley	12.9 (7.1-19.3)	19.9 (13.3-27.3)	18.2 (11.5-25.4)
(I) Pilbara/Central Ranges	11.1 (4.3-17.3)	21 (13.0-30.0)	18.8 (9.8-27.9)
(J) Southern	8.7 (3.4-14.5)	23.1 (15.2-32.2)	21.5 (13.2-30.7)
Calibrations			
Root	uniform 80-150	uniform 80-150	uniform 80-150
Pygopoidea	normal 71.5 (12.5)	normal 71.5 (12.5)	normal 71.5 (12.5)

Discussion

Cryptic species diversity and conservation

Based on the high levels of uncorrected mtDNA divergence (> 15%; even higher if corrected), multiple fixed allozyme differences, reciprocal mtDNA monophyly and deep divergence times we estimate that at least ten lineages of *Crenadactylus*are evolutionarly

divergent, non-interbreeding and warrant recognition as candidate species. Many of these lineages are further defined by multiple nuclear differences. A full taxonomic revision of the genus is currently in preparation, *Crenadactylus ocellatus* will be restricted to the south-west population, the three other recognised subspecies will be elevated to full species, and additional new species will be described. Ongoing analysis and published data also suggests that at least some of these taxa are morphologically diagnosable on the basis of subtle features of scalation and colouration.

Our estimate of total species diversity is almost certainly conservative for several reasons. At least five candidate species are potential short-range endemics [17] (Cape Range, Kimberley A, C, D, and E): thus, *Crenadactylus* lineages have clearly persisted and speciated in relatively small patches of suitable habitat. This would indicate that known and geographically isolated, but genetically unsampled, populations of *Crenadactylus* from the Kimberley and around the Queensland/Northern Territory border (Figure 2C) may include additional unrecognised taxa. *Crenadactylus* are secretive, rare, and difficult to collect (for example four of the five Kimberley taxa were each represented by only a single site in this study), and as large areas across northern and central Australia have not been intensively surveyed, it seems likely that additional populations (potential species) remain undetected. Finally, maximum levels of genetic diversity within the Central Ranges and Pilbara candidate species are moderately high (7.9–9.7% uncorrected), and further work may reveal that these candidate species each comprise complexes of multiple cryptic taxa.

The identification of a clade of five candidate species within the Kimberley region of north-west Australia is also notable. Whereas morphological work has identified

micro-endemic allopatric radiations of species within some Kimberley invertebrate lineages [18], this is the first genetic evidence for moderately extensive *in situ* speciation within the region, and the only documented evidence of a moderately diverse (> 3 species) endemic vertebrate radiation. Few other areas of similar size within Australia contain comparably diverse endemic vertebrate radiations, (examples include the wet tropics (microhylid frogs: *Cophixalus*) and Tasmania (skinks: *Niveoscincus*)) [19, 20]. The results of this and a growing body of work emphasises the biogeographic importance, environmental complexity, high endemism and phylogenetic diversity of the rugged and poorly known Kimberley [21].

Most candidate species of *Crenadactylus* are from areas of low human impact, however such restricted range taxa with potentially narrow climatic tolerances are particularly vulnerable to rapid anthropogenic climate change [22]. The diversity we have uncovered within *Crenadactylus* underlines how an overly conservative taxonomy and patchy sampling may obscure the existence of range-restricted taxa at potentially high risk of extinction. Northern Australia remains relatively poorly sampled, and ongoing studies indicate it probably remains one of the largest remaining frontier areas for modern systematic research in a developed coun try [4, 23]. In light of unprecedented global environmental changes and the apparently high levels of endemism within this area, systematic surveys and genetic assessments of diversity to address this oversight should be a high priority. If not, there is a risk that many deeply divergent, but morphologically conservative lineages will disappear before they are even documented.

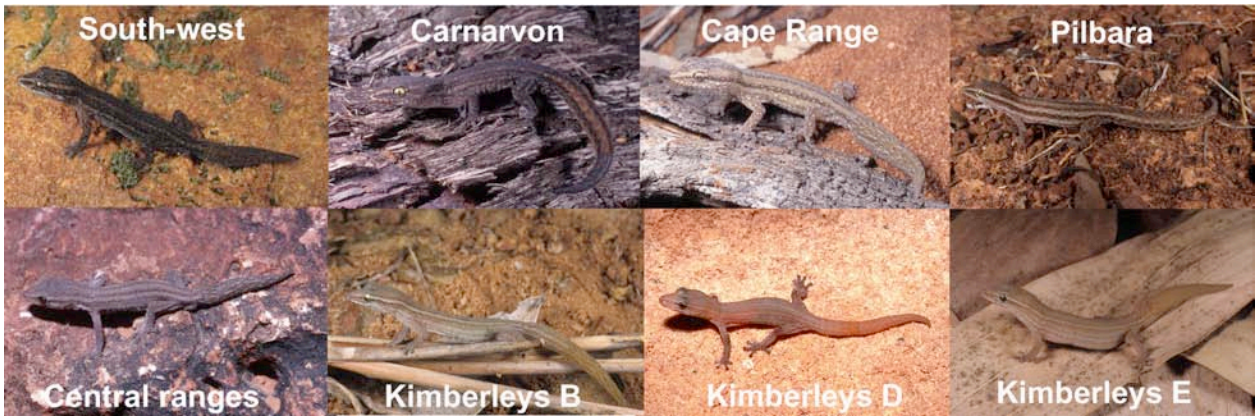

Figure 3. Candidate species of *Crenadactylus*

In life pictures of eight of the ten candidate species currently confounded within the nominal species *Crenadactylus ocellatus*. Photos courtesy Brad Maryan, Glenn Gaikhorst, Glenn Shea.

Divergence dates

Based on our secondary calibrations, the initial diversification of lineages currently confounded within '*Crenadactylus ocellatus*' probably began in the mid to early Miocene (~20 mya) and potentially at least 10 million years earlier. One recognised subspecies of *Crenadactylus* (*C. o. horni*) includes four candidate species (Carnarvon, Cape Range, Pilbara, and Central Ranges) that span the basal divergence of the genus (Figure 2a, b). None of these lineages have been formally recognised or named and they satisfy the definition for cryptic species we are following herein. Given that rigorous molecular dating with direct calibrations is rarely integrated into assessment of cryptic species diversity, it remains to be seen how common such deeply-divergent cryptic lineages are. However, the divergence times between these unrecognised species of *Crenadactylus* are amongst the oldest documented for any cryptic species of tetrapod, and comparable with the oldest cryptic species identified in vertebrates [24], subterranean amphipods [25], and perhaps exceeded only by copepods [10]. In contrast to these studies, our date estimates

are also based on internally calibrated trees, as opposed to generalised and often unreliable global estimates for rates of sequence evolution [26].

The depth of divergences among candidate species of *Crenadactylus* (and within other pygopodoid genera such as *Diplodactylus*, *Lucasium* and *Salturius* [27, 28, 29] are comparable with vertebrate radiations noted for their extreme morphological conservatism (e.g. *Plethodon* salamanders) [30]. The long-term conservatism of these cryptic radiations of geckos is particularly striking in light of the major environmental changes they have experienced (see below) and the great morphological plasticity of related lineages such as the legless lizards (Pygopodidae)[15].

Our date estimates indicate the extensive evolutionary diversity hidden within the nominal species '*Crenadactylus ocellatus*' is as ancient as ecologically diverse crown radiations of many iconic endemic Australian groups: terrestrial venomous snakes (~10 mya, 102+ spp, 26 genera) [31], agamid lizards (~23 mya, 71+ spp, 13 genera) [32], most macropods (~20 mya, 70+ spp, 14 genera) [33] and murine rodents (early Pliocene, ~5 mya 160+ spp, 31 genera) [34]. Likewise, while allozyme data cannot provide reliable divergence dates [35], levels of allozyme genetic divergence found within *Crenadactylus* (mean = 36.7 %FD, range 10–52 %FD; Additional file 1, Table S1) are similar to the entire Australian radiation (17 spp, 5genera) [15] of ‘short-necked’ chelid turtles (mean 35.8 %FD, range = 0–57 %FD) [36]. In contrast to the single recognised 'species' of *Crenadactylus* these are all broadly co-distributed radiations of Australasian vertebrates that are widespread across biomes, include multiple named genera or even families, and show extensive sympatry and ecological diversity across major lineages currently afforded generic or higher rank.

Analysis of nuclear and combined datasets further indicate that initial divergence of the ancestral *Crenadactylus* lineage from other pygopodoids (as opposed to the crown radiation of extant lineages within this genus) was broadly contemporaneous with, or even pre-dated, initial diversification of iconic Gondwanan Australasian clades such as the basal oscine birds [37], most major Australasian marsupial families [38], pelodryad treefrogs [39], many major lineages of the Proteaceae [40], and *Nothofagus* 41]. The divergence of the *Crenadactylus* lineage from other extant geckos also pre-dates current estimates for the initial radiation of most other extant squamate (lizard and snake) families in Australia by at least 10–20 million years [12]. The only comparably divergent Australian squamate genus identified to date are the cave geckos (*Pseudothecadactylus*), however this lineage appears to be (at least distantly) related to an extralimital radiation of geckos in New Caledonia [12]. Thus, *Crenadactylus* is not only unexpectedly diverse, but also the only surviving representative of a relatively ancient lineage. Indeed current evidence indicates that it is the most phylogenetically divergent endemic genus in the diverse Australian squamate fauna of over 870 spp and 115 genera [15].

Ancient vicars across the Australian arid zone

Crenadactylus are among Australia's smallest terrestrial vertebrates. While small body size increases vulnerability to environmental conditions, it also allows access to micro-refugia inaccessible to larger vertebrates [42]. As with other lineages showing outward morphological conservatism over long timescales, an absence of major morphological differentiation since the early Miocene also suggests a relatively constrained ecology [9]. Each of the four major geographic clusters of *Crenadactylus* sampled (Kimberley,

Central Ranges, Pilbara and South-west/Carnarvon/Cape Range) are allopatric and restricted to relatively temperate semi-arid or rocky areas, separated by expanses of arid desert. These geographically isolated and deeply divergent lineages of *Crenadactylus* appear to be relatively ancient relics of a former much wider distribution, now greatly attenuated by the expansion of severe aridity.

Dated phylogenies for many major Australian vertebrate and faunal radiations are now available, and all generally indicate the fauna of the arid zone (the largest biome in Australia and one of the largest arid landforms in the world) is the result of a complete turnover since the estimated onset of aridification around 20 mya, and that most endemic lineages are significantly younger than 20mya [43]. Thus far *Crenadactylus* is the only vertebrate lineage showing strong evidence for a contrasting pattern of the persistence of multiple lineages that pre-date the estimated onset of severe aridification in refugia, both around and *within* the arid zone. Indeed, they are currently the oldest known allopatric sister lineages of Australian vertebrates restricted to isolated ranges and relatively mesic coastal pockets through the semiarid to arid west, centre and north of Australia.

Crenadactylus thus span both the geographic extent and temporal origins of the arid zone, but do not seem to have adapted to it. Like the relatively few other ancient relict lineages present (e.g stygobiontic beetles) [44], this pattern provides rare insights into the spread of aridity. In this case, the timing of diversification of *Crenadactylus* lineages supports the suggestion that semi-arid/seasonally arid conditions (to which the lineage is restricted) date back to at least the mid-Miocene (the basal split within the crown radiation), and that severe aridity dates back to the late Miocene (the oldest splits between multiple major lineages which are now geographically isolated by very arid

desert). Age estimates for the separation of multiple, geographically-isolated candidate species in *Crenadactylus* also provide perhaps the strongest phylogenetic support yet for the hypothesis that significantly arid conditions were already widespread across west and central Australia in the 'Hill gap', 6–10 mya [43], a period where depositional records are poor, making it difficult to assess historical Australian climates.

Conclusion

Our data have revealed that the single nominal species *'Crenadactylus ocellatus'* comprises a moderately diverse and surprisingly ancient complex of numerous unrecognised and highly divergent lineages. The distribution and antiquity of these lineages suggests that with further work incorporating additional sampling, ecological analysis, physiological data and environmental niche modelling, *Crenadactylus* will be an important evolutionary radiation for understanding the deep history of arid Australia. More generally integration of data and techniques from diverse fields into the delimitation of species boundaries is a growing focus of taxonomic work (integrative taxonomy [45]). Our results demonstrate how integration of molecular dating techniques into cryptic species analysis can quantify the depth of phylogenetic divergences and reveal patterns of great evolutionary interest and conservation significance within lineages showing outward morphological conservatism.

Methods

Sampling

Ninety-five *Crenadactylus* specimens were sampled for genetic analysis. Allozyme profiles were successfully scored for 94 individuals and a representative subset of these (N = 53) were sequenced for the *ND2* gene (Additional file 1, Table S5). Based on the results of mitochondrial and allozyme analysis we obtained nuclear data (*RAG1*) for exemplars of the ten most divergent lineages of *Crenadactylus*. For dating analyses we also incorporated published *C-mos* data for three representative deep lineages spanning crown *Crenadactylus* [12]. Outgroups (Additional file 1, Table S6) were selected from published diplodactylid, carphodactylid, pygopodid, gekkonid and sphaerodactylid sequences on GenBank [12].

Allozyme analyses

Allozyme analyses of liver homogenates were undertaken on cellulose acetate gels according to established procedures [46]. The final allozyme dataset (Additional file 1, Table S2) consisted of 94 *Crenadactylus* genotyped at 42 putative loci. The following enzymes displayed banding patterns of sufficient activity and resolution to permit allozymic interpretation: ACON, ACP, ACYC, ADH, AK, DIA, ENOL, EST, FDP, FUM, GAPD, GLO, GOT, GPD, GPI, GSR, IDH, LAP, LDH, MDH, MPI, NDPK, NTAK, PEPA, PEPB, PGAM, 6PGD, PGK, PGM, SOD, SORDH, TPI, and UGPP. Details of enzyme/locus abbreviations, enzyme commission numbers, electrophoretic conditions, and stain recipes are presented elsewhere [46]. Allozymes were labelled alphabetically and multiple loci, where present, were labelled numerically in order of increasing electrophoretic mobility (e.g. $Acp^{a} < Acp^{b}$; *Acon-1* < *Acon-2*).

The genetic affinities of individuals were explored using 'stepwise' Principal Co-ordinates Analysis (PCO), implemented on a pairwise matrix of Rogers' genetic distances. The rationale and methodological details of stepwise PCO are detailed elsewhere [47]. Scatterplots of PCO scores in the first two dimensions were assessed for the presence of discrete clusters of individuals which were diagnosable from all other clusters by the presence of multiple fixed differences (i.e. loci at which the two groups shared no alleles). Separate rounds of PCO were then undertaken individually on these primary groups to assess whether any group harboured additional subgroups which were also diagnosable by multiple fixed differences. Having identified groups of individuals diagnosable from one another by multiple fixed differences, two between-taxon estimates of genetic similarity were calculated; (1) percentage fixed differences (%FD; 1), allowing a cumulative 10% tolerance for any shared alleles, and (2) Nei's unbiased Distance.

DNA laboratory protocols and phylogenetic analyses

DNA extraction and amplification protocols for *ND2* and nuclear loci (*RAG1*, *C-mos*) follow those outlined elsewhere [4, 12, 28]. Newly obtained PCR products for this study were sequenced by the Australian Genome Research Facility in Adelaide using an AB3730 DNA Analyzer (Applied Biosystems) and Big Dye chemistry. New sequences were aligned and compared to pre-existing datasets, and translated to check for substitutions leading to stop codons or frameshifts using standard procedures [4, 12, 28]. Maximum Parsimony (PAUP* vb80) [48], Bayesian Inference (MrBayes v3.1.2) [49] and Maximum Likelihood (RaxML v7.0.4) [50] were used to estimate phylogenetic relationships.

The final *ND2* alignment consisted of 828 sites. All sequences could be translated into protein with no evidence of misplaced stop codons. Within the genus *Crenadactylus* 380 sites were invariable, 32 were variable but not parsimony informative, and 416 were variable and parsimony informative. The final complete nuclear alignment consisted of 2253 sites (1740 *Rag-1* and 513 *C-mos*) of which 88 sites were variable and 28 were parsimony informative within *Crenadactylus*.

We performed both individual and combined analyses for the mitochondrial and nuclear data. The mitochondrial data were partitioned into first, second and third base pair positions as previous studies using the same gene region and many of the same taxa have demonstrated this significantly improves likelihood [28]. The Akaike information criteria in MrModeltest [51] found the GTR+I+G model to have the highest likelihood for all partitions. For our nuclear alignment we did not partition by gene, (see justification given elsewhere [12]) and compared likelihood and topology for three partitioning strategies (unpartitioned; by codon; 1st with 2nds, 3rds separate). Whereas all strategies returned the same topology, likelihood support for the two partition (1st with 2nds, 3rds separate) strategy was highest. Based on the Akaike Information Criterion we used the GTR+I+G model for 1st and 2nd sites, and the GTR+G model for 3rd sites. Combined mitochondrial and nuclear analyses were partitioned by gene, but otherwise partitioned as per the non-combined analysis. As phylogenetic inference has been shown to be robust to such missing data, especially if it is evenly distributed across divergent lineages [52], the combined dataset included some individuals for which nuclear sequence data were unavailable.

Final Bayesian analyses were run for 5 million generations x 4 chains (one cold and three heated) sampling every 200 generations, with a burn-in of 20% (5,000 trees), leaving 20,000 trees for posterior analysis. In all Bayesian analyses, comparison of parallel runs showed posterior probability convergence (standard deviation <0.01) and likelihood equilibrium, were reached within the burn-in phase. The Maximum Likelihood tree was calculated using the -f d search function in RaxML v7.0.4 and Maximum Likelihood bootstrap support values were calculated using the -f i search function for one thousand replicates. We experimented with both simple and complicated models and found that topology, branch lengths and support values were effectively identical. Maximum Parsimony analyses were performed using heuristic searches with 100 random additions of sequences to identify most parsimonious trees. Bootstrap support values for nodes in MP trees were calculated using 100 pseudo replicates.

Molecular dating

Divergences date were estimated using Bayesian dating in BEAST v.1.4 [53]. Dating analyses were performed on three sets of alignment data; RAG1 nuclear data only (nuc), nuclear and mtDNA data combined (comb), and nuc and mtDNA combined with 3rd positions removed from the mtDNA dataset (comb reduced). Mitochondrial data were not analysed alone as the combination of old calibrations and high levels of saturation at this locus would generate significant overestimation of dates. Comparisons between these different analyses focused on variation in both actual and relative date estimates [54], for A) Pygopodoidea, B) Carphodactylidae, C) Pygopodidae, D) Diplodactyidae, E) core Australian Diplodactylidae (as used by Oliver and Sanders [12]), F) crown

Crenadactylus, and (G-J) major geographically isolated clades within *Crenadactylus* (Table 1, Fig. 2A).

Relaxed clock uncorrelated lognormal and GTR+I+G models were applied to all partitions and analyses. Nuclear only dating analyses were run unpartitioned, whereas combined analyses were partitioned into nuclear and mitochondrial data. After multiple initial runs to optimise parameters and priors, final BEAST analyses were run for 10,000,000 generations sampling every 1000 generations using the Yule speciation prior. Adequate sampling and likelihood stability was assessed using TRACER [53]. Two thousand trees (20%) were discarded as burn in. All BEAST runs reached independence and showed no evidence of autocorrelation for all relevant parameters (e.g. branch lengths, topology and clade posteriors).

We used secondary calibrations from two independent studies [11, 12] as broad secondary priors; basal divergences among diplodactyloids (mean 71.5 mya, 95% CI 50–90mya, normal distribution) and a uniform prior at the root of our tree (all geckos 80–150mya). The latter prior was primarily inserted to provide a broad constraint to ensure analyses never converged on unrealistic dates, and was not meant to explicitly reflect current estimates for the age of this radiation. We experimented with incorporation of a potential calibration within crown Pygopodidae, but while this fossil is clearly a pygopod, its position within the extant radiation is uncertain and it thus does not constrain dates very tightly [55], and its incorporation had negligible effect on date estimates, both within the Pygopodidae and amongst other clades (results not shown).

As an independent check of our inferred date estimates, we estimated rates of mitochondrial evolution within *Crenadactylus* using posterior age estimates from the

nuclear and two different combined analyses. A reduced mitochondrial dataset was calibrated with normal priors from reflecting the posterior age estimates for the genus, and the mean and range of rates of variation were then estimated using BEAST with settings outlined above.

Abbreviations

ND2: mitochondrial NADH dehydrogenase subunit 2; *Rag-1*: recombination activating gene 1; *C-mos*: Oocyte-maturation factor.

Authors' contributions

PO and PD conceived the study. PO and MA collected the data. PO and MA wrote the paper. All authors have read and approved the manuscript.

Acknowledgements

We thank Andrew Hugall, Kate Sanders, Adam Skinner, Steve Cooper, Gaynor Dolman and Mike Lee for advice and assistance, Brad Maryan, Claire Stevenson from the West Australian Museum for providing tissues, Ken Aplin for drawing our attention to the need for a systematic revision of *Crenadactylus*, and Brad Maryan, Glen Gaikhorst and Glenn Shea for access to photos. Funding for this project was provided by grants from the Australia Pacific Science Foundation and Australia Biological Resources Survey.

References

1. Cebellos G, Ehrlich P: **Discoveries of new mammal species and their implications for conservation and ecosystem services.** *Proc Natl Acad Sci USA* 2009, **106**: 3841-3846.

2. Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W: **Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator.*** *Proc Natl Acad Sci USA* 2004, **101**:14812-14817.

3. Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmel NJ: **Underestimation of species richness in neotropical frogs revealed by mtDNA analyses.** *Plos One* 2007, **10**: e1109,1-10.

4. Oliver PM, Doughty P, Hutchinson MN, Lee MSY, Adams A: **The taxonomic impediment in vertebrates: DNA sequence, allozyme and chromosomal data double estimates of species diversity in a lineage of Australian lizards (*Diplodactylus*, Gekkota).** *Proc Roy Soc London: B* 2009, **276**:2001-2007.

5. Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I: **Cryptic species as a window on diversity and conservation.** *Trends Ecol Evol* 2007, **22**: 148-55.

6. Crozier R: **Genetic diversity and the agony of choice.** *Biol Cons* 1992, **61**: 11-15.

7. Faith DP: **Phylogenetic diversity and taxonomic priorities for conservation.** *Biol Cons* 1992, **61**:1-10.

8. Roca AL, Bar-Gal G, Eizirik E, Helgen KM, Maria R, Springer MS, O'Brien SJ, Murphy WJ: **Mesozoic origin for West Indian insectivores.** *Nature* 2004, **429**:649-651.

9. Wake DB, Roth G, Wake MH: **On the problem of stasis in organismal evolution.** *J Theor Biol* 1983, **101**:211-224.

10. Rocha-Olivares A, Fleeger JW, Foltz DW: **Decoupling of molecular and morphological evolution in deep lineages of a Meiobenthic Harpactoid Copepod.** *Mol Biol Evol* 2001, **18**:1088-1102.

11. Gamble TP, Bauer AM, Greenbaum E, Jackman TR: **Evidence of Gondwanan vicariance in an ancient clade of gecko lizards.** *J Biogeography* 2008, **35**:88-104.

12. Oliver PM, Sanders K: **Molecular evidence for multiple lineages with ancient Gondwanan origins in a diverse Australian gecko radiation.** *J Biogeography* 2009, **36**:2044-2055.

13. Dixon JR, Kluge AG: **A new gekkonid lizard genus from Australia.** *Copeia* 1964: 174-80

14. Storr GM: **Seven new gekkonid lizards from Western Australia.** *Rec West Aust Mus* 1978, **7**: 337-50

15. Wilson S, Swan G: *A complete guide to reptiles of Australia.* 2nd edn. New Holland Press, Sydney; 2008.

16. Zamudio KR, Greene HW: **Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics, and conservation.** *Biol. J. Linn. Soc.* 1997, **62**:421–442. doi:10.1006/bijl.1997.0162

17. Harvey MS: **Short Range endemism amongst the Australian fauna: some examples from non-marine environments.** *Invertebrate Systematics* 2002, **16**:555-570.

18. Solem A, McKenzie NL: **The composition of land snail assemblages in Kimberley rainforests.** In *Kimberley Rainforests of Australia*. Edited by McKenzie NL, Johnston RB, Kendrick PG: Sydney: Surrey Beatty and sons; 1991:247–263.

19. Hoskin CJ: **Australian microhylid frogs (*Cophixalus* and *Austrochaperina*): phylogeny, taxonomy, calls, distributions and breeding biology.** *Aust J Zool* 2004, **52**:237-269.

20. Melville J, Swain R: **Mitochondrial dna-sequence based phylogeny and biogeography of the snow skinks (Squamata: Scincidae: *Niveoscincus)* of Tasmania.** *Herpetologica* 2000, **56**:196-208.

21. Doughty P, Anstis M, Price P: **A new species of *Crinia* (Anura: Myobatrachidae) from the high rainfall zone of the northwest Kimberley, Western Australia.** *Rec West Aust Mus* 2009, **25**:125-144.

22. Williams SE, Bolitho EE, Fox S: **Climate change and Australian tropical rainforests: an impending environmental catastrophe.** *Proc Roy Soc London* 2003, **270**:1887-1892.

23. Horner P: **Systematics of the snake-eyed skinks, *Cryptoblepharus* Wiegmann (Reptilia: Squamata: Scincidae) – an Australian-based review.** *Rec Mus N Terr* 2007, **Supp 3**:2-98.

24. Colborn J, Crabtree RE, Shaklee JB, Pfeiler E, Bowen BW: **The evolutionary enigma of bonefishes (*Albula* spp.): cryptic species and ancient divergences in a globally distributed shorefish.** *Evolution* 2001, **54**:807-820.

25. Lefébure T, Douady CJ, Gouy M, Trontel P, Briolays J, Gibert J: **Phylogeography of a subterranean amphipod reveals cryptic diversity and dynamic evolution in extreme environments.** *Mol Ecol* 2006, **15**:1797-1806.

26. Martin AP, Palumbi SR: **Body size, metabolic rate, generation time, and the molecular clock.** *Proc Natl Acad Sci USA* 1993, **90**:4087-4091.

27. Couper P, Sadlier R, Shea GM, Worthington Wilmer J: **A reassessment of *Salturius swaini* (Lacertilia: Diplodactylidae) in southeastern Queensland and New South Wales; Two new taxa, phylogeny, biogeography and conservation.** *Rec Aust Mus* 2008, **60**:87-118.

28. Oliver PM, Hugall A, Adams M, Cooper SJB, Hutchinson M: **Genetic elucidation of ancient and cryptic diversity in a group of Australian geckos: the *Diplodactylus vittatus* complex.** *Mol Phyl Evol* 2007, **44**:77-88.

29. Pepper M, Doughty P, Keogh JS: **Molecular systematics and phylogeography of the Australian *Diplodactylus stenodactylus* (Gekkota: Reptilia) species-group based on mitochondrial and nuclear genes reveals an ancient split between Pilbara and non-Pilbara *D. stenodactylus*.** *Mol Phyl Evol* 2006, **41**:539-555.

30. Kozak KH, Weisrock DW, Larson A: **Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders.** *Proc Roy Soc London: B* 2006, **273**:539-546.

31. Sanders KL, Lee MSY: **Molecular evidence for a rapid late-Miocene radiation of Australasian venomous snakes (Elapidae, Colubroidea).** *Mol Phyl Evol* 2008, **46**:1165-1173.

32. Hugall AF, Foster R, Hutchinson M, Lee MSY: **Phylogeny of Australasian agamid lizards based on nuclear and mitochondrial genes: implications for morphological evolution and biogeography.** *Biol J Linn Soc* 2008, **93**:343-358.

33. Meredith RW, Westerman M, Springer MS: **A phylogeny and timescale for the living genera of kangaroos and kin (Macropodiformes: Marsupialia) based on nuclear DNA sequences.** *Aust J Zool* 2008, **56**:395-410.

34. Rowe KC, Reno ML, Richmond DM, Adkins RM, Steppan SJ: **Pliocene colonization and adaptive radiations in Australia and New guinea (Sahul): Multilocus systematics of the old endemic rodents (Muroidea: Murinae)** *Mol Phy Evol* 2008, **47**:84-101.

35. Avise JC, Aquadro CF: **A comparative study of genetic distances in the vertebrates.** *Evol Biol* 1982, **15**:151-185.

36. Georges A, Adams M: **A phylogeny for the Australian chelid turtles based on allozyme electrophoresis.** *Aust J Zool* 1992, **40**:453-476.

37. Barker FK, Cibois A, Schikler P, Feinstein, J, Cracraft, J: **Phylogeny and diversification of the largest avian radiation.** *Proc Natl Acad Sci USA* 2004, **111**:11040-45.

38. Meredith RW, Westerman M, Springer MS: **A phylogeny of the Diprodontia (Marsupialia) based on sequences for five nuclear genes.** *Mol Phyl Evol* 2009, **51**:554-571

39. Roelants K, Gower DJ, Wilkinson M, Loader SP, Biju S.D, Guillaume K, Moriau L, Bossuyt F: **Global patterns of diversification in the history of modern amphibians.** *Proc Natl Acad Sci USA* 2007, **104**:887-892.

40. Sauquet H, Weston PH, Anderson CL, Barker NP, Cantrill DJ, Mast AR, Savolainen V: **Contrasted patterns of hyperdiversification in Mediterranean hotspots.** *Proc Natl Acad Sci USA* 2009, **106**:221-225.

41. Cook LG, Crisp MD: **Not so ancient: the extent crown group of *Nothofagus* represents a post-Gondwanan radiation.** *Proc Roy Soc Lond B* 2004, **272**:2535-2544.

42. Vitt LJ, Sartorius SS, Avila-Pires TCS, Zani PA, Espósito MC: **Small in a big world: Ecology of leaf-litter geckos in new world tropical forests.** *Herp Monographs* 2005, **19**:137-152.

43. Byrne M, Yeates DK, Joseph L, Kearney M, Bowler J, Williams MAJ, Cooper S, Donnellan SC, Keogh JS, Leys R, Melville J, Murphy DJ, Porch N, Wyrwoll K-H: **Birth of a biome: Insights into the assembly and maintenance of the Australian arid zone biota.** *Mol Ecol* 2008, **17**:4398-4417.

44. Leys R, Watts C, Cooper SJB, Humphreys WF: **Evolution of subterranean diving beetles (Coleoptra: Dytiscidae: Hydroporini, Bidessini) in the arid zone.** *Evolution* 2003, **57**:2819-2834.

45. Dayrat B: **Towards integrative taxonomy.** *Biol J Linn Soc* 2005, **85**: 407-415.

46. Richardson BJ, Baverstock PR, Adams MA: *Allozyme electrophoresis: A handbook for animal systematics and population studies.* Sydney: Academic press; 1986.

47. Horner P, Adams M: **A molecular systematic assessment of species boundaries in Australian *Cryptoblepharus* (Reptilia: Squamata: Scincidae) - a case study for the combined use of allozymes and morphology to explore cryptic biodiversity.** *Rec Mus N Terr* 2009, ***Supp* 3**:1-19.

48. Swofford DL: *PAUP*: Phylogenetic Analysis using Parsimony * (and other methods). Version 4.* Sunderland, MA: Sinauer; 2000.

49. Huelsenbeck JP, Ronquist F: **MRBAYES: Bayesian inference of phylogeny.** *Bioinformatics* 2001, **17**:754-755.

50. Stamatakis A: **RaxML-VI-HPC: Maximum Likelihood-based Phylogenetic analyses with thousands of taxa and mixed models.** *Bioinformatics* 2006, **22**:2688-2690.

51. Nylander JAA: *MrModeltest*. 2004 [http://www.abc.se/~nylander/.]

52. Wiens JJ, Fetzner JW, Parkinson CL, Reeder TW: **Hylid frog phylogeny and sampling strategies for speciose clades.** *Syst Biol* 2005, **54**:719-748.

53. Drummond AJ, Rambaut A: *BEAST v 1.4.* 2006 [http://beast.bio.ed.ac.uk/]

54. Loader SP**,** Pisani D, Cotton JA, Gower DJ, Day JJ, Wilkinson M: **Relative timescales reveal multiple origins of parallel disjunct distributions of African caecilian amphibians.** *Biol Letters* 2007, **3**:505-508.

55. Lee MSY, Oliver P, Hutchinson MN: **Phylogenetic uncertainty and molecular clock calibrations: a case study of legless lizards (Pygopodidae: Gekkota).** *Mol Phyl Evol* 2009, **50**:661-666.

Additional files

Additional file 1 - *Supplementary tables*

Table S1. Mean pairwise allozyme distances between taxa. **Table S2**: Mean allozyme frequencies at all loci scored. **Table S3**: Mean interspecific mtDNA divergences between candidate taxa. **Table S4**: Mean intraspecific mtDNA divergences between candidate taxa. **Table S5**: Specimen and sequence details for *Crenadactylus* included in analyses. **Table S6**: Outgroup sequence details.

Additional File 1.

Table S1. Specimen, locality and Genbank details for all individuals of *Crenadactylus 'ocellatus'* sequenced for either ND2, RAG1 or C-mos, and/or included in allozyme analyses.

Taxon	LOCALITY	Exnum	STATE	ND2	RAG1	C-mos	allozymes	LAT	LONG
South West	Norseman	DV355	WA	_	_	_	x	320923S	1214424E
South West	Walganna Rock	DV361	WA	x	_	_	x	272400S	1172800E
South West	4km N Ravensthorpe	DV379	WA	_	_	_	x	333200S	1200300E
South West	Yorkrakine Rock	DV380	WA	x	_	_	x	312600S	1173100E
South West	Bindoon Military Training Area	DV381	WA	x	_	_	x	311344S	1161738E
South West	West Wallabi Island	DV382	WA	_	_	_	x	282900S	1134100E
South West	West Wallabi Island	DV421	WA	_	_	_	x	282900S	1134100E
South West	Spalding Park,Geraldton	DV423	WA	_	_	_	x	284600S	1143700E
South West	Murray Island	DV424	WA	_	_	_	x	285347S	1135352E
South West	Irwin R	DV425	WA	_	_	_	x	285800S	1152900E
South West	Bungalbin Woodland Camp	DV426	WA	_	_	_	x	301812S	1194346E
South West	Esscape Island	DV427	WA	_	_	_	x	302002S	1145904E
South West	55km NNW Norseman	DV428	WA	_	_	_	x	314600S	1214000E
South West	Old Badgingara Townsite	DV429	WA	_	_	_	x	302500S	1153400E
South West	North Cervantes Island	DV430	WA	_	_	_	x	303200S	1150300E
South West	Bindoon Military Training Area	DV431	WA	_	_	_	x	311553S	1161519E
South West	Eglinton	DV432	WA	_	_	_	x	313900S	1154100E
South West	Neerabup	DV433	WA	_	_	_	x	314000S	1154500E
South West	Darling Ra. Behind Brigadon Estate	DV434	WA	_	_	_	x	314600S	1160700E
South West	Darlington	DV435	WA	_	_	_	x	315500S	1160400E
South West	7KM NE Kellerberrin	DV437	WA	_	_	_	x	313600S	1174600E
South West	Boodaring Rock	DV438	WA	_	_	_	x	313621S	1194827E
South West	Yellowdine	DV439	WA	_	_	_	x	311800S	1193900E

South West	Dedari	DV440	WA	_	_	_	x	310500S	1204500E
South West	Nr Carracarrup Pool	DV443	WA	_	_	_	x	334425S	1195835E
South West	Kordinrup Dam, 6KM ESE Ravensthorpe	DV444	WA	_	_	_	x	333700S	1200700E
South West	Spalding Park,Geraldton	DV594	WA	x	_	_	x	284600S	1143700E
South West	Mcdermid Rock	DV596	WA	x	_	_	x	320100S	1204400E
South West	Ravensthorpe	DV598	WA	x	x	_	x	333500S	1200200E
South West	Dryandra	DV601	WA	x	_	_	x	324702S	1165514E
South West	Murray Island	NA	WA	_	_	_	x	285347S	1135352E
Pilbara	Burrup Peninsula	DV288	WA	x	_	_	x	203645S	1164737E
Pilbara	Deepdale oustation, Robe River	DV362	WA	x	_	_	x	214300S	1161100E
Pilbara	80 km s Telfer	DV363	WA	_	_	_	x	222000S	1220500E
Pilbara	20KM WSW Pannawonica	DV399	WA	_	_	_	x	214400S	1161000E
Pilbara	5km South Mount Tom Price Mine	DV400	WA	x	_	_	x	224834S	1174640E
Pilbara	5km South Mount Tom Price Mine	DV401	WA	x	_	_	x	224834S	1174640E
Pilbara	Hope Downs	DV402	WA	x	_	_	x	225800S	1190700E
Pilbara	Burrup Peninsula	DV403	WA	x	_	_	x	203645S	1164737E
Pilbara	Burrup Peninsula	DV404	WA	x	_	_	x	203645S	1164737E
Pilbara	Burrup Peninsula	DV405	WA	x	x	_	x	203534S	1164758E
Pilbara	58 KM ESE Meentheena Outcamp	DV446	WA	x	_	_	x	22535S	118.977E
Pilbara	26 KM WSW Mt Marsh	DV447	WA	x	_	_	x	213219S	121.002E
Pilbara	Burrup Peninsula	NA	WA	_	_	_	x	203534S	1164758E
Pilbara	Burrup Peninsula	NA	WA	_	_	_	x	203534S	1164758E
Pilbara	Burrup Peninsula	NA	WA	_	_	_	x	203534S	1164758E
Kimberleys E	Koolan Island	DV365	WA	x	x	_	x	160718S	1234312E
Kimberleys E	Koolan Island	DV406	WA	_	_	_	x	160821S	1234453E
Kimberleys E	Koolan Island	DV407	WA	x	_	_	x	160814S	1234529E
Kimberleys D	Mitchell Falls	DV285	WA	x	x	x	x	144900S	1254100E
Kimberleys C	Augustus Is (NE Corner)	DV366	WA	x	x	_	x	152700S	1243800E
Kimberleys B	Bream Gorge-Osmond Valley	DV286	WA	x	_	_	x	171500S	1281800E
Kimberleys B	Calico Spring Mabel Downs Stn	DV367	WA	x	_	_	x	171700S	1281100E

Kimberleys B	25 km se Kununurra	DV368	WA	x	x	_	x	155600S	1285400E
Kimberleys B	Bream Gorge-Osmond Valley	DV408	WA	x	_	_	x	171500S	1281800E
Kimberleys B	Mount Parker	DV409	WA	x	_	_	x	171004S	1281823E
Kimberleys B	25 km se Kununurra	DV410	WA	x	_	_	x	155600S	1285400E
Kimberleys B	25 km se Kununurra	DV411	WA	x	_	_	x	155600	1285400E
Kimberleys A	24 Km N Tunnel Creek	DV364	WA	x	x	_	x	172841S	1250118E
Central Ranges	10km S of Barrow Creek	NA	NT	AY369016	AY662627	x	_	213800S	1335300E
Central Ranges	1.9k SW Sentinel Hill	DV283	SA	x	_	_	_	260533S	1322605E
Central Ranges	38k ESE Amata	DV369	SA	x	_	_	x	261714S	1312930E
Central Ranges	Bagot Ck Watarrka NP NT	DV370	NT	x	_	_	x	242200S	1314800E
Central Ranges	1.9k SW Sentinel Hill	DV412	SA	x	_	_	x	260533S	1322605E
Central Ranges	Lawrence Gorge	DV413	NT	x	_	_	x	240100S	1332400E
Central Ranges	Ellery Creek	DV414	NT	x	_	_	x	235000S	1325800E
Central Ranges	38k ESE Amata	DV415	SA	x	_	_	x	261714S	1312930E
Central Ranges	11.2k SW Sentinel Hill	DV416	SA	x	_	_	x	260828S	1322133E
Central Ranges	4k SSW Mt Cuthbert	DV417	SA	_	_	_	x	260809S	1320360E
Central Ranges	2.5k SW Womikata Bore	DV418	SA	_	_	_	x	260641S	1320759E
Central Ranges	Lawrence Gorge	DV419	NT	x	_	_	x	240100S	1332400E
Central Ranges	36k W junct Namatjira/Larapinta Drv	DV420	NT	x	_	_	x	234600S	1331000E
Carnarvon	False Entrance Well	DV289	WA	x	x	x	x	262300S	1131900E
Carnarvon	Kalbarri	DV357	WA	_	_	_	x	274200S	1141000E
Carnarvon	Carnarvon Basin	DV358	WA	x	_	_	x	271541S	1140148E
Carnarvon	70k S Exmouth	DV359	WA	x	_	_	x	223500S	1140700E
Carnarvon	East Yuna Nature Reserve	DV383	WA	_	_	_	x	282800S	1151300E
Carnarvon	10k NW Wandina HS	DV384	WA	_	_	_	x	275600S	1153300E
Carnarvon	Kalbarri N.P.	DV385	WA	_	_	_	x	275200S	1141000E
Carnarvon	Kalbarri N.P.	DV386	WA	_	_	_	x	274200S	1141300E
Carnarvon	Carnarvon Basin	DV387	WA	x	_	_	x	27249S	1143423E
Carnarvon	False Entrance Well	DV388	WA	x	_	_	x	262300S	1131900E
Carnarvon	False Entrance Well	DV389	WA	x	_	_	x	262300S	1131900E

Carnarvon	Carnarvon Basin, WA -sector CU6	DV390	WA	_	_	_	x	241818S	1132645E
Carnarvon	5KM s Quobba Homestead	DV391	WA	x	_	_	x	242535S	1132410E
Carnarvon	Red Bluff	DV392	WA	x	_	_	x	240024S	1132747E
Carnarvon	Warroora Station	DV393	WA	x	_	_	x	233900S	1134800E
Carnarvon	Bullara HS, WA	DV394	WA	_	_	_	x	224100S	1140200E
Carnarvon	4k W Bullara HS	DV395	WA	_	_	_	x	224100S	1140200E
Carnarvon	70k S Exmouth	DV396	WA	x	_	_	x	223500S	1140700E
Carnarvon	False Entrance Well	NA	WA	_	_	_	x	262300S	1131900E
Cape Range	Shothole Canyon Cape Range NP	DV397	WA	x	_	_	x	220300S	1140100E
Cape Range	Shothole Canyon Cape Range NP	DV398	WA	x	_	_	x	220300S	1140100E
Cape Range	Vlaming Head, WA	Dv595	WA	x	_	_	x	215000S	1140500E
Cape Range	Shothole Canyon Cape Range NP	DV599	WA	x	x	_	x	220300S	1140100E

Table S2. Specimen and sequence details for species used as outgroups in phylogenetic and molecular analyses.

Taxon	**Specimen**	**Locality**	*RAG-1*	*c-mos*	***ND2***
Carphodactylids					
Carphodactylus laevis	QMJ8944	Lake Barrine, Qld, Australia	FJ855442	AF039467	AY369017
Nephurus milii	SAMA R38006	17 km SE Burra, South Australia	FJ571622	FJ571637	xxxxx
Nephurus stellatus	SAMA R36563	19.3 km NE Courtabie, South Australia	FJ855446	FJ855466	xxxxx
Nephrurus asper	SAMAR55649	10 km W Isaac R, Qld, Australia	FJ855445	FJ855465	xxxxx
Phyllurus platurus	ABTC51012	Bents Basin, Sydney, Australia	FJ855443	_	xxxxx
Phyllurus platurus	NA	NA	_	AY172942	_
Saltuarius swaini	SAMAR29204	Wiangaree, NSW, Australia	FJ855444	FJ855464	AY369023
Diplodactylids					
Bavayia sauvagei	AMSR125814	Mare Island, New Caledonia.	FJ855448	FJ855468	xxxxx
Diplodactylus granariensis	WAMR127572	Goongarrie, Western Australia	FJ855452	FJ855473	xxxxx
Diplodactylus granariensis	**WAMRxxxxxx**	Mt Jackson, Western Australia	_	_	EF532870
Diplodactylus tessellatus	SAMAR41130	Nr Stuart Hwy, South Australia	FJ571624	FJ571639	AY134607
Lucasium byrnei	SAMA R52296	Camel Yard Spring, South Australia	FJ855453	FJ855474	EF681801
Luscasium stenodactylum	NTMR26116	Mann River, Northern Territory	FJ855454	FJ855475	xxxxx
Oedura marmorata	SAMAR34209	Lawn Hill NP, Qld, Australia	FJ571623	FJ571638	AY369015
Oedura reticulata	SAMA R23035	73 km E. Norseman, Western Australia	FJ855450	FJ855471	EF681803
Oedura rhombifer	SAMA R34513	Townsville area, Qld, Australia	FJ855451	FJ855472	xxxxx
Pseudothecadactylus australis	QMJ57120	Heathlands, Qld, Australia	FJ855449	FJ855470	xxxxx
Pseudothecadactylus lindneri	AMS90915	Liverpool R, NT, Australia	AY662626	FJ855469	AY369024
Rhychoedura ornata	SAMAR36873	Mern Merna Station, South Australia	FJ855455	FJ855476	_
Rhychoedura ornata	ANWCR6141	Native Gap, Stuart Hwy, Northern Territory	_	_	AY369014
Strophurus intermedius	SAMAR28963	Gawler Ranges, South Australia	FJ571625	FJ571640	_
Strophurus intermedius	SAMAR22768	Uro Bluff, South Australia	_	_	AY369001
Strophurus jeanae	SAMAR53984	11 km S. of Wycliffe Well	FJ855456	FJ855477	_
Pygopodids					

Aprasia inaurita	SAMAR40729	2 km E of Burra, South Australia	FJ571632	FJ571646	_
Aprasia inaurita	SAMAR47087	ST Peters Island	_	_	AY134574
Delma australis	SAMAR22784	Mt Remarkable NP, South Australia	FJ571633	FJ571647	AY134582
Delma molleri	SAMAR23137	Mt Remarkable NP, South Australia	FJ571635	FJ571649	AY134593
Lialis jicari	TNHC59426	NA	AY662628	_	_
Lialis jicari	NA	Irian Jaya	_	AY134564	AY134600
Ophidiocephalus taeniatus	SAMAR44653	Todmorden Stn, South Australia	FJ571630	FJ571645	AY134601
Pletholax gracilis	WAM R104374	Victoria Park, Western Australia	FJ571631	_	AY134602
Pletholax gracilis	WBJ-2483	Lesueur National Park, Western Australia	_	AY134566	_
Paradelma orientalis	QMJ56089	20 km N Capella, Qld, Australia	FJ571626	FJ571642	AY134605
Pygopus lepidopodus	WAM R90378	Walpole-Nornalup NP, Western Australia	FJ571627	FJ571643	_
Pygopus lepidopodus	WBJ-1206	Lesueur National Park, Western Australia	_	_	AY134603
Other gekkonids					
Gehyra variegata	SAMAR54022	Brunette Downs, NT, Australia	FJ855439	FJ855460	_
Gehyra variegata	ANWCR6138	Old Andado Homestead, Northern Territory	_	_	AY369026
Gekko gekko	MVZ215314	NA	AY662625	_	AF114249
Gekko gekko	FMNH258696	NA	_	AY444028	_
Teratoscincus przewalski	CAS171010	South Gobi Desert Mongolia	AY662624	AY662569	U71326
Sphaerodactylus shreveri	SBH194572	Haiti	AY662623	AY662547	AY662547

Table S3. Matrix of pairwise genetic distances from allozyme data among 10 candidate species of *Crenadactylus*. Lower left triangle = number of fixed differences (%FD in brackets); upper right triangle = unbiased Nei D.

Taxon	**South West**	**Carnarv Basin**	**Cape Range**	**Pilbara**	***Kimb A***	**Kimb B**	**Kimb C**	**Kimb D**	**Kimb E**	**Central Ranges**
South West	-	0.304	0.355	0.619	0.560	0.657	0.640	0.542	0.623	0.447
Carnarv Basin	10 (24%)	-	0.446	0.794	0.572	0.728	0.810	0.597	0.740	0.531
Cape Range	9 (21%)	13 (31%)	-	0.700	0.593	0.557	0.629	0.591	0.607	0.637
Pilbara	18 (43%)	21 (50%)	20 (48%)	-	0.439	0.386	0.479	0.491	0.498	0.574
Kimb A	18 (44%)	18 (44%)	17 (41%)	13 (32%)	-	0.194	0.404	0.253	0.314	0.534
Kimb B	20 (48%)	22 (52%)	15 (36%)	13 (31%)	7 (17%)	-	0.368	0.414	0.435	0.593
Kimb C	20 (48%)	22 (52%)	18 (43%)	16 (38%)	14 (34%)	11 (26%)	-	0.321	0.262	0.689
Kimb D	16 (39%)	17 (41%)	17 (41%)	16 (39%)	9 (22%)	13 (32%)	12 (29%)	-	0.120	0.525
Kimb E	18 (43%)	21 (50%)	16 (38%)	16 (38%)	10 (24%)	12 (29%)	8 (19%)	4 (10%)	-	0.575
Central Ranges	15 (36%)	17 (40%)	17 (40%)	16 (38%)	16 (39%)	19 (45%)	20 (48%)	16 (39%)	17 (40%)	-

Table S4. Allozyme frequencies for 10 candidate species of *Crenadactylus* at 37 variable loci. For polymorphic loci, the frequencies of all but the rarer/rarest alleles are expressed as percentages and shown as superscripts (allowing the frequency of each rare allele to be calculated by subtraction from 100%). Alleles joined without being separated by a comma all shared the frequency indicated. A dash indicates no genotypes were assignable at this locus. The maximum number of individuals sampled for each taxon is shown in brackets. Invariant loci: *Ak-1*, *Enol*, *Lap*, *Npdk-1*, and *Pgam*.

Locus	**South West** (35)	**Carnarv Basin** (16)	**Cape Range** (4)	**Pilbara** (15)	**Kimb A** (1)	**Kimb B** (7)	**Kimb C** (1)	**Kimb D** (1)	**Kimb E** (3)	**Central Ranges** (11)
Acon-1	d^{99},b	a	d^{75},a^{13},c	a	d	d	e	f	e	a
Acon-2	h^{90},e^{7},k^{2},i	l^{63},m^{18}, k^{13},g	f^{83},h	f^{93},a	d	h^{43},f^{36}, b^{14},c	f	f	f	j^{91},f
Acp	d^{97},b	d	c	e^{87},d	d	c	e	b^{50},d	e	d^{95},a
Acyc	b	c^{80},b	d	b	b	a	b	b	b	a
Adh-1	b^{93},c^{5},a	b	d^{75},b	b^{79},e	b	b	b	b	b	b
Adh-2	d^{96},g	d^{97},a	d	c^{73},b^{17},e^{7},f	a^{50},b	d^{93},b	d	a	a	a
Ak-2	a^{97},b^{3}	a	a^{75},c^{25}	a	a	a	a	a	a	a
Dia	f^{91},c^{4},h^{2}, abd^{1}	g^{53},d^{33}, e^{7},h^{4},f	f	h^{47},c^{37}, f^{7},g^{6},i	g	h	h	g	g^{67},h	f^{95},c
Est	c^{69},b^{20},e^{9},a	c	e	e	e	e^{50},g^{29},f	c	c	c	c^{70},d^{25},e
Fdp	b^{79},a^{16},c	b	a	a	a	a	a	a	a	b^{82},a
Fum	a	e	c^{75},d	c	c^{50},g	c	c	e	c^{83},f	c^{95},b
Gapd	a^{98},b^{2}	a	a^{88},b^{12}	a	a	a	a	a	a	a
Glo	b	b^{91},a	b	b^{96},d	b	b^{93},c	b	b	b	b
Got-1	b^{54},e^{31}, c^{14},a	e	e	e^{60}, b^{37},g	e	e^{93},d	d	e	e	e^{95},f
Got-2	b^{97},a^{3}	b	b	c	d	d	d	d	d	b
Gpd-1	d^{98},a	d^{90},f	d^{67},e^{17},g	b	b	b^{93},e	b	b	b	c

Gpd-2	b[96],c	b[45],c[42],a	c	b	e	c[70],d	c	b	a[50],c	b
Gpi	b	b	b[87],a	b	b	b	b	b	b	b
Gsr	h[31],k[17],l[16], n[14],m[11],j[9],i	h[31],f[25], j[25],c[13],g	h	g[93],i[4],d	a	a[93],b	c	b	b	c[95],e
Idh	e[97],d	d	b	c	c[50],g	c[93],f	a	c	c	e
Ldh-1	a	a	a	a	a	a	a	a	a	a[95],b
Ldh-2	a	b	a	a	b	b	a	a	a	a
Mdh-1	h[60],d[27], g[7],b[3],a	e	d	f	f	f	f	f	d[67],f	f[95],c
Mdh-2	e[91],c	d	e[88],f	e[93],b	e	e	e	e	e	e[50],c[45],a
Mpi	d[91],f	d[87],e	d	c[93],e[4],b	c	c[86],a	c	b	b	d[86],f[9],e
Ndpk-2	c[99],a	c	a	c	c	c[79],a	a	a	b[83],a	c
Ntak	b[79],c[19],af[1]	b	b[75],d	e	e	e	e	e	e	g[91],f[5],h
PepA	b[98],d	b	b[83],e	c	e	e	e	e	e	e[95],a
PepB	c[94],e[4],a	c	c	c	f	d[64],f[29],b	f	f	f	c
6Pgd	e[98],c	e[83],b	e	d[86],a[11],f	e	e	g	e	b[50],e	e[95],g
Pgk	c[98],a	c	c[75],d	b	b[50],c	b	c	c	c	b
Pgm-1	d[70],a[22],f[7],b	e[87],c	e	h[68],i[25],j	e	e[64],f[22],b	f	d[50],e	d	h[68],g
Pgm-2	b[99],a	b	b	b	b	b[86],c	b	b	b	b
Sod	e[99],d	e	e	c[97],a	e	c[93],b	e	e	e	e[95],d
Sordh	b	c[81],d	e	b[96],a	-	b	b	-	a	a
Tpi	b[97],d	b	b	a	c	c	c	c	c	c
Ugpp	a	a	a	a	a	a	a	a	a	b

Table S5. Corrected (GTR+I+G) and uncorrected genetic distances between ten candidate species confounded within '*Crenadactylus ocellatus*', calculated using 828 bp of ND2 data.

	N	1	2	3	4	5	6	7	8	9	10
1. South-west	7	_	0.235	0.183	0.212	0.219	0.256	0.217	0.245	0.239	0.222
2. Cape Range	4	0.623	_	0.201	0.246	0.254	0.289	0.255	0.264	0.262	0.267
3. Carnarvon	10	0.359	0.456	_	0.205	0.205	0.243	0.22	0.24	0.232	0.225
4. Pilbara	10	0.505	0.709	0.494	_	0.171	0.206	0.192	0.209	0.199	0.185
5. Central Ranges	11	0.512	0.718	0.457	0.294	_	0.203	0.202	0.221	0.217	0.207
6. Kimberly A	1	0.704	1.059	0.673	0.445	0.415	_	0.191	0.174	0.153	0.153
7. Kimberly B	1	0.557	0.872	0.577	0.421	0.443	0.347	_	0.181	0.174	0.153
8. Kimberly C	2	0.657	0.923	0.698	0.47	0.491	0.281	0.333	_	0.161	0.165
9. Kimberly D	1	0.68	0.981	0.686	0.463	0.504	0.221	0.326	0.266	_	0.139
10. Kimberly E	7	0.615	0.997	0.636	0.405	0.479	0.248	0.281	0.281	0.227	_

Table S6. Uncorrected and corrected (GTR+I+G) genetic distances within ten candidate species of *Crenadactylus*, calculated from 828 bp of ND2 data.

	N	Uncorrected	corrected
1. South-west	7	0.002 (0.000-0.005)	0.002 (0.000-0.004)
2. Cape Range	4	0.001 (0.000-0.001)	0.000 (0.000-0.001)
3. Carnarvon	10	0.013 (0.000-0.022)	0.012 (0.000-0.020)
4. Pilbara	10	0.062 (0.002-0.097)	0.071 (0.000-0.116)
5. Central Ranges	11	0.056 (0.002-0.079)	0.059 (0.002-0.090)
6. Kimberly A	1	NA	NA
7. Kimberly B	1	NA	NA
8. Kimberly C	2	NA	NA
9. Kimberly D	1	NA	NA
10. Kimberly E	7	0.021 (0.001-0.034)	0.019 (0.003-0.032)

Additional file 2 - *Figure S1.* Bayesian tree from combined *RAG1* and *ND2* dataset.

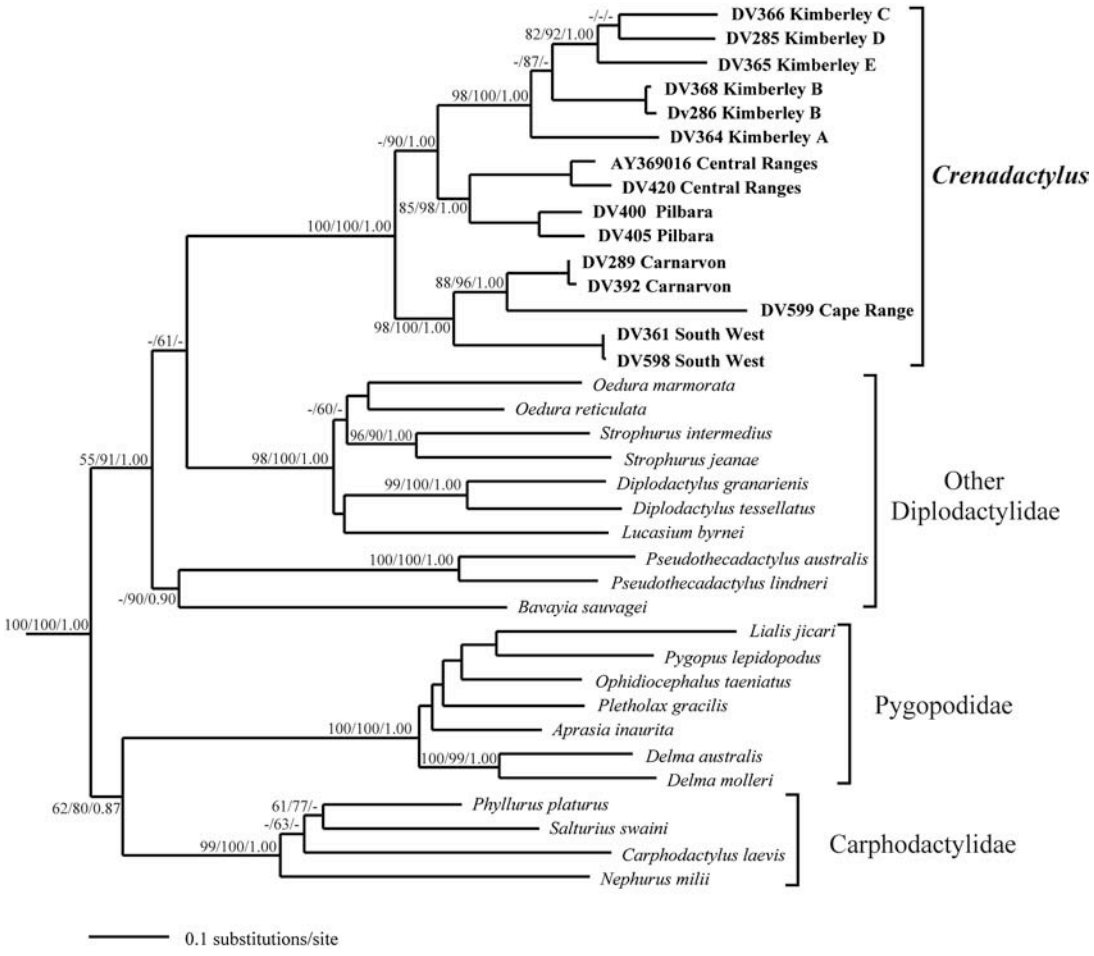

Figure S1. Representative estimate of phylogenetic relationships between 10 candidate species confounded within *Crenadactylus 'ocellatus*' based on combined analysis of 978bp RAG1 and 828bp ND2 for a subset of ingroup specimens spanning major divergences. Consensus phylogram of 20,000 trees from 5 million generation bayesian analyses with a burnin of 20%, support values at major nodes are respectively maximum parsimony (PAUP), maximum likelihood (RaxML) and Bayesian posterior probabilities (MrBayes). See methods and materials for further details of analyses. All analyses supported the same relationships between the major geographically isolated lineages of *Crenadactylus*.

CHAPTER 6

Molecular phylogeny for the Australian knob-tail geckos (*Nephrurus*, Carphodactylidae, Gekkota): progressive biome shifts through the Miocene.

P.M. OLIVER* & A.M. BAUER†

*Australian Centre for evolutionary Biology and Biodiversity, Unversity of Adelaide, Adelaide, SA, Australia, 5005, and Herpetology Section, South Australian Museum, Adelaide, SA, Australia, 5000.

†Department of Biology, Villanova University, 800 Lancaster Avenue, Villanova, PA 19085, USA.

Correspondance: Paul M. Oliver, Darling Building, School of Earth and Environmental Sciences, University of Adelaide, Adelaide, 5005, Australia.
Tel.: +61 8207 7473 ; fax: +61 8207 7222
e-mail: Paul.Olver@adelaide.edu.au

Journal of Evolutionary Biology (2010), submitted paper.

Evolution of the Australian knob-tail geckos (*Nephrurus*, Carphodactylidae, Gekkota): progressive biome shifts through the Miocene

P.M. OLIVER* & A.M. BAUER†

*Australian Centre for evolutionary Biology and Biodiversity, Unversity of Adelaide, Adelaide, SA, Australia, 5005, and Herpetology Section, South Australian Museum, Adelaide, SA, Australia, 5000.

†Department of Biology, Villanova University, 800 Lancaster Avenue, Villanova, PA 19085, USA.

Correspondance: Paul M. Oliver, Darling Building, School of Earth and Environmental Sciences, University of Adelaide, Adelaide, 5005, Australia.
Tel.: +61 8 ; fax: +61 8
e-mail: Paul.Olver@adelaide.edu.au

Keywords: adaptation, arid biome, Australia, Bayesian Inference, Bayesian dating, gecko, Maximum Likelihood, *Underwoodisaurus, Uvidodactylus* gen. nov.

Abstract

Lineages distributed across major biomes provide opportunities to examine both when major environmental changes occurred, and how clades of organisms adapted to these changes. The family Carphodactylidae is an ancient Gondwanan lineage of geckos that occurs across all major Australian biomes. We present the results of a multilocus (ND2, Rag-1, C-mos) phylogenetic and dating analysis of the most ecologically diverse clade within this group, the genus *Nephrurus* (*sensu* Bauer 1990). Two of three major morphological taxa historically recognised within the clade (the 'spiny knob-tails' and '*Underwoodisaurus*') appear to represent pleisomorphic basal grades that diversified through the late Oligocene and early Miocene. These lineages are species depauperate and concentrated in seasonally arid to arid areas towards the coast, but are largely absent from sandy habitats that now dominate the vast central Australian arid zone. Based on their deep divergence and morphological distinctiveness we recognise the two most basal lineages (*milii* and *sphyrurus*) as monotypic genera, one of which is named herein **(***Uvidodactylus* nov. gen.). In contrast, a third group, the 'smooth knob-tails,' is a monophyletic lineage restricted to sandy deserts within the arid zone that has radiated into five species relatively recently (mid Miocene). We hypothesise that amongst other adaptations, an initial shift to terrestriality, and the eventual evolution of burrowing specialisations have allowed *Nephrurus* to successfully colonise and diversify within a novel and challenging biome.

Keywords: adaptation, arid zone, Australia, Bayesian Inference, Bayesian dating, gecko, Maximum Likelihood

Introduction

Over geological time major environmental transitions are relatively common; in contrast, clades of organisms tend to retain their ancestral ecologies, and successful colonisation of completely new biomes is relatively rare (Losos *et al.*, 2003; Crisp *et al*., 2009). Over the last 30 million years Australia has undergone a profound environmental transition; from relatively mesic, to dominated by one of the largest continuous arid zones on the planet (Martin, 2006; Byrne *et al*., 2008). It has been suggested that that intermediate environments such as rocky areas and seasonal sclerophyll habitats have played an important role in allowing elements of an originally mesic biota to persist in and adapt to increasingly arid biomes (Crisp and Cook, 2004; Couper and Hoskin, 2009; Crisp *et al.*, 2009). In light of the poor fossil record of arid Australia (Hill, 1994; Byrne *et al*., 2008), phylogenetic data provide one of the few means available to test both when, and how, elements of the Australian biota adapted to this newly emerging biome. However, while ongoing work is beginning to provide an insight into the complex history of the biota of the vast Australian arid zone (Byrne *et al*., 2008), species level dated phylogenies demonstrating a clear correspondence between ecological shifts within clades and successful adaptation to the developing arid biomes are few.

Lizards are the most diverse and abundant vertebrate group in the Australian arid zone. Australia's gecko fauna is especially species rich (160+ species), highly endemic and morphologically diverse (Wilson and Swan, 2008). Of the four gecko families present, only the Gekkonidae is widespread outside the Australasian region; the three remaining families (Carphodactylidae, Diplodactylidae and Pygopodidae *sensu* Han *et al*., 2004) are part of an ancient East Gondwanan radiation originating in the late Cretaceous (Gamble *et al*., 2008; Oliver and Sanders, 2009), recently named the Pygopodoidea (Vidal and Hedges, 2009). These ancient lineages have successfully adapted to the changing environment, and a significant proportion of diversity in all three families is now found in arid Australia.

Relative to other Australian gekkotans, the Carphodactylidae have a relatively unique distribution. Over half the species diversity, and most of the generic diversity (*Carphodactylus*, *Orraya*, *Phyllurus* and *Saltuarius*) within this family is concentrated within temperate and mesic areas of Australia (the aseasonal wet biome (Crisp *et al.*, 2004)), where other gekkotan families are relatively depauperate. In striking contrast, the 11 described species of *Nephrurus* (*sensu* Bauer, 1990), the only other recognised genus of Carphodactylid, occur across all other Australian biomes (temperate, monsoonal and arid) and have a wider environmental distribution than most other Australian gecko genera.

Nephrurus are morphologically highly aberrant geckos; the tail is variably quite reduced (autonomy has been completely lost in three species) (Holder, 1960); the head shows varying degrees of disproportionate enlargement with respect to the body; and a number of species have lost phalanges and evolved specialised subdigital scalation to assist burrowing (Bauer and Russell, 1988; 1991). However, the most distinctive feature of the genus is the caudal knob of all but two species (see below), which is characterised by a thickened dermis, hypervascularisation, and an aggregation of sensory organs. The function of the knob is uncertain, but it has been suggested that it is involved in mechanoreceptive monitoring of the environment (Russell and Bauer, 1988) or in pheromonal transfer (Annable, 2004).

Three major groups of *Nephrurus* have been recognised based on morphological similarity (Greer, 1989): 1) the 'smooth' knob-tails, which can be further broken into small-tailed (*N. deleani*, *N. laevissimus*, *N. stellatus*) and the big-tailed groups (*N.levis* (with three subspecies) and *N. vertebralis*), 2) the 'spiny' knob-tails (*N. amyae*, *N. asper*, *N. sheai* and *N. wheeleri* (with two subspecies)), and 3) a two species lacking a caudal knob on the tail, frequently placed in the separate genus *Underwoodisaurus* (e.g., Cogger, 2000; Wilson and Swan, 2008), comprising *N. milii* and *N. sphyrurus* (but following Bauer, 1990, here treated as part of *Nephrurus*). Bauer (1990) presented a comprehensive morphological cladistic analysis of *Nephrurus* and found support for the monophyly of the smooth knob-tails, but not for the other two groups. He regarded the two

‘*Underwoodisaurus*’ species as plesiomorphic members of the group, lacking the characteristic knob-tail.

Many taxa within these three groups of *Nephrurus* share similar ecologies. Most notably, the smooth knob-tails, have the widest distribution, but are restricted to the arid zone, and occur predominately in sandy deserts across arid central and western Australia. The spiny knob-tails are largely restricted to rocky ranges and plains in predominantly summer rainfall, arid to seasonally arid areas across north and central Australia. The two species of '*Underwoodisaurus*' have perhaps the most contrasting distribution, *N. sphyrurus*, is restricted to a small area of cool upland woodland in the New England tableland, while *N. milii* ranges from similar temperate areas, through semi-arid and into arid areas spanning the southern third of the continent (Wilson and Swan 2007).

The wide environmental distribution of lineages within this ancient Gondwanan clade of geckos provides unique opportunity to examine hypothesises about the timing of aridification and the nature of biotic responses to it. In this study we examine phylogenetic relationships between the 11 described species of *Nephrurus* and other carphodactylines using a combination of nuclear (*RAG1*, *C-mos*) and mitochondrial data (*ND2*), and use this data to examine the trajectory and temporal scale of evolution within the genus, with particular focus on (a) testing the monophyly and relationships of morphologically recognised groups (b) the temporal and environmental distribution of lineages spanning the evolutionary transition from mesic to arid areas, and (c) the evolution of key adaptive features which may have mediated the ecological success of this lineage across such a broad range of Australian environments.

Methods

Taxon sampling, DNA extraction and amplification

DNA was extracted from frozen or alcohol preserved liver and tail tissue using Gentra protocols. A full list of all carphodactylid geckos included in analyses is

given in Appendix 1. We amplified portions of ND2 (~1000bp), *RAG-1* (~1700 bp from the 3' end in two fragments) and *c-mos* (~530 bp) for a single examplar of each nominal species and most subspecies of *Nephrurus* using primers given in Appendix 2. We sequenced ND2 from additional specimens from across the distribution of most nominal taxa to provide an assessment of within taxon genetic diversity, and an additional five ND2 sequences of *Nephrurus* amplified by Melville *et al*. (2004) were also downloaded from GenBank. Nuclear and combined analyses were rooted with outgroups spanning the extent gekkotan radiation, especially Pygopoidea, and used data from Oliver and Sanders (2009). Data for outgroups outside Carphodactylidae is summarised in Appendix 3.

PCR products were amplified following protocols and primers outlined elsewhere (Appendix III; Pepper *et al*. 2007; Oliver *et al*. 2007; Oliver and Sanders; 2009). Products were amplified using standard polymerase chain reaction protocols for TAQgold and buffer at temperatures ranging from 50-63 ºC for 34-38 cycles. PCR products were visualised using acrylimide gels, cleaned using a vacuum clean-up kit, and sequenced using ABI Prism BigDye Terminator technology and an ABI 3700 Automated sequencer at the Australian Genome Research facility (AGRF) in Adelaide.

Phylogenetic analysis

Mitochondrial data was initially aligned using clustal X V1.81 (2000) and subsequently edited by eye using Maclade V. 4.0 (Maddison and Maddison, 2005). Nuclear data were aligned with a pre-existing alignment of same two genes used by Oliver and Sanders (2009). All sequences were translated into amino acids to check for nonsense mutations using MacClade V. 4.0 (Maddison and Maddison, 2005).

Phylogenetic analyses were performed on three different combinations of alignment data; 1) a nuclear gene only alignment comprising *RAG-1* (1725 bp) and *c-mos* (521 bp*)* including exemplars of all 11 recognised *Nephrurus* species, five other carphodactylids including all recognised genera except *Orraya*, 24 other pygopoids and six other gekkonids; 2) 957 base pairs from the coding region of

ND2 gene from approximately 52 *Nephrurus* including multiple exemplars spanning the range of most recognised species and seven other carphodactylids; and 3) a combined nuclear and ND2 dataset including all *Nephrurus* and outgroup samples used in nuclear analyses and five additional taxa for which mitochondrial samples but not nuclear data was available, and which represented deep intraspecific divergences within *Nephrurus* or carphodactylid outgroups. Inclusion of a small number of taxa for which there is missing data does not necessarily impede phylogenetic reconstruction, provided this data is not concentrated in particular portions of the tree (Wiens *et al*., 2005).

Each dataset was analysed using Bayesian inference and Maximum Likelihood (ML) phylogentic techniques. Bayesian analyses were implemented using MrBayes V 3.1 (Huelsenbeck and Ronquist, 2001). Final Monte Carlo Markov chains of 5,000,000 generations with a burn in of 20% were run for each dataset. Maximum Likelihood bootstrap support values were calculated using 100 iterations of the - f i function in RaxML V 7.0.4 Stamakikis (2006) and bootstrap support values were then drawn onto a maximum likelihood tree calculated using the - f a or - f t functions.

We experimented with the following partitioning strategies for both nuclear and mitochondrial datasets; unpartitioned, partitioned by codon, and partitioned into first plus seconds versus thirds. Preliminary Bayesian analyses of all strategies returned similar topologies, node supports and overall likelihoods. Based on the Bayesian information criterion (Posada and Buckley, 2004) and observed stability of parameter estimates and estimated samples sizes in Bayesian runs we choose the three partition strategy for mitochondrial data and the two partition strategy for nuclear data. For Bayesian analyses we choose models of sequence evolution using the Aikaike Information criteria as implemented in MrModeltest (Nylander, 2004); relevant models chosen were the GTR+I+G for mitochondrial first and seconds, and combined nuclear 1st and 2nds, and GTR+G for both mitochondrial thirds and mitochondrial 3rds. For likelihood analyses we only used the GTR+G model as recommended by Stamakakis (2006). In combined analysis we partitioned nuclear

and mitochondrial data, but otherwise used the same partitions and models as the other analyses.

We used the combined dataset to test support for the monophyly of the following seven putative phylogenetic groupings of *Nephrurus* using the Shimodaira-Hasegawa (1999) (S-H) test: 1) *Nephrurus s.l.*, 2) the nominal genus '*Underwoodisaurus*', 3) the knob-tailed *Nephrurus* plus *milii*, 4) the 'spiny' knob-tails, 5) the 'small-tailed smooth' knob-tails, 6) the 'big-tailed smooth' knob-tails and 7) the 'smooth' knob-tails. The S-H test was implemented using the -f h function in Rax-ML to simultaneously compare ML trees satisfying and violating each of the above constraints; the partitioning schemes and models used above were employed.

Estimation of divergence dates.

Divergence ages for major nodes within *Nephrurus* were estimated using Bayesian inference implemented in BEAST v 1.4 (Drummond and Rambaut, 2006). Mitochondrial data were not included in this analysis, as it is strongly suspected that the combination of relatively old (early Miocene or older) calibration points (see below) and highly saturated mitochondrial loci, can severely bias branch length and age estimates (e.g., Jansa *et al.*, 2006). Our nuclear dataset was significantly overlapping with that used by Oliver and Sanders (2009) and dating methodologies were similar. Following Oliver and Sanders (2009) we used the relaxed clock uncorrelated lognormal molecular clock model. A Yule branching process (appropriate to interspecific data) and uniform root height was adopted. As per likelihood analyses the nuclear data were partitioned by codon position (1st + 2nd vs. 3rd). Final MCMC chains were run for 10,000,000 generations sampling every 1000 steps. TRACER 1.2 was used to determine appropriate burn-in (10%) and confirm that acceptable effective sample sizes had been attained. Multiple independent chains were run to confirm consistency of date estimates.

There are no reliable within clade calibrations for the Pygopoidea (see Lee *et al.*, 2009 for discussion of the Miocene pygopodid *Pygopus hortulanus*). However, three studies have independently estimated that the basal divergence of

the three extent families began around 70 million years ago (Mya) (King, 1987; Gamble *et al*., 2008; Oliver and Sanders, 2009). We applied to the combined C-mos and RAG-1 nuclear dataset a broad uniform root prior (l00-250mya) and two calibrations with normal distributions 1) the basal split of the Pygopoidea from all other geckos at 120mya with the standard deviation of 14.0, and 2) the basal split of the three recognised families of Pygopoidea at 70mya with a standard deviation of 12.0 (reflecting the 95% posterior distribution of age estimates for this divergence (Oliver and Sanders, 2009).

Results

Phylogenetic analyses

The mitochondrial alignment consisted of carphodactylid geckos only, and included 957 sites of which 701 were variable and 536 were parsimony informative. The nuclear dataset included 2249 sites of which 943 were variable and 549 were parsimony informative. The final combined dataset included a reduced number of mitochondrial samples and comprised 3193 sites of which 1664 were variable and 1205 were parsimony informative (mitochondrial dataset of 926 characters, 706 variable, 640 parsimony informative: nuclear dataset identical to above). The combined sequence for *Nephrurus sheai* is a chimera of nuclear and mitochondrial data from different specimens. We were also unable to amplify C-mos for *Nephrurus sphyrurus*. Independent analyses based on different loci supported the same phylogenetic positions for both these taxa, indicating that the missing data and concatenation are unlikely to have affected our overall conclusions about phylogenetic relationships between species of *Nephrurus*.

Likelihood and Bayesian analyses of nuclear, mitochondrial and combined analyses all returned broadly similar topologies and support for key nodes, especially within *Nephrurus* (Figs. 1-3). In combined and nuclear analyses the monophyly of the three recognised families of Pygopodoidea was strongly supported (ML bootstrap support (ML)=100, Bayesian Posterior Probabilities

(PP)=1.00). Intrafamilial relationships were similar to Oliver and Sanders 2009. Within the Carphodactylidae exclusive of *Nephrurus*, nuclear data did not resolve leaf-tailed geckos as a whole, and specifically the genus *Phyllurus*, as monophyletic groups, while the combined and mitochondrial data analyses united these groupings with weak to strong support. The relationship of the monotypic genus *Carphodactylus* to other carphodactylid genera was unresolved.

All analyses strongly supported the monophyly of *Nephrurus* (*sensu* Bauer 1990) (ML>89, PP=1.00) and identified the same five deeply divergent lineages within this clade. Of these, *Nephrurus sphyrurus* was the most basal, sister to all other *Nephrurus*. *Nephrurus milii* was the next most basal and sister to a clade containing the eight knob-tailed species. Within the knob-tailed clade there were three strongly supported monophyletic groupings: *N. wheeleri,* the *asper* group and the 'smooth' knob-tails; combined analyses and mitochondrial data strongly supported *N. wheeleri* as the most divergent of these three lineages (ML>74, PP >0.90, while nuclear data did not strongly support any order of branching**.**

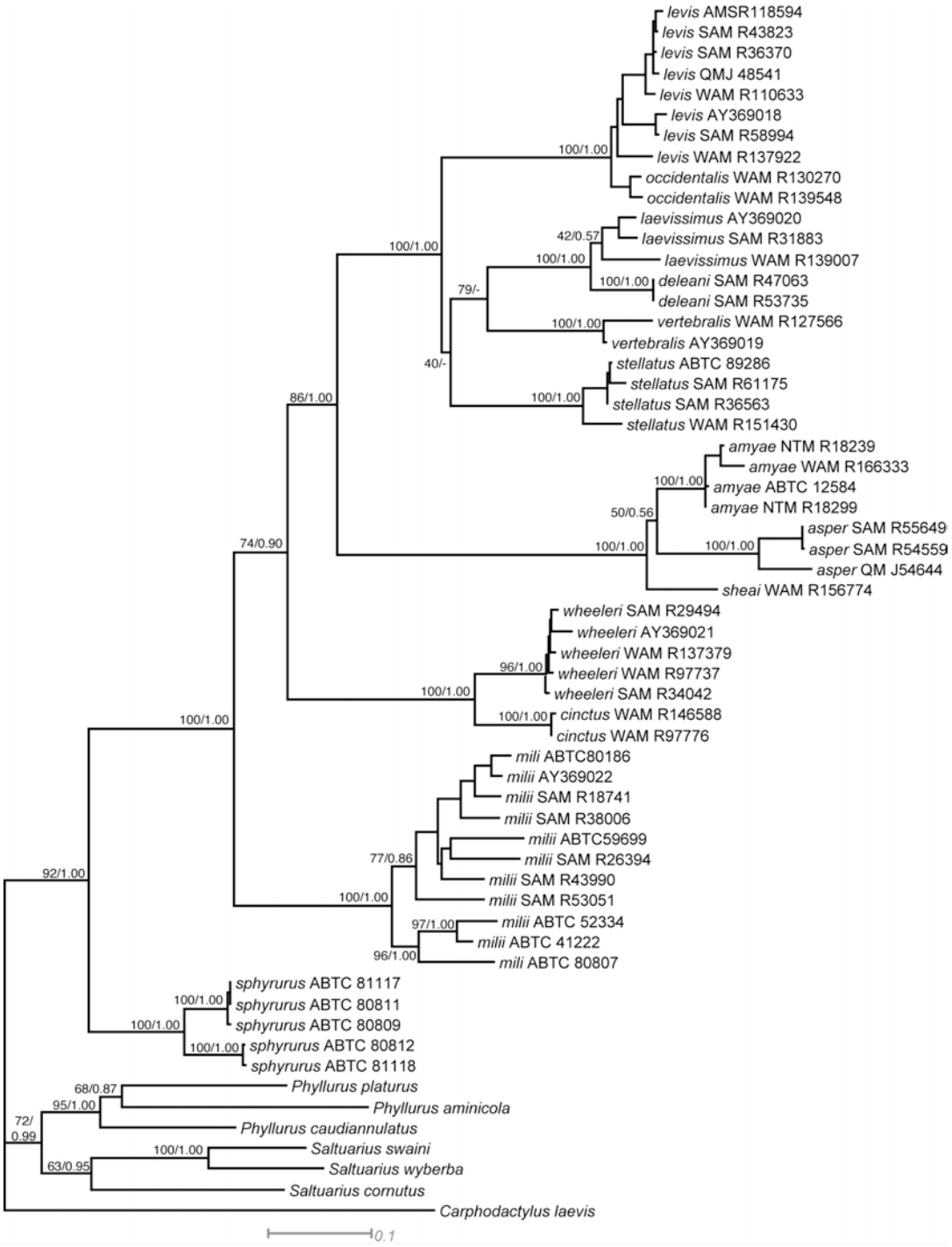

Figure 1. Maximum likelihood (RaxML) tree of relationships amongst Carphodactylid geckos based on mitochondrial *ND2* datasets. Maximum Likelihood Bootstrap (RaxML) and Bayesian Posterior Probability support values for key nodes are shown.

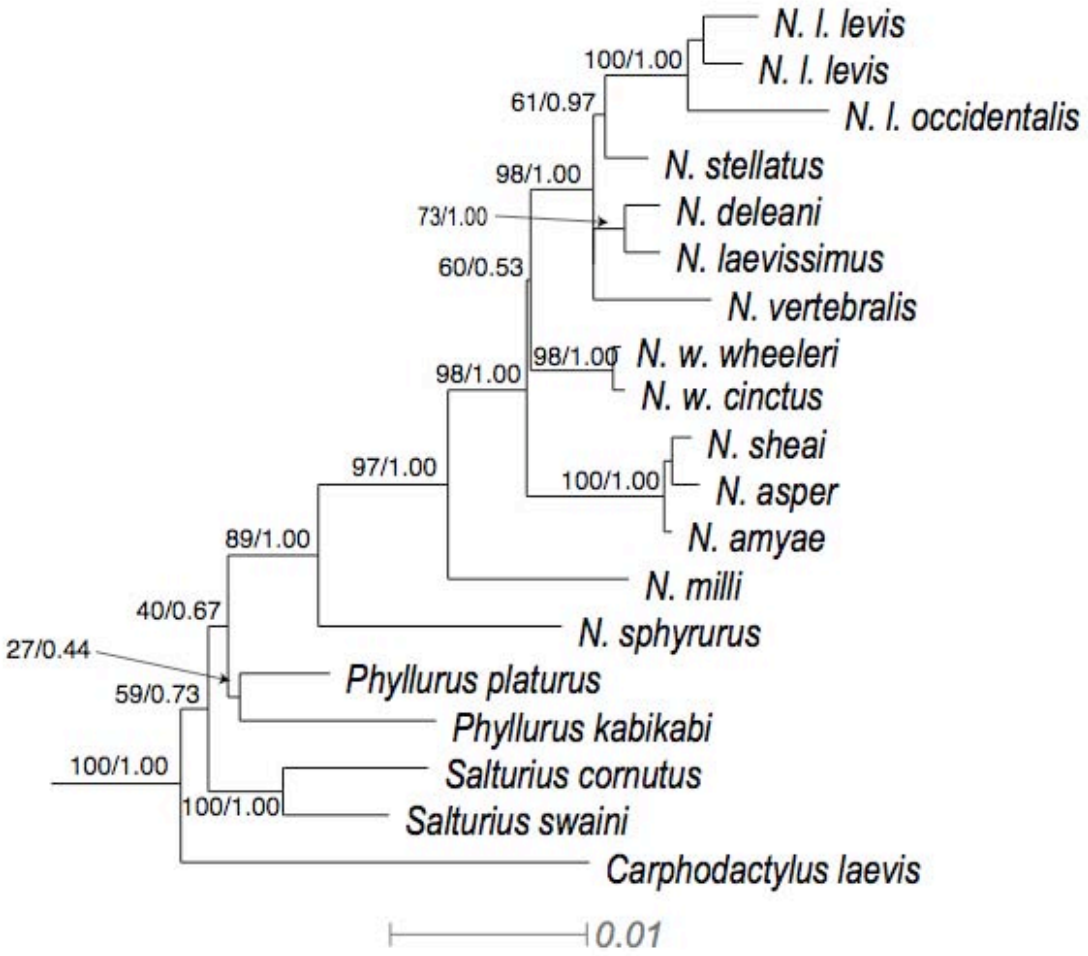

Figure 2. Maximum likelihood (RaxML) tree of relationships amongst Carphodactylid geckos based on nuclear (*RAG-1*, *c-mos*) ND2 datasets. Maximum Likelihood Bootstrap (RaxML) and Bayesian Posterior Probability support values for key nodes are shown.

Of the described species of *Nephrurus* only the three species in the *N. asper* group, and *N. laevissimus* and *N. deleani*, consistently formed strongly supported clades (ML>73, PP=1.00), although combined analyses supported a sister taxon relationship between the *N. laevissimus*/*N. deleani* clade and *N. vertebralis* (ML=72, PP=0.99). The relationships among the three taxa within the *asper* group were unresolved.

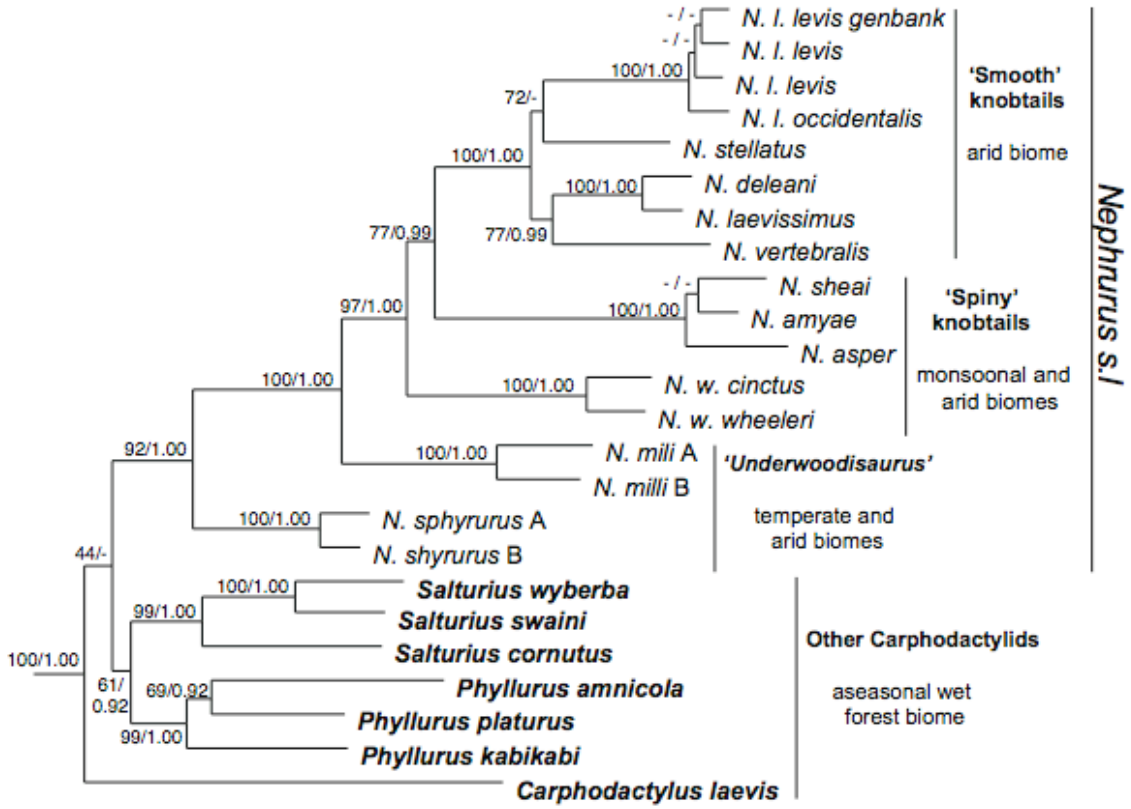

Fig. 1. Maximum likelihood (RaxML) tree of relationships amongst Carphodactylid geckos based on combined nuclear and mitochondrial datasets. Maximum Likelihood Bootstrap (RaxML) and Bayesian Posterior Probability support values for key nodes are shown.

Results of the S-H test (Table 1) indicated that constraining '*Underwoodisaurus*' (i.e., *sphyrurus* and *milii*) to be monophyletic significantly reduced likelihood. In contrast, the other three monophyly constraints inconsistent with the ML tree, 'spiny knob-tails', 'big-tailed smooth knob-tails' and the 'small-tailed smooth knob-tails', did not significantly reduce overall likelihood. Of the three phylogenetic hypothesis that were consistent with our unconstrained ML tree, non-monophyly of both the 'smooth' knob-tails, and *N. milii* and all knob-tails significantly reduced the tree likelihood. In contrast non-monophyly of *Nephrurus s.l.*, (which placed *N. sphyrurus* as a basal lineage within Carphodactylidae) did not significantly reduce likelihood.

Table 1. Results of SH tests for seven different phylogenetic hypotheses of relationships within *Nephrurus s.l.*

Hypothesis	Diff - ln L	significantly worse
Monophyly of:		
"*Underwoodisaurus*"	-42.9	yes
"spiny Knobtails"	-4.62	no
"small tail smooth knobtails"	-7.93	no
"big tail smooth knobtails"	-10.46	no
Non-monophyly of:		
Nephrurus s.l.	-14.63	no
milii and knobtails	-41.83	yes
"smooth Knobtails"	-52.49	yes

Intraspecific genetic diversity

We found evidence for significant intraspecific divergences in the ND2 gene for most taxa sampled (Table 2, Fig 4.). The deepest divergences were found within *N. sphyrurus*, *N. wheeleri* (corresponding to the two recognised subspecies) and *N. milii*. In the case of the latter two taxa divergences were deeper than between the allopatric sister species *N. deleani* and *N. laevissimus* (mean uncorrected distance 8.1%). Within the most widespread species *Nephrurus levis*, we found evidence for significant geographic structure, but also for low genetic diversity over very large areas. While at least one deeply divergent mitochondrial lineage corresponds with a named subspecies, *N. levis occidentalis*, similarly divergent populations elsewhere are currently all ascribed to *N. levis levis*. The uncorrected genetic divergence between two allopatric populations of *N. stellatus* across southern Australia (either side of the Nullarbor Plain) was also comparatively low (~5.3%).

Table 2. Mean and range of ND2 uncorrected distances within recognised species of *Nephrurus*.

	N	P-distance
N. amyae	4	0.020(0.003-0.030)
N. asper	3	0.048 (0.002-0.071)
N. deleani	2	0
N. laevissimus	3	0.068 (0.029-0.086)
N. levis	10	0.046 (0.005-0.064)
N. milii	11	0.094(0.026-0.130)
N. sheai	1	NA
N.sphyrurus	5	0.046 (0.000-0.075)
N. stellatus	4	0.031 (0.004-0.053)
N. vertebralis	2	0.042
N. wheeleri	7	0.053 (0.003-0.104)

Dating

Bayesian dating using BEAST yielded high effective sample sizes (>500) for key parameters such as topology, branch lengths and posterior support values. The maximum credibility tree was similar to that produced by the maximum likelihood and Bayesian analyses. Mean and 95% posterior age distributions for key nodes including priors and posteriors for the calibrations are shown in Table 3. The posterior age estimates of the Pygopodoidea, and for major divergences within this radiation were similar to estimates from Gamble *et al*. (2008) and Oliver and Sanders (2009).

Table 3. Mean and 95% confidence intervals for divergence dates estimates for key nodes.

	Calibration priors normal distribution: zero offset [95% CI]	Posterior probability density: median [95%, HPD]	Oliver and Sanders 2009 Posterior probability density: mean [95%, HPD]
All geckos	120 [97.0, 143.0]	115.8 [94.14, 138.22]	118.1 [88.9, 147.3]
Pygopoids	71.5 [55.9, 87.1]	69.69 [55.38, 83.78]	71.5 [53.2, 91.2]
Diplodactylidae	_	57.67 [43.55, 72.79]	56.9 [41.0, 73.2]
Pygopodidae	_	28.66 [19.27, 39.47]	31.3 [20.4, 44.9]
Carphodactylidae	_	35.1 [23.99, 47.43]	33.3 [20.8, 46.1]
sphyrurus vs other *Nephrurus*	_	24.66 [16.5, 33.67]	_
milii vs knobtails	_	18.65 [12.9, 25.71]	16.48 [NA]
knobtails	_	14.38 [9.73, 19.73]	_
smooth knobtails	_	10.18 [6.61, 14.51]	_

Our relative dating indicated that crown *Nephrurus* (the divergence between *N. sphyrurus* and the other ten species of *Nephrurus*) (95% CI of 16.5-33.7 Mya) is as old or older than almost all other genera of Pygopodoidea, except perhaps the leaf-tail genus *Phyllurus* (Figure 2), while the divergence of *N. milii* from the knob-tailed clade is significantly younger but still relatively old (95% CI of 12.9-25.7 Mya). The three major lineages of knob-tailed species (*N. wheeleri*, the *asper* group and the smooth knob-tails) are all estimated to have diverged through the early to mid Miocene (95% CI of 9.7-19.7 Mya). The crown radiation of major lineages within the smooth knob-tailed geckos appears to have occurred relatively rapidly (short branch lengths and poor support for interrelationships) around the mid to late Miocene (95% CI 6.61-14.51 Mya).

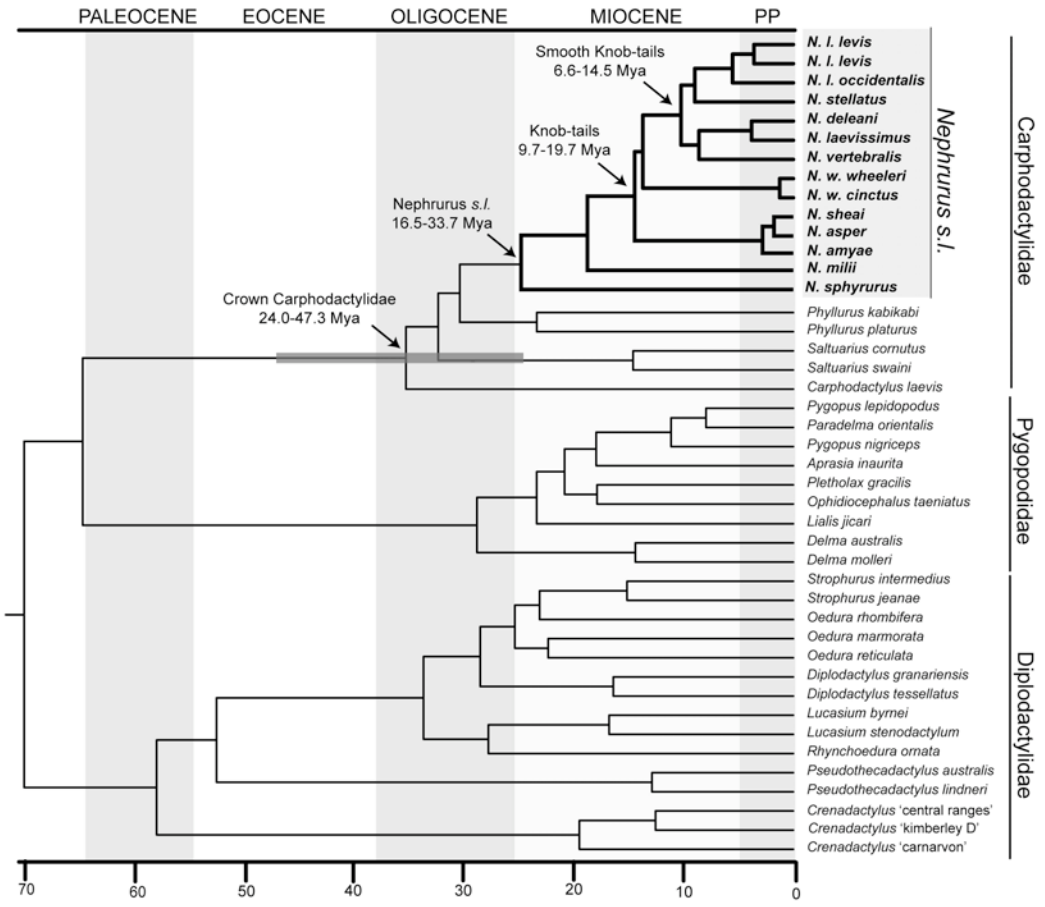

Figure 4. BEAST maximum credibility ultrametric tree for Australian diplodactyloids geckos, including all recognised species of *Nephrurus s.l.*, derived from nuclear dataset and calibrated with secondary basal gekkotan priors. Node bar at base of the Carphodactylidae corresponds to 95% confidence interval for age estimates for the current radiation.

Discussion

The molecular dataset assembled in this paper provides the first robust estimate of phylogenetic diversity and relationships within and between the 11 nominal species of *Nephrurus* (*sensu* Bauer, 1990). These data provide important insight into levels of intraspecific genetic structure; putative morphological groups and generic relationships; the temporal and geographic distribution of major lineages; and the evolution of some key behavioural and morphological characters.

Intraspecific genetic structure and cryptic species

Our data suggests that additional sampling will reveal significant phylogeographic structure in most species of *Nephrurus*. However, in comparison with both relatively closely related pygopoid lineages such as the leaf-tailed geckos (*Phyllurus and Saltuarius*) (Couper *et al.*, 2000, 2008) and broadly co-distributed lineages such as *Lucasium, Diplodactylus,* and *Crenadactylus* (Pepper *et al.*, 2006; Oliver *et al.*, 2009), there is little evidence of deeply divergent, morphologically cryptic species. While there are lineages that require taxonomic assessment (*N. levis, N. milii, N. wheeleri* — most of which have either recognised subspecies or potentially available synonyms) all of the most deeply divergent lineages of *Nephrurus* are both morphologically distinct and named. There is also generally strong support for their monophyly, and the lengths of the intraspecific branches are relatively short compared to the basal branches. Compared to many other pygopoid gecko genera, most speciation events within the *Nephrurus* lineage appear to have been accompanied by at least some relatively obvious morphological and/or ecological differentiation.

'The smooth knob-tails'

Morphological analyses (Bauer, 1990) and molecular evidence both strongly support the monophyly of this group. The five recognised species (*N. deleani, N. laevissimus, N. levis* (with three subspecies), *N. stellatus* and *N. vertebralis*) are characterised by an array of derived morphological characters including the most reduced phalangeal formula in the genus (2.3.3.3.3/2.3.3.3.3), elongate metatarsals relative to phalanges (metatarsals I-IV approximately twice the length of longest phalanx in corresponding digit), relatively smooth skin, and a dorsal colour pattern incorporating three dark dorsal bands across the head, nape and shoulders. These same morphological analyses (Bauer, 1990) failed to strongly support any relationships of species within this group. Molecular data also failed to strongly

resolve relationships within this clade, with the exception of *N. laevissimus* and *N. deleani* which are clearly allopatric sister taxa. Combined likelihood analyses suggest a relationship between *N. stellatus* and *N. levis*, and between *N.deleani*/*N.laevissimus* and *N. vertebralis*, however these were only weakly supported, and further data are required before further conclusions can be drawn.

'The spiny knob-tails'

Bauer (1990) and others (e.g., Greer, 1989) postulated a relationship between two highly rugose knob-tailed lineages; *N. asper* (now including two additional species *N. amyae* and *N. sheai* (Couper and Gregson, 1994)) and *N. wheeleri*. These lineages share extremely rugose skin, have a common phalangeal formula (2.3.4.4.3/2.3.4.4.4) and are generally found in rocky ranges and plains across northern and central Australia. However, while our data could not reject the monophyly of this group, it did not support it. The morphological and ecological characters that unite this group are also either potentially pleisomorphic (preference for rocky ranges and phalangeal formula that is derived, but still potentially ancestral to the more derived smooth knob-tails)), or evolutionarily plastic within the Carphodactylidae and potentially also strongly correlated with ecology (rugosity of skin for camouflage in rocky habits). These two lineages also have many morphological differences, including the configuration of the parietal bones (Bauer, 1990), body size (≤ 103 mm SVL in *N. wheeleri* versus over 135 mm SVL in the *N. asper* group; Annable 2004), and conspicuously different tail morphologies (greatly reduced and lacking autotomy planes in the *asper* group and relatively large and basally autotomic in *wheeleri*). Our combined phylogenetic analysis suggests that *N. wheeleri* is the most basal of these two lineages, (a hypothesis consistent with its less derived tail morphology), however, support was not unequivocal.

'Underwoodisaurus'

Our molecular data unequivocally reject the monophyly of this group, and indicate that these two lineages represent a basal grade to the knob-tailed *Nephrurus*. Contrary to Bauer (1990) many workers have placed *milii* and *sphyrurus* in the genus *Underwoodisaurus* (e.g., Greer 1989; Cogger, 2000; Wilson and Swan, 2008), implicitly suggesting that they represent a monophyletic group. However, in large part the grouping of these taxa appears to have been based a number of shared plesiomorphic characters, such absence of a terminal knob on the tail, enlarged labial scales, transverse subdigital lamellae and the generalised squamate phalangeal formula (2.3.4.5.3/2.3.4.5.4). If these sympleisomorphies are disregarded, there are a number of significant differences in tail morphology (shape, degree of tuberculation and number of vertebrae), scalation (especially around the snout), colouration and ecology between these two species, which further support the relative distinctiveness of these two taxa (Bauer, 1990). In addition, *milii* shares a number of derived features with the knob-tailed taxa that are absent in *sphyrurus*, including the reduction or loss of pleurapophysis on the post-pygal caudal vertebrae, and a perforated interclavicle.

Generic taxonomy

While our data strongly reject the monophyly of *milii* and *sphyrurus*, the two species frequently placed together in the genus '*Underwoodisaurus*', are morphologically divergent from the knob-tailed *Nephrurus* (see above; Bauer, 1990). Relative dating also indicates that *sphyrurus* and *milii* diverged from each other, and from other '*Nephrurus'* before the crown radiation within many other recognised Australian pygopodoid gecko genera (Fig. 2). To best reflect the phylogenetic and morphological divergence of these two lineages we propose a revised generic taxonomy restricting *Nephrurus* to the knob-tailed clade and *Underwoodisaurus* to *milii*, and erect a new genus for *sphyrurus*.

In addition to further skeletal characters given in Bauer (1990) all *Nephrurus s.l* differ from other carphodactylid genera by a suite of external characters. The leaf-tailed geckos (*Orraya*, *Phyllurus* and *Saltuarius*) are

diagnosable by the combination of depressed body and tail, long laterally compressed toes, kinked proximal joints of manus and pes, and enlarged subdigital lamellae. Monotypic *Carphodactylus* has an elongate laterally compressed tail without spinose scales, enlarged mid-dorsal scales forming a low crest, transverse subdigital lamellae and weakly developed preanal organs (against no preanal organs).

All further morphological descriptions below are for comparison between *Nephrurus s.l.* only. Synonymies are abbreviated to primary synonyms. Complete summaries of citations, spelling lapses and emendations for older names are provided in Bauer and Henle (1994). All definitions below are branch-based (http://www.ohio.edu/phylocode/).

Nephrurus Günther 1876

Type species *Nephrurus asper* Günther, 1876 by monotypy. Type locality "Peak Downs", Queensland, Australia.

Included species: *amyae* Couper 1994, *asper* Günther 1876, *deleani* Harvey 1983, *laevissimus* Mertens 1958, *levis* De Vis 1886, *sheai* Couper 1994, *stellatus* Storr 1968, *vertebralis* Storr 1963, *wheeleri* Loveridge 1932.

Definition: The most inclusive clade containing *Nephrurus asper* Günther 1876, but not *Underwoodisaurus milii* or *Uvidodactylus* gen. nov. *sphyrurus*

Diagnosis: A genus of moderately to very large (adult SVL 80-137mm) carphodactylid geckos; ventral toe scalation spinose; toes relatively short and rounded in cross section; phalangeal formula reduced (2.3.4.4.3/2.3.4.4.4 or 2.3.3.3.3/2.3.3.3.3); anterior loreals much smaller than posterior loreals, labial scales only slightly larger than neighbouring scales, 25-26 presacral vertebrae, original tail highly variable, ranging from extemely vestigal to relatively large and fat with 20-32 postsacral vertebrae, post-pygal pleurapophysis absent or reduced, but always terminating in a small knob.

Underwoodisaurus Wermuth, 1965

Type species *Phyllurus milii* Bory de Saint-Vincent 1823 by original designation. Original *t*ype locality “Australasie sur les rives de la baie des Chiens-Marins” [= Shark Bay, Western Australia]. Neotype locality: “Bernier Island, Shark Bay”. Note: Shea (2002) demonstrated that the type of *P. milii* was, in fact, a specimen of *Nephrurus levis occidentalis* but maintained current usage of the epithet *mili* for the species of *Underwoodisdaurus* to which it has uniformly been applied, and designated a neotype to fix the name.

Anomalurus Fitzinger, 1843 (non *Anomalurus* Waterhouse 1843 (Mammalia)). Type species *Gymnodactylus Miliusii* Duméril and Bibron, 1836 = *Phyllurus milii* Bory de Saint-Vincent 1825 by monotypy.

Definition: The most inclusive clade containing *Underwoodisaurus milii* (Bory de Saint-Vincent 1823), but not *Nephrurus asper* or *Uvidodactylus* gen. nov. *sphyrurus*.

Diagnosis: A monotypic genus containing only *milii* (Bory de Saint-Vincent 1825). A moderately large (Adult SVL to 100mm) genus of carphodactylid geckos with transverse subdigital lamellae, anterior loreals minute and strongly differentiated from posterior loreals, labial scales much larger than neighbouring scales, mean of 26 presacral vertebrae, phalangeal formula unreduced (2.3.4.5.3/ 2.3.4.5.4), and original tail long with 33-42 postsacral vertebrae, post-pygal pleurapophysis absent or reduced, rounded in cross section, and gradually tapering to tip lacking a terminal 'knob'.

***Uvidodactylus* gen. nov.**

Type species *Gymnodactylus sphyrurus* Ogilby, 1892 here designated. Type locality “interior of New South Wales (Tumut? [in error, fide Cogger et al. 1983])”, Australia.

Etymology: Derived from the Latin *uvidus*, meaning moist or humid, and the Greek *dactyl*, meaning fingers. In reference to the restricted range of this gecko in relatively mesic and cool highland areas of the central Great Dividing Range of eastern Australia.

Definition: The most inclusive clade containing *Uvidodactylus sphyrurus* Ogilby, 1892 but not *Nephrurus asper* or *Underwoodisaurus milii*.

Diagnosis: A monotypic genus containing only *sphyrurus* Ogilby 1892. A small (adult SVL to 70mm) genus of carphodactylid geckos with transverse subdigital lamellae, anterior loreals only slightly smaller than posterior loreals, labial scales much larger than neighbouring scales, mean of 26 presacral vertebrae, phalangeal formula unreduced (2.3.4.5.3/ 2.3.4.5.4), and original tail short with 26 or fewer postsacral vertebrae, pleurapophyses borne on basal post-pygal vertebrae, depressed proximally, rectangular in dorsal view and sharply tapering to tip lacking terminal 'knob'.

Temporal and ecological transition into the arid zone

While their precise relationships to the *Nephrurus* clade (*s.l.*) are incompletely resolved, the distribution of all other extant lineages of carphodactylids (*Carphodactylus*, *Orraya*, *Phyllurus* and *Saltuarius*) is centred upon the relatively cool areas of 'aseasonal wet forest' biome on the Australian east coast (Wilson and Swan, 2007; Crisp *et al*., 2004). *Uvidodactylus* is restricted to similar, although potentially slightly more arid and strongly seasonal habitats in the cool temperate uplands of the New England Plateau. In contrast *Underwoodisaurus* has a very wide distribution spanning temperate to southern arid areas. The distribution of these lineages, and especially *Underwoodisaurus*, suggests that the ancestors of *Nephrurus* clade were already accumulating adaptations to significantly arid conditions through the very late Oligocene and early to mid-Miocene.

Away from few areas of eastern Australia, the next most derived lineages, the *asper* group and *N. wheeleri* are almost entirely restricted to areas with extensive rocky outcropping in both arid and seasonally arid areas across northern Australia. These lineages are clearly somewhat adapted to aridity, but have been unable to colonise habitats that lack at least some hard rocky substrate.

In contrast to other lineages of *Nephrurus*, the most diverse and derived clade, the smooth knob-tails, have a massive distribution centred on vast sandy deserts of arid Australia. This lineage also displays significant intraclade morphological diversity and broad sympatry of least two, and sometimes more, species (the only instance of extensive sympatry of congeneric species in the family Carphodactylidae); suggesting some ecological displacement and diversification has occurred within the arid zone. Our phylogenetic reconstruction and date estimates suggest this group underwent a relatively rapid radiation of extant species groups (basal polytomy) around in the late Miocene around 6.7-14.6 Mya. This estimate is consistent with recent suggestions that the Australia arid zone underwent a major expansion in the late Miocene, approximately 10-6 Mya (Byrne *et al.*, 2008).

A number of recent papers have presented a temporal scale for the transition of the Australian environment from predominantly mesic to predominantly arid (Martin, 2006; Byrne *et al.*, 2008). These have emphasised that this transition began as early as the mid Miocene (~20 Mya), and probably involved a gradual progression through a range of increasingly arid climatic regimes, ranging through ancestral wet, seasonally arid and semi-arid, with the current expansive, sandy arid-zone viewed as a relatively recent endpoint of the process (Martin, 2006; Byrne, *et al*. 2008). The temporal scale of divergence between lineages of carphodactylid gecko broadly mirrors our current understanding of this environmental transition; and provides a conceivable model for how other ancient mesic clades may have gradually accumulated adaptations to persist, and subsequently diversify, in this changing environment.

Pre-adaptations to aridity?

Successful colonisation of new biomes is evolutionarily rare, and often requires novel ecological and morphological traits (Lamb and Bauer, 2006; Jordan *et al.*, 2008). A number of studies have indicated that the Australian arid zone is dominated by a small number of ancestrally mesic lineages that have probably developed specific adaptations to aridity (Martin, 2006; Rabosky *et al.*, 2007; Jordan *et al.*, 2008). In addition to less obvious physiological and behavoural adaptations, in the case of *Nephrurus* there are two major aspects of their ecology that may have played a key role.

Most extant Carphodactylidae are largely arboreal or saxicolous, and have specialised adaptations for such an existence, including robust decurved claws and (usually) a depressed body-plan (Bauer, 1990). In contrast, *Nephrurus s.l*, were clearly terrestrial by the late Oligocene to early Miocene. As terrestrial niches are relatively more abundant within the arid biome than within other regions of Australia, and often less vulnerable to desiccation than arboreal niches; this exadaptation may have favoured the success of *Nephrurus* in the expanding arid biome. While geckos in general are climbing lizards, the transition to a predominately terrestrial lifestyle has occurred convergently in several lineages, and is particularly well documented in several arid zone radiations (Lamb and Bauer, 2006). Dating analyses, fossils and the distribution of divergent taxa also indicate that some of the most successful vertebrate radiations in the Australian arid zone are probably ancestrally terrestrial (Rabosky *et al.*, 2007), or terrestrial lineages within largely arboreal/saxicolous clades (e.g., the macropods (Meredith *et al.*, 2008) and the gecko clade comprising *Diplodactylus*, *Lucasium* and *Rhynchoedura* within the Diplodactylidae (Oliver and Sanders, 2009)).

Access to relatively cool and moist refugia is also of critical importance to many arid zone taxa in avoiding desiccation and/or heat stress. Field observations indicate the many Australian pygopoid geckos (including the smooth knob-tails) are most active on warm humid overcast nights (Pianka and Pianka, 1976), and captive observations indicate that smooth knob-tails are highly susceptible to desiccation if not given suitably moist refugia (Porter, 2008). The smooth knob-

tails have a suite of morphological specialisations such as digital reduction, spinose subdigital scales and a significantly expanded brillar fold above the eye, that allow them to dig their own refugia in sandy substrates (Bauer, 1990; Bauer and Russell, 1991). During times of dryness and inactivity they can even seal the entrance to their burrows; this is likely to reduce the risk of both predation and desiccation. The spiny *Nephrurus, Underwoodisaurus* and *Uvidodactylus* lack the ability to dig well and are largely restricted to habitats within arid and semi-arid Australia that provide suitable refugia for large bodied geckos, such as rocky outcrops or scree fields. It seems likely that the relative success of smooth knob-tails in the arid zone is significantly attributable to their unique ability to create and maintain their own micro-refugia in an otherwise inhospitable environment.

The deep divergence between lineages of smooth knob-tail geckos, and other Australian arid-zone lizards (e.g *Lerista*, Skinner *et al.*, 2009) with specialisations for burrowing in loose substrates, suggests that extensive areas of sand have been present since the mid to late Miocene. However, while large fields of stablised or mobile dunes are now a dominant feature of the central Australian arid zone, there is little historical evidence of these habitats from before the late Pleistocene to early Pliocene (Byrne *et al.*, 2008). However, representatives of *Nephrurus* (*N. levis* and *N. stellatus*) and *Lerista* (Wilson and Swan *et al*., 2007) now occur extensively in coastal dune systems and these areas may have been the source of origin for this suite of lineages preadapted to sandy deserts (as been hypothesised elsewhere; Martin, 2006; Byrne *et al.*, 2008). An additional, and not necessarily exclusive explanation is simply that sand dunes are a relatively ancient part of the arid zone, but evidence of these highly mobile and unstable environments has not preserved well.

Evolution of the tail

The most distinctive morphological feature of *Nephrurus* is the unique enlarged terminal 'knob' on the tail (Russell and Bauer 1988). The knob-tailed *Nephrurus* form a strongly supported monophyletic clade based on both molecular (this study)

and morphological (Bauer 1990) data; indicating this feature has evolved only once within *Nephrurus*. However, assuming that the tail is primitively relatively wide and used in fat storage (as it is in both *U. milii* and *U. sphyrurus*, many other carphodactylids, and other geckos) our study indicates that three independent lineages of *Nephrurus* have evolved greatly reduced tails; the '*N. asper*' group, and two lineages of smooth knob-tails, the sister taxon pair *N. deleani* and *N. laevissimus*, and the relatively distantly related *N. stellatus.* Whatever the exact trajectory of evolution, this plasticity (and a well documented reduction in the frequency of autonomy) suggests the tail has lost its traditional functions of defense and fat-storage on a number of separate occasions. However, while this independent reduction is probably associated with the terminal knob, the function (if indeed it has one) of this bizarre structure remains an enduring mystery.

Acknowledgements

We thank Paul Doughty, Brad Maryan (Western Australian Museum), Terry Bertozzi and Steve Donnellan for providing access to specimens, and Adam Skinner, Kate Sanders, Mark Hutchinson, Mike Lee and especially Andrew Hugall, for advice and various assistances. This work was supported by grants from the Australia Pacific Science Foundation to Paul Doughty, Paul Oliver, Andrew Hugall, Mike Lee and Mark Adams, and from the Australian Biological resources survey to Mark Hutchinson, Paul Oliver, Paul Doughty and Mark Adams.

References

Annable, T.J. 2004. Reproductive biology of *Nephrurus* and *Underwoodisaurus* geckos (Reptilia: Gekkonidae: Carphodactylini). Ph.D. dissertation, University of Sydney.

Bauer, A. M. 1990. Phylogenetic systematics and biogeography of the Carphodactylini (Reptilia: Gekkonidae). *Bonner Zoologische Monographien.* **30**: 1-218.

Bauer, A. M. and Henle, K. 1994. *Das Tierreich. Gekkonidae (Vol 1: Australia and the Pacific).* Walter De Gruyter Publishers, Berlin. 306p.

Bauer, A. M. and Russell, A. P. 1988. Morphology of gekkonid cutaneous sensilla, with comments on function and phylogeny in the Carphodactylini (Reptilia: Gekkonidae). *Can. J. Zool.* **66**: 1583-1588.

Bauer, A. M. and Russell, A. P. 1991. Pedal specialisations in dune-dwelling geckos. *J. Arid Environm.* **20**: 43-62.

Byrne, M., Yeates, D.K., Joseph, L., Kearney, M., Bowler, J., Williams, M.A.J., Cooper, S., Donnellan, S.C., Keogh, J.S., Leys, R., Melville, J., Murphy, D.J., Porch, N. & Wyroll, K.H. 2008 Birth of a biome: insights into the assembly and maintenance of the Australia arid zone biota. *Mol. Ecol*. **17**: 4398-4417.

Cogger, H.G. 2000. *Reptiles and amphibians of Australia.* (6th Edn). (Reed New Holland: Sydney).

Couper, P.J. & Gregson, R.A.M. 1994. Redescription of *Nephrurus asper* Gunther, and description of *N. amyae* sp. nov. and *N. sheai* sp. nov. *Memoirs of the Queensland Museum*. **37**: 53-67.

Couper, P.J., Schneider, C.J., Hoskin, C.J. & Covaecevich, J.A. 2000. Australian leaf-tailed geckos: phylogeny, a new genus, two new species and other new data. *Memoirs of the Queensland Museum*. **45**: 253-265.

Couper, P., Sadlier, R., Shea, G.M., Worthington Wilmer, J. 2008. A reassessment of *Salturius swaini* (Lacertilia: Diplodactylidae) in southeastern Queensland and New South Wales; Two new taxa, phylogeny, biogeography and conservation. *Rec. Aust. Mus*. **60**: 87-118.

Couper, P.J. & Hoskin, C.J. 2009. Litho-refugia: the importance of rock landscapes for the long-term persistence of Australian Rainforest fauna. *Australian Zoologist*. **34**: 554-560.

Crisp, M., Cook, L. & Steane, D. 2004. Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present day communities? *Phil. Trans. Roy. Soc. B.* **359**: 1559-1571.

Crisp, M.D., Arroyo, M.T.D., Cook, L.G., Gandolfo, M.A., Jordan, G.J., CcGlone, M.S., Weston, P.H., Westoby, M., Wilf, P. & Linder, H.P. 2009. Phylogenetic biome conservatism on a global scale. *Nature*. **458**: 754-756.

Drummond, A.J. & Rambaut, A. 2006. BEAST v 1.4. Available from http://beast.bio.ed.ac.uk/ (last accessed 27/7/08).

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology*. **4**:5, e88 doi:10.1371/journal.pbio.0040088.

Gamble, T.P., Bauer, A.M., Greenbaum, E. & Jackman, T.R. 2008. Evidence of Gondwanan vicarience in an ancient clade of gecko lizards. *J. Biogeog.* **35**: 88-104.

Greer, A. E. 1989. *The biology and evolution of Australian lizards.* Surrey Beatty and Sons, Sydney.

Han, D., Zhou, K. & Bauer, A. M. 2004. Phylogenetic relationships among gekkotan lizards inferred from *C-mos* nuclear DNA sequences and a new classification of the Gekkota. *Biol. J. Linn. Soc.* **83**: 353-368.

Hill, R.S. 1994. The history of selected Australian taxa. In 'History of the Australian Vegetation: Cretaceous to recent (ed. Hill RS), pp 390-419. Cambridge University Press, Cambridge, UK.

Holder, L.A. 1960. The comparative morphology of the axial skeleton in Australian Gekkonidae. *Zool. J. Linn. Soc.* **44**: 300-35.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*. **17**: 754-755.

Jansa, S.A., Barker, F.K., & Heaney, L.R. 2006. The pattern and timing of diversification of Phillipene endemic rodents: evidence from mitochondrial and nuclear gene sequences. *Syst. Biol*. **55**: 73-88.

Jordan, G.J., Weston, P.H., Carpenter, R.J., Dillon, R.A. & Brodribb, T.J. 2008. The evolutionary relations of sunken, covered, and encrypted stomata to dry habitats in Proteaceae. *Amer. J. Bot.* **95**: 521-530.

King, M. 1987. Origin of the Gekkonidae: chromosomal and albumin-evolution suggests Gondwanaland. *Search*. **18**: 252-254.

Lamb, T. & Bauer, A.M. 2006. Footprints in the sand: independent reduction of subdigital lamellae in the Namib-Kalahari burrowing geckos. *Proc. Roy. Soc. B.* **273**: 855-864.

Lee, M.S.Y., Oliver, P.M. & Hutchinson, M.N. 2009. Phylogenetic uncertainty and molecular clock calibrations: a case study of legless lizards (Pygopodidae, Gekkota). *Mol. Phylogenet. Evol.* **50**: 661-666.

Losos, J.B., Leal, M., Glor, R.E., de Queiroz, K., Hertz, P.E., Rodríquez Schettino, L., Jackman, T.R. & Larson, A. 2003. Niche lability in the evolution of a Caribbean lizard community. *Nature*. **424**: 542-545.

Maddison, D.R. & Maddison, W.P. 2005. MacClade V. 4.0. Sinauer Associates, Sunderland, MA.

Martin, H.A. 2006. Cenzoic climate changes and the development of the arid vegetation of Australia. *Journal of arid environments*. **66**: 533-563.

Melville, J., Schulte, J. A. & Larson, A. 2004. A molecular study of phylogenetic relationships and the evolution of antipredator strategies in Australian *Diplodactylus* geckos, subgenus *Strophurus*. *Biol. J. Linn. Soc.* **82**: 123-138.

Meredith , R.W., Westerman, M. & Springer, M.S. 2008. A phylogeny and timescale for the living genera of kangaroos and kin (Macropodiformes: Marsupialia) based on nuclear DNA sequences. *Aust. J. Zool*. **56**: 395-410.

Nylander, J.A.A. (2004) *MrModeltest*. Avaliable at: Avaliable at ://www.abc.se/~nylander (last accessed 18/03/2002).

Oliver, P., Hugall, A., Adams, M., Cooper, S. J. B. & Hutchinson, M. 2007. Genetic elucidation of ancient and cryptic diversity in a group of Australian geckos: the *Diplodactylus vittatus* complex. *Mol. Phylogenet. Evol.* **44**: 77-88.

Oliver, P., Doughty, P., Hutchinson, M.N., Lee, M.S.Y, & Adams, A. 2009. The taxonomic impediment in vertebrates: DNA sequence, allozyme and chromosomal data double estimates of species diversity in a lineage of Australian lizards (*Diplodactylus*, Gekkota). *Proc. Roy. Soc. B.* **276**: 2001-2007.

Oliver, P.M. & Sanders, K.L. 2009. Molecular evidence for multiple lineages within a diverse Australasian gecko radiation. *J. Biogeog*. **36**: 2044-2055.

Pianka, E.R. & Pianka, H.D. 1976. Comparative ecology of twelve species of nocturnal lizards (Gekkonidae) in the Western Australian desert. *Copeia.* **1976**: 125-142.

Pepper, M., Doughty, P. & Keogh, J. S. 2006. Molecular systematics and phylogeography of the Australian *Diplodactylus stenodactylus* (Gekkota: Reptilia) species-group based on mitochondrial and nuclear genes reveals an ancient split between Pilbara and non-Pilbara *D. stenodactylus*. *Mol. Phylogenet. Evol.* **41**: 539-555.

Porter, R. 2008. *Keeping Australian Geckos*. Australian Reptile Keeper Publications, Bendigo, Victoria.

Posada, D. & Buckley, T.R. 2004. Model selection and model averaging in phylogenetics: advantages of AIC and Bayesian approaches over likelihood ratio tests. *Syst. Biol*. **53**: 793-808.

Rabosky, D.L., Donnellan, S.C., Talaba, A.L. & Lovette, I.J. 2007. Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. *Proc. Roy. Soc. B.* **274**: 2915-23.

Russell, A.P. & Bauer, A.M. 1988. Caudal morphology of the knob-tailed geckos, genus *Nephrurus* (Reptilia: Gekkonidae), with special reference to the tail tip. *Aus. J. Zool.* **35**: 541-551.

Sanders, K.L. & Lee, M.S.Y. 2008. Molecular evidence for a rapid late-Miocene radiation of Australasian venomous snakes (Elapidae, Colubroidea). *Molecular Phylogenet. Evol*. **46**: 1165-1173.

Shea, G.M. 2002. The identity of *Phyllurus milii* Bory de Saint Vincent, 1823 (Squamata: Pygopodidae: Diplodactylinae). *Rec. W. Aust. Mus.* **20**: 431-436.

Shimodaira, H. & Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* **16**: 114-1116.

Skinner, A., Lee, M.S.Y., Hutchinson, M.N. 2008. Rapid and repeated limb loss in a clade of scincid lizards. BMC Evolutionary Biology **8**: 310,1-9.

Stamatakis, A. 2006. RaxML-VI-HPC: Maximum Likelihood-based Phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*. **22**: 2688-2690.

Vidal, N. & Hedges S.B. 2009. The molecular evolutionary tree of lizards, snakes, and amphisbaenians. *C.R.Biologies*. **332**: 129-139.

Wiens JJ, Fetzner JW, Parkinson CL, Reeder TW (2005) Hylid frog phylogeny and sampling strategies for speciose clades. *Syst. Biol*. **54**: 719-748.

Wilson, S. & Swan, G. 2008. *A complete guide to reptiles of Australia*, 2nd edn. New Holland Press, Sydney.

Appendix I. Specimen and sequence details for Carphodactylid geckos sampled in this study.

Species	Specimen	Tissue	Locality	State	*ND2*	*RAG-1*	*C-mos*
Carphodactylus laevis	QMJ8944	ABTC16380	Lake Barrine, QLD	QLD	AY369017	FJ855442	AF039467
Nephrurus amyae	NTMR18239	ABTC30224	Ross River Hwy 9k N	NT	xxx	_	_
Nephrurus amyae	NTMR18299	ABTC30282	Finke Gorge NP	NT	xxx	xxx	xxx
Nephrurus amyae	NA	ABTC12584	Kings Creek Stn	NT	xxx	_	_
Nephrurus amyae	WAMR166333	ABTC91586	Kutjuntari Rockhole	**WA**	xxx	_	_
Nephrurus asper	QMJ54644	ABTC31875	Heathlands	QLD	xxx	_	_
Nephrurus asper	SAMAR55649	ABTC76958	10k W Isaac R on Suttor Developmental Rd	QLD	xxx	FJ855445	FJ855465
Nephrurus asper	SAMAR54559	ABTC72987	Dawson Development Rd 18k E Alpha T/off	QLD	xxx	_	_
Nephrurus deleani	SAMAR53735	ABTC70030	Bellamy Creek	SA	xxx	_	_
Nephrurus deleani	SAMAR47063	ABTC58420	Edge L MacFarlane	SA	xxx	xxx	xxx
Nephrurus laevissimus	SAMAR31893	ABTC64053	11km NE Mt. Finke	SA	xxx	xxx	xxx
Nephrurus laevissimus	WAM R139007	WAM R139007	Mandora	WA	xxx	_	_
Nephrurus laevissimus	WAM R146821	WAM R146821	Yeo Lake Road	WA	AY369020	_	_
Nephrurus levis levis	SAMAR36370	ABTC00575	Cooper Ck	SA	xxx	_	_
Nephrurus levis levis	SAMAR58994	ABTC80692	57.4 Km WNW Oak Valley	SA	xxx	_	_
Nephrurus levis levis	QMJ48541	ABTC16333	Naccowlah, 36km WNW Jackson	QLD	xxx	_	_
Nephrurus levis levis	AMSR118594	ABTC59745	Coonbah	NSW	xxx	xxx	xxx
Nephrurus levis levis	SAMAR43823	ABTC59642	41K W Vokes Hill Jtn,Unamed CP	SA	xxx	_	_
Nephrurus levis levis	SAMAR19968	ABTC52254	Indooroopilly Outstn	SA	AY369018	_	_
Nephrurus levis levis	WAM R110633	WAM R110633	Tanami Desert	WA	xxx	_	_

Nephrurus levis levis	WAM R137922	WAM R137922	Telfer	WA	xxx	xxx	xxx
Nephrurus levis occidentalis	WAM R139548	WAM R139548	Giralia Station	WA	xxx	xxx	xxx
Nephrurus levis occidentalis	WAM R130270	WAM R130270	Northhampton	WA	xxx	_	_
Nephrurus sheai	WAM R156744	WAM R156744	Oscar Range	WA	xxx	_	_
Nephrurus sheai	QMJ57515	ABTC31893	10k SE Oenpelli	NT	_	xxx	xxx
Nephrurus stellatus	SAMA R61175	ABTC87145	14.9k E Pidinga Tank	SA	xxx	_	_
Nephrurus stellatus	NA	ABTC89286	8k SW Moonabie HS	SA	xxx	_	_
Nephrurus stellatus	SAMA R36563	ABTC56784	7.5k N Courtabie	SA	xxx	FJ855446	FJ855466
Nephrurus stellatus	WAM R151430	WAM R151430	Bungalbin Hill	WA	xxx	_	_
Nephrurus vertebralis	WAM R127566	WAM R127566	Goongarrie	WA	xxx	_	_
Nephrurus vertebralis	WAM R146822	WAM R146822	Banjawarn Station	WA	AY369019	_	_
Nephrurus wheeleri cinctus	WAM R146588	WAM R146588	231km ssw Port Hedland	WA	xxx	xxx	xxx
Nephrurus wheeleri cinctus	WAM R97776	ABTC15021	Coolawanyah, WA.	WA	xxx	_	_
Nephrurus wheeleri wheeleri	WAM R146823	WAM R146823	Wydgee Station	WA	AY369021	_	_
Nephrurus wheeleri wheeleri	WAM R137379	WAM R137379	Yuinmery Station	WA	xxx	xxx	xxx
Nephrurus wheeleri wheeleri	SAMAR29494	ABTC52440	47k NNE Leonora	WA	xxx	_	_
Nephrurus wheeleri wheeleri	SAMAR34042	ABTC11715	Yoothapinna H/S. WA.	WA	xxx	_	_
Nephrurus wheeleri wheeleri	WAMR97737	ABTC15093	Cunyu H/S. WA.	WA	xxx	_	_
Underwoodisaurus mili	SAMAR22885	ABTC52334	Canning Dam, Darling Rngs	WA	xxx	_	_
Underwoodisaurus mili	SAMAR18741	ABTC52247	3k S Penneshaw Kangaroo Is	SA	xxx	_	_
Underwoodisaurus mili	SAMAR19395	ABTC52248	Taylors Is	SA	AY369022	_	_
Underwoodisaurus mili	NA	ABTC59699	Nyngan tip	NSW	xxx	_	_
Underwoodisaurus mili B	SAMAR38006	ABTC57066	17k SE Burra	SA	xxx	FJ571622	FJ571637

Underwoodisaurus mili	SAMAR53051	ABTC68211	20k NW Leigh Creek	SA	xxx	_	_
Underwoodisaurus mili	SAMAR26394	ABTC40641	24km N Hughes	SA	xxx	_	_
Underwoodisaurus mili	SAMAR58455	ABTC80186	9.2 km NNE Round Hill	SA	xxx	_	_
Underwoodisaurus mili	SAMAR43990	ABTC12818	Melville Caves Kooyoora State Pk	VIC	xxx	_	_
Underwoodisaurus mili	NA	ABTC41222	38km ENE Laverton, WA.	WA	xxx	_	_
Underwoodisaurus mili A	NA	ABTC80807	Packsaddle Range 100Km NW of Newman	WA	xxx	_	_
Uvidodactylus sphyrurus B	NA	ABTC81117	Attunga SF	NSW	xxx	xxx	_
Uvidodactylus sphyrurus	NA	ABTC81118	Bolivia Hill	NSW	xxx	_	_
Uvidodactylus sphyrurus A	NA	ABTC80812	Bolivia Hill	NSW	xxx	_	_
Uvidodactylus sphyrurus	NA	ABTC80811	Attunga SF	NSW	xxx	_	_
Uvidodactylus sphyrurus	NA	ABTC80809	Attunga SF	NSW	xxx	_	_
Phyllurus amnicola	QMJ64406	ABTC80478	Mt Elliot	QLD	xxx	_	_
Phyllurus kabikabi	NA	ABTC51253	Crediton	QLD	xxx	xxx	_
Phyllurus platurus	NA	ABTC51012	Bents Basin	NSW	xxx	FJ855443	_
Phyllurus platurus	NA	NA	NA	NSW	_	_	AY172942
Saltuarius cornutus	QMJ60629	ABTC32154	Mt Boolbun South QLD	QLD	xxx	xxx	xxx
Saltuarius swaini	SAMAR29204	ABTC11519	Wiangaree	NSW	AY369023	xxx	xxx
Saltuarius wyberba	QMJ61542	ABTC32175	Girrawen NP, QLD	QLD	xxx	_	_

Appendix II. Other gekkotans sequences included in this study.

Taxon	Specimen	*RAG-1*	*c-mos*	*ND2*
Diplodactylids				
Crenadactylus o. horni	SAMA R22245	AY662627	FJ57641	AY369016
Crenadactylus o. naso	AMS R126186	FJ855458	FJ855479	xxxx
Crenadactylus o. ocellatus	WAM R135495	FJ855457	FJ855478	xxxxx
Diplodactylus granariensis	WAMR127572	FJ855452	FJ855473	_
Diplodactylus granariensis	WAMR144551	_	__	EF532870
Diplodactylus tessellatus	SAMAR41130	FJ571624	FJ571639	AY134607
Lucasium byrnei	SAMA R52296	FJ855453	FJ855474	EF681801
Luscasium stenodactylum	NTMR26116	FJ855454	FJ855475	xxxxx
Oedura marmorata	SAMAR34209	FJ571623	FJ571638	AY369015
Oedura reticulata	SAMA R23035	FJ855450	FJ855471	EF681803
Oedura rhombifer	SAMA R34513	FJ855451	FJ855472	xxxxx
Pseudothecadactylus australis	QMJ57120	FJ855449	FJ855470	xxxxx
Pseudothecadactylus lindneri	AMS90915	AY662626	FJ855469	AY369024
Rhychoedura ornata	SAMAR36873	FJ855455	FJ855476	_
Rhychoedura ornata	ANWCR6141	_	_	AY369014
Strophurus intermedius	SAMAR28963	FJ571625	FJ571640	_
Strophurus intermedius	SAMAR22768	_	_	AY369001
Strophurus jeanae	SAMAR53984	FJ855456	FJ855477	_
Pygopodids				
Aprasia inaurita	SAMAR40729	FJ571632	FJ571646	_
Aprasia inaurita	SAMAR47087	_	_	AY134574
Delma australis	SAMAR22784	FJ571633	FJ571647	AY134582
Delma molleri	SAMAR23137	FJ571635	FJ571649	AY134593
Lialis jicari	TNHC59426	AY662628	_	_
Lialis jicari	NA	_	AY134564	AY134600
Ophidiocephalus taeniatus	SAMAR44653	FJ571630	FJ571645	AY134601
Pletholax gracilis	WAM R104374	FJ571631	_	AY134602
Pletholax gracilis	WBJ-2483	_	AY134566	_
Paradelma orientalis	QMJ56089	FJ571626	FJ571642	AY134605
Pygopus lepidopodus	WAM R90378	FJ571627	FJ571643	_
Pygopus lepidopodus	WBJ-1206	_	_	AY134603
Other gekkonids				
Gehyra variegata	SAMAR54022	FJ855439	FJ855460	_

Gehyra variegata	ANWCR6138	_	_	AY369026
Gekko gekko	MVZ215314	AY662625	_	AF114249
Gekko gekko	FMNH258696	_	AY444028	_
Teratoscincus przewalski	CAS171010	AY662624	AY662569	U71326
Sphaerodactylus shreveri	SBH194572	AY662623	AY662547	AY662547
Other squamates				
Dibamus sp.	MVZ	AY662645	AY662574	AY662562

Appendix III. Primers used in this study.

Gene(s)	Primer	Reference
ND2	5'- AAGCTTTCGGGGCCCATACC -3'	see Pepper et al. 2006
	5'- CTAAAATRTTRCGGGATCGAGGCC -3'	
RAG-1	G755 – AAG TTT TCA GAA TGG AAG TTY AAG CTN TT	see Oliver and Sanders, 2009
	G756 – TCT CCA CCT TCT TCY TTN TCA GCA AA	
	G1278 – TGA TGC AAR AAY CCT TTC AGA	
	G1279 – TCT CCA CCT TCT TCT TTC TCA G	
	G889 – AAA GGT GGA CGC CCT AGG CAR CA	
	G883 – TCA TGG TCA GAT TCA TCA GCN ARC AT	
c-mos	G303 5'– ATT ATG CCA TCM CCT MTT CC-3'	see Oliver and Sanders, 2009
	G74 5'– TGA GCA TCC AAA GTC TCC AAT C-3'	
	G708 5'– GCT ACA TCA GCT CTC CAR CA-3'	
	G1092 5'– CTTTTGTCCGATGGCTGAGTC-3'	

CHAPTER 7: CONCLUDING DISCUSSION

This thesis comprises a compilation of published or submitted papers. As each of these papers already includes a discussion of the significance of key findings, in the following chapter I attempt to synthesise the broader relevance of my work in terms of the overall aims of this thesis, and to highlight areas in which there is obvious scope for further research.

7.1 Summary of aims of thesis

In this thesis I set out to examine the diversity, relationships and history of the Australian pygopodoid geckos. In particular I sought to (1) examine interfamilial and intergeneric divergences across the clade, (2) resolve interspecific and intrageneric relationships, and generic boundaries, in the problematic genera *Diplodactylus* and *Nephrurus*; (3) use molecular genetic techniques to assess levels of cryptic diversity within the genera *Diplodactylus* and *Crenadactylus*; and (4) use this data to understand how historical environmental events may have affected evolution within this clade of geckos.

7.2 Phylogenetic relationship of the pygopodoids to other gekkotans.

The results presented in Chapter 2, in addition to two other papers published while data for this thesis were being gathered (Gamble *et al.* 2008a,b), unequivocally indicate that the pygopodoids (*sensu* Chapter 1) are both monophyletic, and the sister lineage to all other living geckos. This is in contrast to older morphologically based classifications which regarded the basal split amongst extant gekkotans to be between the eyelid geckos (Eublepharidae) and all other lineages (Kluge 1987). As eublepharids possess eyelids, the sister relationships between this lineage and clade containing the gekkotan families Gekkonidae, Phyllodactylidae and Sphaerodactylidae (all lacking eyelids), exclusive to the Pygopodoids (again lacking eyelids) suggests convergent loss of eyelids in the two most diverse gecko lineages. Alternatively, eyelids could have been lost in gekkotans and re-evolved in eublepharids. Another lineage of largely nocturnal lizards, the Xantusidae, have also lost their eyelids and evolved eye-licking, and on this basis were once associated with

gekkotans, but are now known to be unrelated (Vicario *et al.* 2003; Townsend *et al.* 2004). These results suggest that there is a strong correlation between a predominantly nocturnal lifestyle and lidless, tongue-cleaned eyes in lizards, and that these important taxonomic characters are more plastic than has been widely recognised.

7.3 Family level relationships of the Pygopodoidea

Chapter 2 presents the most complete analysis of intergeneric relationships amongst the Australian pygopodoids using nuclear loci yet published. This analysis included 30 species and exemplars of all recognised Australian genera (except *Orraya* and *Uvidodactylus*). Maximum parsimony and Bayesian analyses unequivocally supported the monophyly of the three pygopodoid families recognised by Han *et al.* (2004); the Carphodactylidae, the Diplodactylidae, and Pygopodidae. Two exemplars of an extralimital radiation of pygopodoids from New Caledonia were also included and grouped unequivocally with the Diplodactylidae. The only major lineage missing from our analysis was the New Zealand Diplodactylidae; analyses underway elsewhere indicate that these are a deeply divergent lineage of uncertain relationships within the family Diplodactylidae (S Neilson in prep.).

In contrast to the strong support for the monophyly of the three Pygopodoid families, there was no support for any order of branching between them. While additional data may resolve the order of branching, a long branch at the base of all three clades, and the very short internodes between them suggests these three morphologically disparate lineages diverged within a short time period.

7.4 Generic boundaries and relationships in Pygopodidae

The Pygopodidae currently comprise seven morphologically diverse genera (Kluge 1976; Greer 1989; Wilson and Swan 2008). Given the morphological and ecological distinctness of most of these genera it is not surprising that analyses published elsewhere (Jennings *et al.* 2003) and in chapter 2 strongly support their relative distinctiveness and monophyly. There is some argument for uniting the monotypic *Paradelma* within its sister lineage *Pygopus* (Kluge 1976; Jennings *et al*. 2003) however given the morphological, ecological and genetic distinctiveness of this lineage I follow most other authors and continue to recognise both genera (Greer

1989; Cogger 2000; Wilson and Swan 2008). Avaliable data supports placing *concinna* within *Delma* (Kluge 1976; Jennings *et al.* 2003), and not in a separate monotypic genus *Aclys*.

In contrast to the strong support for the monophyly of most genera, the interrelationships of pygopod genera have proven difficult to resolve (Kluge 1976; Jennings *et al*. 2003). Analyses presented in chapter two and Appendix 5 provide strong phylogenetic support for a basal dichotomy within the family; between the 20+ relatively generalised and morphologically similar species of *Delma*, and six other morphologically diverse, relatively specialised, but less species-rich genera (*Aprasia*, *Lialis*, *Ophidiocephalus*, *Pletholax*, *Paradelma* and *Pygopus*). This relationship was also recovered, although with lower support by combined morphological and molecular studies (Jennings *et al.* 2003). Within the clade of five genera, only a close relationship between *Pygopus* and *Paradelma* was supported, and all other generic relationships are largely unresolved.

Pygopodids have been highlighted on many occasions as a spectacular example of adaptive diversification within a lineage (Shine 1986, Patchell and Shine 1986; Greer 1989; Webb and Shine 1994). Current phylogenies confirm that this ecological diversity is distributed very differently in the two main clades of Pygopodids. One clade is remarkable for its very high levels of ecological and morphological specialisation. It includes two apparently independent burrowing lineages (*Aprasia*, a specialist ant and termite predator, and *Ophidiocephalus*), three predominantly terrestrial lineages (*Pygopus* are apparently specialist although not exclusive predators of arachnids; and *Lialis*, unique amongst pygopods in being a specialist vertebrate predator and possessing striking convergent adaptations to snakes), a semi-arboreal lineage that feeds extensively on nectar and arthropods (*Paradelma*), and one grass-swimming lineage (*Pletholax*) (Patchell and Shine 1986; Greer 1989; Tremul 2000). The poor resolution at the base of this clade also suggests there was a fairly rapid radiation of these morphologically and ecologically divergent types.

In contrast, although showing significant variation in size and body shape, the equally species-diverse and ancient sister lineage (*Delma*) is comparatively homogenous in body form (particularly head morphology) and all species are relatively generalised arthropod predators. Possible intrinsic morphological or

ecological factors that may have mediated the contrasting patterns of evolution of these two sister clades of pygopodids warrant investigation.

7.5 Generic boundaries and relationships in the Carphodactylidae

Five genera of carphodactylid geckos were recognised at the start of the study, monotypic *Carphodactylus*, three genera of leaf-tail geckos (*Orraya*, *Phyllurus* and *Saltuarius*), and an ecologically and morphologically diverse assemblage within *Nephrurus s.l.*

A phylogeny of recognised species of *Nephrurus s.l.* (Chapter 6) strongly supports the monophyly of this group. Nonetheless it also identifies three highly divergent lineages; thus we restrict *Nephrurus* to clade of species diagnosed by the possession of a caudal knob (consistent with the association of this genus name and vernacular term "knob-tailed geckos"), and advocate the recognition of two additional monotypic genera; *Underwoodisaurus* (*milii*) and *Uvidodactylus* (*sphyrurus*). These three genera are diagnosable by a suite of morphological characters, ecologically divergent and appear to be lineages of equivalent or greater age and antiquity to most other recognised genera of pygopodoid geckos. This new phylogeny and taxonomy resolves the long-standing systematic issue of the relationships and generic status of the species *milii* and *sphyrurus*.

Within redefined *Nephrurus s.s*, three major monophyletic and morphologically diagnosable groupings are recognised - the *wheeleri* group (comprising a single species with two deeply divergent subspecies that probably warrant specific recognition), the *asper* group (*asper*, *amyae* and *sheai*) and the smooth knob-tails (*deleani*, *laevissumus*, *levis*, *stellatus* and *vertebralis*). The relationships of these three lineages are poorly resolved; although combined and mitochondrial data suggest that the *wheeleri* group is sister to the rest.

While not a focal group for this study, and not discussed in chapter 6, six leaf-tail geckos were included in the phylogenetic analysis of *Nephrurus*. The nuclear sequence data did not strongly support or reject the monophyly of the leaf-tail geckos, however the mitochondrial data and a suite of morphological synapomorphies (Bauer 1990) strongly suggest that they are a clade. Our data indicate that a number of deeply divergent lineages are currently placed within *Phyllurus*, and further work is required to confirm the monophyly of this genus as it is currently construed. The phylogenetic

divergence and position of *Orraya* has also thus far only been examined using mitochondrial data (Couper *et al.* 2000) and requires further examination.

As a number of key lineages of leaf-tail geckos were missing from our dataset the inter-generic relationships of the Carphodactylidae were not discussed. However the data presented suggests that there is moderate to strong support for three major lineages within the Carphodactylidae 1) *Nephrurus*, *Underwoodisaurus* and *Uvidodactylus* 2) the leaf-tail geckos *Orraya*, *Phyllurus* and *Saltuarius* and 3) *Carphodactylus*. These same three lineages were identified by a thorough morphological analysis (Bauer 1990), and in a further similarity with this study, the relationships amongst these three groups also were poorly resolved, suggesting rapid cladogenesis at the base of Carphodactylidae.

A comparison of both nuclear and mitochondrial branch lengths between *Nephrurus* and the sampled leaf-tail geckos suggests that overall rates of molecular evolution within *Nephrurus* have been significantly higher (see figures in Chapter 6). While it has often been suggested that different life histories and demographic patterns may affect rates of nucleotide evolution, quantative demonstrations are few (Smith and Donoghue 2008). The possibility that rate variation within these two related, but ecologically divergent lineages, can be attributed to contrasting aspects of their life history and demographics warrants investigation. A relatively slow rate of molecular evolution within the leaf-tails may also explain the poor support for the monophyly of this group in nuclear only analyses, and the difficulty resolving basal nodes within the Carphodactylidae. Sampling of additional leaf-tail geckos and nuclear loci across all Carphodactylids is required to generate an improved understanding of the relationships of the basal lineages, and to examine possible rate variation between them.

7.6 Generic boundaries and relationships in the Diplodactylidae

The nuclear dataset presented in chapter 2 identified three highly divergent lineages of Australian Diplodactylidae. I review our current understanding of the systematics of these three groups independently.

'Core Diplodactylidae'

With over sixty recognised species (and many more undescribed, Chapter 3), by far the most diverse radiation of Australian geckos is a diverse monophyletic radiation of over sixty species in the five genera *Diplodactylus*, *Lucasium*, *Oedura*, *Rhynchoedura* and *Strophurus* (Wilson and Swan 2008). For convenience we herein refer to this group as the 'core Diplodactylidae'.

Phylogenetic and morphological data presented in chapter three provide the justification for the formal resurrection of *Lucasium* (type species *damaeum*) and the reassignment of nine species formally placed in the genus *Diplodactylus*. These data also strongly support the monophyly of the redefined *Diplodactylus*, and the relative distinctiveness of *Rhynchoedura*.

While a comprehensive analysis of the arboreal genera *Oedura* and *Strophurus* was not performed in this work, samples spanning the diversity of both genera were included in analyses. Melville *et al.* 2004 suggested that *Strophurus taenicauda* might be relatively divergent from other *Strophuru*s and render the genus paraphyletic with respect to *Oedura*. Subsequent work has revealed this result was due to a sequence mix-up with a specimen of *Oedura robusta* (Oliver pers obs.), and that *S. taenicauda* is actually nested within a clade of spiny-tailed climbing species (Worthington Wilmer pers. com.). Data from the divergent lineages of *Strophurus* included in chapters 2 and 3, morphology (Russell and Rosenberg 1981), and the other species included in Meville *et al.* 2004, strongly suggest that *Strophurus* is monophyletic.

In contrast, no analyses strongly supported the monophyly of three divergent lineages of *Oedura* included in chapter 2. These three taxa, *O. marmorata*, *O. reticulata* and *O. rhombifera*, are morphologically divergent and in the case *O. reticulata*, biogeographically disjunct. *Oedura* is a relatively generalised genus without any clear synapomorphies (Kluge 1967; Greer 1989). It seems likely that further data may reveal that additional lineages are divergent enough to warrant generic recognition, and that the assemblage itself is not be monophyletic with respect to other genera within the 'core Diplodactylidae'.

Analyses presented in Chapters 2 and 3 demonstrate that the three relatively arid-adapted, small and terrestrial genera *Diplodactylus*, *Lucasium* and *Rhynchoedura* form a relatively strongly supported clade, within which a sister relationship between *Lucasium* and *Rhynchoedura* is also supported. No other intergeneric relationships within the core Diplodactylidae' are strongly supported, and current data indicates that the base of this radiation is bested viewed as a polytomy consisting of *Strophurus*, at

least three different lineages of *Oedura*, and a relatively diverse clade including three genera of small terrestrial species.

Pseudothecadactylus

We sampled two of the three recognised species of *Pseudothedactylus*, including examplars of the two most morphologically and ecological diverse groups; the tree geckos (*P. australis*) and the cave geckos (*P. lindneri*). Our data strongly supported a sister taxa relationship between these two taxa. While moderately deeply divergent, they were apparently no more so than species within a number of other recognised diplodactylid genera, thus at this stage there does not seem to be any strong argument to resurrect the genus name *Torresia* Brongersma from synonymy (for *australis*).

Nuclear data strongly indicated that *Pseudothecadactylus* was both highly divergent from all other Australian Diplodactylidae, and most closely related to an extralimital radiation in New Caledonia. Morphological data also supports to this relationship, however our data does not support the synonymy of *Pseudothecadactylus* with the New Caledonia genus *Rhacodactylus*, despite the ecological similarities of these two genera (Bauer 1990).

Crenadactylus

Dixon and Kluge (1964) demonstrated that the tiny geckos of the monotypic genus *Crenadactylus* were morphologically distinctive from all other geckos. Perhaps because of this have been largely overlooked in systematic examinations of Australian geckos, although at least some authors have suggested an affinity between this genus and *Diplodactylus* (Greer 1989). The evolution and systematics of this genus are discussed in detail in chapter 5. This data underlines the distinctiveness of *Crendactylus* from all other extant lineages of geckos and indicates that, while far more species-rich than previously recognised, the genus is clearly a natural evolutionary grouping.

Basal relationships of the Diplodactylidae

Data presented in Chapter 2, published (Bauer 1990) or in prep (Bauer, Neilson and Jackman pers com) all indicate that the Diplodactylidae includes five highly divergent lineages; the 'core Diplodactylidae', *Pseudothecadactylus*, *Crenadactylus*, and relatively recent monophyletic radiations in New Zealand and New Caledonia. These lineages have a unique distribution amongst vertebrates, and are one of the few ancient Gondwanan clades distributed across, but restricted to, these three island landmasses. Many authors have interpreted this distribution as the result of vicariance subsequent to the break-up of east Gondwana (Kluge 1967; Main 1987; Bauer 1990; Couper *et al*. 2000; Han *et al*. 2004). The pattern of diversification amongst these basal lineages within the Diplodactylidae has important ramifications for understanding the role of Australia in the initial diversification of lineages, and the process and timing of colonisation of New Zealand and New Caledonia.

The only sister clade relationship within these five lineages that is strongly support thus far is between the New Caledonia radiation and *Pseudothecadactylus*. While geographically disjunct, the distribution of both lineages is centred upon the broadly similar habitats in seasonally to perpetually humid tropical areas, and they are also both relatively active and arboreal clades (Bauer 1990; Bauer and Sadlier 2000). In spite of this phylogenetic relatedness, these two clades have undergone remarkably different radiations, the Australian lineage is largely relictual and species depauperate, while the New Caledonian lineage is ecologically and specifically diverse (Bauer and Sadlier 2000). This disparity in overall diversity may reflect a combination of environmental contingencies (absence of other gecko lineages in New Caledonia) and the increasing aridity and unsuitability of the Australian environment for humid adapted geckos through the Miocene.

The phylogenetic analyses presented in Chapter 2 strongly indicate that the largest proportion of phylogenetic diversity (although not necessarily ecological diversity) within the Diplodactylidae is preserved within Australia. Most notably, *Crenadactylus* is potentially the most divergent extant lineage within the Diplodactylidae, although this relationship is not unequivocally supported. The preservation of older lineages within Australia is not surprising given the much larger size and stable history of this landmass.

The importance of Australia for early diplodactylid evolution is further emphasised by the relatively young estimated age of divergence between Australian and New Caledonian diplodactylids, which suggest that at least limited overwater

dispersal from Australia may have been involved in their origin (Chapter 2). However, as the New Zealand Diplodactylidae were missing from our analyses, more data is clearly required before further conclusions about the relationships and history of these five major lineages of Diplodactylidae can be drawn.

7.7 The higher-level systematics of pygopodoids - future directions.

Work presented in this thesis has made considerable progress in understanding the phylogenetic relationships of extant Australian pygopodoids. It has supported the monophyly and distinctness of the three families currently recognised, improved resolution of intergeneric relationships, resulted in the redefinition and resurrection of two genera, and description of one new genus. Based on this work the following three priorities for further molecular systematic work are highlighted.

1. *The relationships and monophyly of* Oedura.
Evidence suggests that this 'genus' may be a basal grade within the 'core Diplodactylidae'.

2. *A complete phylogeny of the Carphodactylidae, including missing taxa and genera.*
The relationships of the three major lineages of Carphodactylids are poorly resolved in all analyses, as were the inter-relationships and monophyly of the leaf-tail geckos.

3. *The basal relationship of the Diplodactylidae.*
The pattern of branching within these five lineages has important ramifications for understanding the timing of potential vicariance or dispersals in east Gondwana, and will provide important insight into the relative phylogenetic divergence of the three major Australian lineages.

7.8 Intrageneric relationships

The first species level molecular phylogenetic analyses of Australian pygopodoid geckos were produced around a decade ago, and phylogenies for a number of further groups were published in the following years (Couper *et al*. 2000; Jennings *et al.*

2003; Melville *et al*. 2004; Pepper *et al.* 2006). Work presented in this thesis has added relatively complete (one or no described species missing) species level phylogenies for four additional genera; *Lucasium*, *Diplodactylus* (Chapter 3), *Nephrurus* (Chapter 6) and *Crenadactylus* (Chapter 5). These phylogenies are discussed in detail in the relevant chapters, while the taxonomic and evolutionary ramifications of our results will be discussed in more detail below. However, based on the work done, some clear priorities for further research are worth noting.

Phylogenetic studies have now addressed intrageneric relationships within all Australian pygopodoid genera except *Oedura*. As mentioned above this genus is morphologically diverse and biogeographically interesting. A species level phylogeny for this group is necessary to both resolve how many deeply divergent lineages are contained within this genus, and to provide a comparative framework for understanding species level diversification in related but ecologically or environmentally divergent clades such as the terrestrial Diplodactylidae and *Strophurus*.

A second major problem is that many species level datasets have been produced using non-comparable loci, and in several cases (including one chapter in this thesis) only a single mitochondrial locus has been employed. This situation is clearly less than ideal, both for determination of accurate species trees as opposed to gene trees, and for comparing patterns of diversification. An integrated multi-locus species level dataset for the Australian pygopodoids would have obvious benefits for comparing and understanding patterns of diversification within this diverse and widespread Australian lizard radiation, and should be a priority for ongoing research.

7.9 Cryptic species diversity and the taxonomic impediment

The problem of cryptic taxonomic diversity in the Australian herpetofauna was flagged and nicely summarised by Donnellan *et al*. 1993. Chapters 3 and 4 present detailed genetic assessments of species diversity in two distantly related genera within the family Diplodactylidae. These chapters reveal that more than a decade after this seminal paper, the problem remains severe. Within *Diplodactylus* estimates of species diversity were increased from 13 to 29. The description of an additional species of *Diplodactylus* that we sampled, but did not count as a candidate species, is also in press (Doughty, Pepper and Keogh. Zootaxa). Even more remarkably, the single

recognised species of *Crenadactylus* was found to comprise a radiation of at least ten candidate species. In total estimates of species diversity within these two genera have increased from 14 to 40+. Our data also provided evidence of unrecognised deeply divergent lineages within *Nephrurus* and *Underwoodisaurus*, however in this case analyses did not include multi-locus data appropriate to the identification of cryptic taxa.

Similar molecular genetic evaluations have significantly increased estimates of species diversity in a number of other pygopodoid genera (e.g Leaf-tail geckos (Couper *et al*. 2008), *Lucasium* (Pepper *et al*. 2006), the New Caledonian genus *Bavayia s.l* (Bauer et al. 2006, *pers. com.*), and some *Delma* (Maryan *et al.* 2007)). In total, genetic studies since the late 1990s have helped to identify at least fifty previously unrecognised pygopodoid gecko species.

These results highlight the importance and ongoing relevance of integrated multilocus studies combining both molecular and morphological studies to identify and diagnose species. It seems very likely that a large proportion of Australian unrecognised vertebrate species will only be delimited, and potentially also diagnosed, by molecular datasets.

If comprehensive assessments of just two genera can identify over 25 unrecognised species, it seems reasonable to assume that at a minimum, hundreds more Australian reptile taxa potentially remain undescribed, especially in poorly sampled areas of northern and central Australia. Even within pygopodoids, several diverse genera of (namely *Delma*, *Oedura* and *Strophurus*) remain relatively poorly assessed for cryptic diversity.

These results also suggest that recent estimates that the Australian reptile fauna probably consists of approximately 950 species (as opposed to the 920 species currently recognised) must be treated has highly conservative (Chapman 2009). A total substantially above this seems more likely in light of the evidence presented here. As emphasised in Chapters 4 and 5, this high level of unrecognised species diversity has profound ramifications for all fields of biological inquiry and conservation management, and there remains an urgent need to comprehensively and systematically evaluate other groups of Australian vertebrates to assess the scale and extent of this problem.

7.10 Historical Biogeography of the Pygopodoidea

7.10.1 Initial diversification and origins

As outlined in Chapter 2, pygopodoid geckos are currently the only squamate lineage endemic to the east of Wallace's Line that show unequivocal evidence of a long history pre-dating the final break-up of east Gondwana (similar claims for Australian agamids and varanids have been convincingly refuted: Hugall and Lee 2004). Indeed, the pygopodoid geckos are in many ways comparable to lineages such as the marsupials or myobatrachid frogs; all are ancient Gondwanan lineages that have diversified largely in isolation from potential competitors and/or ecological analogues that now dominate faunas outside Australasia (Keast 1981; Heatwole 1987; Roelants *et al.* 2007). The distribution and age of these lineages powerfully underlines the fundamental divide that once existed between faunas to the east and west of Wallace's Line.

7.10.2 The timing and pattern of evolutionary radiations

Dating analyses presented in Chapters 2 and 6 suggest that the timing of initial diversification within the Carphodactylidae, the 'core Diplodactylidae' and the Pygopodidae, all occurred in the Oligocene to early Miocene. All three lineages are also characterised by single long stems at the base, followed by a rapid accumulation of poorly resolved lineages separated by short internodes. In all three families these poorly resolved nodes encompass several ecologically divergent lineages. This congruent pattern is suggestive of fairly rapid and broadly concurrent adaptive radiation.

A number of other studies have also indicated that the accumulation of most extant diversity within many other major Australian vertebrate lineages began, at the earliest, in the late Oligocene. Apart from the five lineages of pygopodoid geckos there is almost no evidence for any diversity within extent lineages of Australian squamates that pre-date the Oligocene (reviewed in Chapter 2), and even the relatively ancient marsupials contain comparatively few deep lineages that pre-date the Oligocene (Nilsson *et al*. 2004; Beck 2008; Meredith *et al*. 2009).

It is tempting to suggest that this relative dearth of lineages pre-dating the Oligocene reflects radiation following significant environmental change within

Australia. An obvious candidate explanation is massive environmental change as the Australian plate separated from Antarctica, gradually moved north and the circumpolar current formed. It seems likely that this process caused major changes in Australian environment (Martin 2006; Byrne *et al*. 2008); prior to isolation conditions may have been have been more mesic, temperate and potentially strongly seasonal (i.e the polar winter). With respect to squamates such conditions are not ideal, and are generally dominated by relatively derived live-bearing taxa (Hutchinson *et al.* 2001; Pianka and Vitt 2004; Jewell 2008). Pygopodoid geckos are currently depauperate to absent in temperate southern Australia, and are most diverse in warm arid and semi-arid areas, lending further support to the hypothesis that the environment of Australia at southern latitudes may not have been favourable to the lineages that dominate the extant fauna.

An alternative hypothesis is that the timescales involved are so large that relatively few lineages are likely to persist across them, and that the observed 'pattern' is simply an artefact of largely stochastic extinction over time. In addition the confidence intervals involved in date estimates are very large, thus events separated by millions of years may artificially appear to significantly overlap. To further examine the basal radiation of the pygopodoid geckos, additional nuclear datasets and coalescent analyses are necessary. This may help to resolve and more precisely date the order of cladogenesis. A more complete and comprehensive phylogenetic tree would also provide considerable scope to test whether there is any evidence for significant correlations in the rate and timing of diversification across lineages. This work within the pygopodoids would obviously also benefit from and inform comparison with many other major Australian radiations.

7.10.3 Pygopodoid phylogeny and aridification

The development and vast expansion of severely arid conditions has been the single most significant environmental change within Australia over the last twenty million years (Martin 2006; Byrne *et al.* 2008). A recent review of this process made a number of key predictions about the origins of the diverse biota of this relatively new biome; relevant here are hypotheses that the arid zone biota is derived from an ancestral mesic biota, and that there was a significant expansion of this biome and

corresponding upturn in diversification of arid zone lineages around 6-10 million years ago (Byrne *et al.* 2008).

This study has unequivocally demonstrated that at least five extant lineages of pygopodoids had diverged well before the putative onset of widespread severe aridification. With the notable exception of *Pseudothecadactylus*, a significant proportion of diversity within each of these lineages is now restricted, or largely restricted, to arid environments (Wilson and Swan 2008). However the overall distribution of diversity across these lineages is quite contrasting.

Within the Carphodactylidae a majority of basal lineages are concentrated within isolated patches of aseasonal wet forest biome of eastern Australia. Only the *Nephrurus s.l.* clade is now found outside this area. Within this clade there is strong evidence that lineages such as *Underwoodisaurus*, *Uvidodactylus* and *Nephrurus* were already adapting to relatively or seasonally arid conditions over 20 million years ago. Nonetheless the observed pattern of diversification supports hypotheses that lineages restricted to the arid biome are the most derived, and that these specialist arid zone lineages diversified within the last 10 million years. The observed phylogenetic patterns within this genus are thus relatively concordant with both predictions outlined above.

In contrast, the distribution of both the Diplodactylidae and Pygopodidae suggests that mesothermal environments (which are often viewed as the historical standard for Australia: e.g White 1994) have played little role in their evolution. While some clades range into the aseasonal wet forest or temperate biomes, based on current data there is no clear evidence of a consistent environmental shift from mesic to arid environments (Chapter 3; Jennings *et al.* 2003). Available data also suggest that some of these predominantly arid radiations are surprisingly old: most strikingly the radiation of the small terrestrial diplodactylids is estimated to have begun around 30 million years ago, and that the almost exclusively arid *Lucasium*/*Rhynchoedura* clade is estimated to be around 20 million years old (Chapter 2). A number of deeply divergent lineages in genera such as *Oedura*, *Pygopus*, *Delma* and *Diplodactylus* are, however, restricted to seasonally arid, semi-arid or tropical environments (Chapters 1, 2; Jennings *et al*. 2003). This suggests, as with *Nephrurus*, that at least seasonally arid environments have had a long history within Australia, and may have been an important source of lineages to colonise the putatively much younger arid zone.

The phylogeny and age of divergences within *Crenadactylus* (the other genus examined in detail) further support the idea that at least moderately arid conditions have had a long history within Australia. This lineage is almost completely absent from mesic coastal and temperate zones and is largely restricted to relatively moist microclimates in semi-arid or seasonally arid areas. Nonetheless basal divergences within *Crenadactylus* are estimated to be over twenty million years old, again suggesting that significantly arid habitats, and lineages adapted to these conditions, had have a long history within Australia.

Unfortunately, most radiations within the Diplodactylidae and Pygopodidae have not yet been properly dated using reliable calibrations and multiple loci. In both families there are also strong evidence for serious difficulties with underestimation of species diversity across Australia (chapters 3, 4; K. Aplin, pers com); thus the available data do not yet allow us to assess the possibility that there has been significant upturn in diversification within arid zone lineages of these radiations over the last ten million years (sensu Rabosky *et al*. 2007).

Nonetheless, despite contrasting patterns across Families, the data presented in this thesis seem to broadly support many of the predictions outlined by Bryne *et al*. 2008. Most notably, the genera examined in detail support the contention that the arid landforms that now characterise much of Australia have spread significantly over the last 10 million years. *Nephrurus s.l.* does so through evidence of recent radiation of a specialist arid zone lineage and *Crenadactylus* does so through vicariant speciation events as suitable semi-arid habitat became fragmented. The data presented here also build on Bryne *et al*. (2008) by providing strong evidence that lineages adapted to semi-arid and seasonally arid habitats potentially date back to the Miocene or earlier, and were an important source of arid zone diversity.

7. 11 Key evolutionary trends within the Pygopodoids

7.11.1 The arboreal to terrestrial shift

The major ecological split within both the Diplodactylidae and the Carphodactylidae is between predominantly terrestrial and arboreal genera. In both cases many arboreal taxa possess specific adaptations associated with this lifestyle such as greatly expanded subdigital lamellae and a dorsoventrally flattened body, while the terrestrial

taxa tend to lack these features. Data presented in Chapters 2, 3 and 6 indicate that the predominantly terrestrial species in both families each form strongly supported clades, suggesting each have evolved terrestriality only once. In contrast the arboreal lineages are more deeply divergent, not obviously monophyletic, and may represent pleisomorphic grades at the base of each radiation.

Thus, our data indicate that the majority of diversity in the pygopodoids is terrestrial (especially given that almost all Pygopodidae are terrestrial), but that this is unlikely to be the ancestral condition for at least two families, and potentially the entire Pygopodoidea. Studies in African gecko groups have indicated that increasing aridification and the formation of sandy deserts is followed by convergent shifts from arboreal to terrestrial ecologies (Lamb and Bauer 2006). The convergent radiation of terrestrial pygopodoid lineages almost certainly also correlates with an increase in the relative abundance of terrestrial habitats through aridification. Within Australia, the most successful marsupial radiation (the macropods) has also shifted from an arboreal to a terrestrial lifestyle (Meredith 2009). It might be predicted that these convergently evolving terrestrial lineages would have much higher rates of speciation and ecological diversification than their arboreal ancestors. The Australian biota clearly provides a number of parallel opportunities to test this hypothesis further.

7.11.2 Non-adaptive diversification

As speciation is not necessarily an adaptive process, the existence of cryptic species is not surprising, however the apparently high percentage of morphologically similar pygopodoid species is notable, and raises the question of whether some of these lineages are undergoing unusually high levels of non-adaptive diversification (Kozak et al. 2005). One prediction of adaptive radiation theory is that morphologically similar species will accumulate more towards the end of an adaptive radiation, through an increased tendancy towards non-adaptive diversification (McPeek 2008). Relative to many other Australian lizard groups pygopodoids are old and seem to contain a notably high diversity of cryptic species. Even within pygopodoid geckos, it seems that cryptic taxa are more frequent in clades such as the leaf-tail geckos and *Crenadactylus,* which are restricted to older environments, and less frequent in lineages such as *Nephrurus*, which are most diverse in the younger arid zone. Pygopodoid geckos potentially provide an opportunity to test predictions about the

evolution of morphological diversity more precisely, both by mapping rates and patterns of morphological change onto phylogenies for the relevant groups, and by comparing frequencies of "cryptic species" (however identified) across clades from different habitats or biomes, or with different ages of origin. Further testing of these hypotheses may also have important ramifications for predicting clades and biomes in which cryptic species diversity may be concentrated.

7.12 Concluding comments

The work completed in this thesis has made important steps forward in our understanding of the systematics and evolution of Australian pygopodoid geckos. It is now clear that they are both a relatively old, and unexpectedly diverse component of the terrestrial biota. Dating analyses also suggests that they are likely to be a key group for examining patterns of environmental change and evolution within Australia since its isolation from Antarctica. However there is still significant scope for basic systematic work and evolutionary research. The priorities must be to produce a complete a species-level dated phylogeny for the entire radiation, and to establish an accurate estimate of total species diversity. Such a dataset will provide excellent opportunities to examine patterns of evolutionary and environmental change within Australia from the Oligocene to the present day.

CHAPTER 8: REFERENCES FOR CHAPTERS 1 & 7

Aplin KP, Adams M (1998) Morphological and genetic discrimination of new species and subspecies of geckonid and scincid lizards (Squamata: Lacertilia) from the Carnarvon Basin region of Western Australia. *Journal of the Royal Society of Western Australia* **81**, 201-224.

Barker FK, Cibois A, Schikler P, Feinstein J, Cracraft J (2004) Phylogeny and diversification of the largest avian radiation. *Proceedings of the National Academy of Sciences* **101**, 11041-45.

Bauer AM (1990) Phylogenetic systematics and biogeography of the Carphodactylini (Reptilia: Gekkonidae). *Bonner Zoologische Monographien* **30**,1-220.

Bauer AM, Jackman T, Sadlier R, Whitaker A (2006) A revision of the *Bavayia validiclavis* group (Squamata: Gekkota: Diplodactylidae), a clade of New Caledonian geckos exhibiting microendemism. *Proceedings of the California Academy of Sciences* **18**, 503-547.

Bauer AM, Sadlier RA (2000) The herpetofauna of New Caledonia. Society for the study of reptiles and amphibians, Ithaca, New York, USA. 310pp.

Beck RM (2008) A dated phylogeny of marsupials using a molecular supermatrix and multiple fossil constraints. *Journal of Mammology* **89**, 175-189.

Bowler JM (1982) Aridity in the late Tertiary and Quaternary of Australia. In "Evolution of the flora and fauna of arid Australia." (Eds WR Barker, PJM Greenslade) pp. 35-45 (Peacock Publications: Adelaide)

Byrne M, Yeates DK, Joseph L, Kearney M, Bowler J, Williams MAJ, Cooper S, Donnellan SC, Keogh JS, Leys R, Melville J, Murphy DJ, Porch N, Wyroll KH (2008) Birth of a biome: insights into the assembly and maintenance of the Australia arid zone biota. *Molecular Ecology* **17**, 4398-4417.

Chapman AD (2009) Numbers of living species in Australia and the world. 2nd Edition. A Report for the Australian Biological Resources Study. Australian Biodiversity Information Services, Toowoomba.

Cogger HG (2000) The reptiles and amphibians of Australia. Reed New Holland. Sydney.

Cogger HG, Heatwole H (1981) The Australian reptiles: origins, biogeography, distribution patterns and island evolution. In "Ecological biogeography of Australia." (Ed. A Keast) pp. 1393-1434 (Dr W.Junk: The Hague)

Couper PJ, Covacevich JA, Moritz C (1993) A review of the Australian leaf-tailed geckos endemic to eastern Australia: a new genus, two new species and other new data. *Memoirs of the Queensland Museum* **34**, 95-124.

Couper PJ, Schneider C, Covacevich JA (1997) A new species of *Saltuarius* (Lacertilia: Gekkonidae) from granite-based, open forests of eastern Australia. *Memoirs of the Queensland Museum* **42**, 91-16.

Couper PJ, Schneider CJ, Hoskin CJ, Covacevich JA (2000) Australian leaf-tailed geckos: phylogeny, a new genus, two new species and other new data. *Memoirs of the Queensland Museum* **45**, 253-265.

Couper PJ, Sadlier RA, Shea GM, Worthington-Wilmer J (2008a) A reassessment of *Salturius swaini* (Lacertilia: Diplodactylidae) in southeastern Queensland and New South Wales; Two new taxa, Phylogeny, Biogeography and Conservation. *Rec. Aust. Mus.* **60**, 87-118.

Couper PJ, Hamley B, Hoskin CJ (2008b) A new species of *Phyllurus* (Lacertilia: Gekkonidae) from the Kilkivan district of south-eastern Queensland. *Memoirs of the Queensland Museum* **52**, 139-147.

Crisp M, Cook L, Steane D (2004) Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present day communities? *Philosophical transactions of the Royal Society B* **359**, 1551-1571.

Dixon J, Kluge AG (1964) A new gekkonid lizard genus from Australia. *Copeia* **1964**, 174-80.

Donnellan SC, Adams M, Hutchinson MN, Baverstock PR (1993) The identification of cryptic species in the Australian herpetofauna: a high research priority. In "Herpetology in Australia: A diverse discipline." (Eds D Lunney, D Ayers) pp. 121-126 (Surrey Beatty and Sons: Sydney)

Donnellan SC, Hutchinson MN, Saint KM (1999) Molecular evidence for the phylogeny of Australian gekkonid lizards. *Biological Journal of the Linnean Society* **67**, 97-118.

Fitch AJ, Goodman AE, Donnellan SC (2006) A molecular phylogeny of the Australian monitor lizards (Squamta:Varanidae) inferred from mitochondrial DNA sequences. *Australian Journal of Zoology* **54**, 253-269.

Gamble T, Bauer AM, Greenbaum E, Jackman TR (2008a) Evidence of Gondwanan vicarience in an ancient clade of gecko lizards. *Journal of Biogeography* **35**, 88-104.

Gamble T, Bauer AM, Greenbaum E, Jackman TR (2008b) Out of the blue: A novel, trans-atlantic calde of geckos (Gekkota, Squamata). *Zoologica scripta* **37**, 355-366.

Greer AE (1989) Biology and Evolution of Australian Lizards. Surrey Beatty & Sons: NSW

Hall R (2001) Cenozoic reconstructions of SE Asia and the SW Pacific: changing patterns of land and sea. *Faunal and floral migrations and evolution in SE Asia–Australasia* (ed. by I. Metcalfe, J. M. B. Smith, M. Morwood and I. Davidson) pp. 35-56. Balkema Publishers, Lisse.

Han D, Zhou K, Bauer AM (2004) Phylogenetic relationships among gekkotan lizards inferred from *C-mos* nuclear DNA sequences and a new classification of the Gekkota. *Biological Journal of the Linnean Society* **83**, 353-368.

Heatwole H (1987) Major components and distributions of the terrestrial fauna. In "Fauna of Australia. Volume 1A: General articles." (Ed. GR Dyne) pp. 69-100 (Australian Government Publishing Service: Canberra)

Horner P, Adams M (2009) A molecular systematic assessment of species boundaries in Australian *Cryptoblepharus* (Reptilia: Squamata: Scincidae) - a case study for the combined use of allozymes and morphology to explore cryptic biodiversity. *Records of the Museum of the Northern Territory* 2009, ***Supp* 3**, 1-19.

Hoskin CJ, Couper PJ, Schneider CJ (2003) A new species of *Phyllurus* (Lacertilia: Gekkonidae) and a revised phylogeny and key for the Australian leaf-tailed geckos. *Australian Journal of Zoology* **51**, 153-164.

Hugall AF, Lee SYM (2004) Molecular claims of Gondwanan age for Australian agamid lizards are untenable. *Molecular Biology and Evolution* **21**, 2102-2110.

Hugall AF, Foster R, Hutchinson M, Lee, MSY (2008) Phylogeny of Australasian agamid lizards based on nuclear and mitochondrial genes: implications for morphological evolution and biogeography. *Biological Journal of the Linnean Society* **93**, 343-358.

Hutchinson MN (1997) The first fossil pygopod (Squamata, Gekkota), and a review of mandibular variation in living species. *Memoirs of the Queensland Museum* **41**, 355-366.

Hutchinson M, Swain R, Driessen M (2001) Snakes and lizards of Tasmania. Fauna of Tasmania Handbook no. 9. University of Tasmania, Hobart.

Jennings WB, Pianka ER, Donnellan S (2003) Systematics of the lizard family Pygopodidae with implications for the diversification of Australia temperate biotas. *Systematic Biology* 52, 757-780

Jewell T (2008) A photographic guide to reptiles & amphibians of New Zealand. New Holland, Auckland, New Zealand. 143 p. (with photographs by R Morris)

Keast A (1981) Origin and relationships of the Australian biota. *Ecological biogeography of Australia* (ed. by A. Keast) pp. 1999-2059. Junk, The Hague.

King M (1987) Origin of the Gekkonidae: Chromosomal and albumin evolution suggests Gondwanaland. *Search* **18**, 252-254.

Kluge AG (1967) Systematics, phylogeny, and zoogeography of the lizard genus *Diplodactylus* Gray (Gekkonidae). *Australian Journal of zoology* **15**, 1007-1108.

Kluge AG (1974) Taxonomic revision of the lizard family Pygopodidae. *Miscellaneous publications of the Museum of Zoology, University of Michigan* **147.**

Kluge AG (1976) Phylogenetic relationships of the lizard family Pygopodidae: an evaluation of theory, methods and data. *Miscellaneous publications of the Museum of Zoology, University of Michigan* **152.**

Kluge AG (1987) Cladistic relationships in the Geckonoidea (Squamata, Sauria). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* **173**.

Kozak KH, Weisrock DW, Larson A (2006) Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders. *Proceedings of the Royal Society London B* **273**, 539-546.

Lamb T, Bauer AM (2006) Footprints in the sand: independent reduction of subdigital lamellae in the Namib-Kalahari burrowing geckos. *Proceedings of the Royal Society London B* **273**, 855-864.

Main B (1987) Evolution and radiation of the terrestrial fauna. In “Fauna of Australia. Volume 1A: General articles.” (Ed. GR Dyne) pp. 69-100 (Australian Government Publishing Service: Canberra)

Martin H.A (2006) Cenzoic climate changes and the development of the arid vegetation of Australia. *Journal of arid environments*. **66**, 533-563.

Maryan B, Aplin K, Adams M (2007) Two new species of the *Delma tincta* group (Squamata: Pygopodidae) from northwestern Australia. *Records of the Western Australian Museum* **23**, 273-305

McPeek MA (2008) The ecological dynamics of clade diversification and community assembly. *American Naturalist* **172**, 270-284.

Melville JE, Schulte JA, Larson A (2002) A molecular phylogenetic study of ecological diversification in the Australian lizard genus *Ctenophorus*. *Journal of experimental Zoology* **291**, 339-353.

Melville JE, Schulte JA, Larson A (2004) A molecular study of phylogenetic relationships and the evolution of antipredator strategies in Australian *Diplodactylus* geckos, subgenus *Strophurus*. *Biological Journal of the Linnean Society* **82,** 123-138.

Meredith RW, Westerman M, Springer MS (2009) A phylogeny of the Diprotodontia (Marsupialia) based on sequences for five nuclear genes. *Molecular Phylogenetics and Evolution* **51**, 554-571

Nilsson MA, Arnason U, Spencer PBS, Janke A (2004) Marsupial relationships and a timeline for marsupial radiation in South Gondwana. *Gene* **340**, 189-196.

Oliver PM, Hugall A, Adams M, Hutchinson M, Cooper S (2007) Genetic elucidation of ancient and cryptic diversity in a clade of Australian lizards: the *Diplodactylus vittatus* complex. *Molecular Phylogenetics and Evolution* **44**, 77-88.

Patchell FC, Shine R (1986) Food habits and reproductive biology of the Australian legless lizards (Pygopodidae). *Copeia* **1986**, 30-38.

Pepper M, Doughty PD, Keogh JS (2006) Molecular Phylogeny and phylogeography of the Australian *Diplodactylus stenodactylus* species-group based on mitochondrial and nuclear genes reveals an ancient split between Pilbara and non-Pilbara *D. stenodactylus*. *Molecular Phylogenetics and Evolution* **41**, 539-555.

Pianka ER (1972) Zoogeography and speciation of Australian desert lizards: an ecological perspective. *Copeia* **1972**, 127-145.

Pianka ER (1981) Diversity and adaptive radiations of Australian desert lizards. In "Ecological biogeography of Australia." (Ed. A Keast) pp. 1393-1434 (Dr W.Junk: The Hague).

Pianka ER, Vitt L (2004) Lizards; windows on the evolution of diversity. University of California Press, California.

Rawlings LH, Rabosky DL, Donnellan SC, Hutchinson MN (2008) Python phylogenetics: inference from morphology and mitochondrial DNA. *Biological Journal of the Linnean Society* **93**, 603-619.

Rabosky DL, Donnellan SC, Talaba AL, Lovette IJ (2007) Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. *Proceedings of the Royal Society B, Biological Sciences* **274**, 2915-23.

Reeder TW (2003) A phylogeny of the Australian *Spenomorphus* group (Scincidae: Squamata) and the phyogenetic placement of the crocodile skinks (*Tribolonotus*):

Bayesian approaches to assessing congruence and obtaining confidence in maximum likelihood inferred relationships. *Molecular Phylogenetics and Evolution* **27**, 157-170.

Roelants K, Gower DJ, Wilkinson M, Loader SP, Biju SD, Guillaume K, Moriau L, Bossuyt F (2007) Global patterns of diversification in the history of modern amphibians. *Proceedings of the National Academy of Sciences USA* **104**, 887-892.

Russel AP, Rosenberg J (1981) Subgeneric classification in the gekkonid genus *Diplodactylus*. *Herpetologica* **37**, 86-92.

Sanders KL, Lee MSY, Leys R, Foster R, Keogh JS (2008) Molecular phylogeny and divergence dates for Australasian elapids and sea snakes evidence from seven genes for rapid evolutionary radiations. Journal of evolutionary Biology **21**, 682-695.

Schulter D (2000) The ecology of adaptive radiation. Oxford Univ. Press, Oxford, U.K.

Shine R (1986) Evolutionary advantages of limblessness: Evidence from the pygopodid lizards. *Copeia* **1986**, 525-529.

Skinner AW (2007) Phylogenetic relationships and rate of early diversification of Australian Sphenomorphus group scincids (Scincoidea, Squamata). *Biological Journal of the Linnean Society* **92**, 347-366.

Smith SA, Donoghue MJ (2008) Rates of molecular evolution are linked to life histry in flowering plants. *Science* **322**, 5898, 86-89.

Townsend TM, Larson A, Lousi E, Macey JM (2004) Molecular phylogenetics of squamates: the position of snakes, amphisbaenians, and dibamids, and the root of the squamate tree. *Systematic Biology* **53**, 735-757.

Tremul PR (2000) Breeding, feeding and arboreality in *Paradelma orientalis*: a poorly known, vulnerable pygopodid from Queensland, Australia. *Memoirs of the Queensland Museum* **45**, 599-609.

Webb JK, Shine R (1994) Feeding habits and reproductive biology of Australian pygopodid lizards of the genus *Aprasia*. *Copeia* **1994**, 390-398.

White ME (1994) After the Greening: The browning of Gondwana. Kangaroo Press, NSW.

Wilson S, Swan G (2008) A complete guide to reptiles of Australia. 2nd Edition. Reed New Holland, Sydney.

Vicario S, Caccone A, Gauthier J (2003) Xantusiid 'night' lizards: a puzzling phylogenetic problem revisted using likelihood-based methods on mtDNA sequences. *Molecular Phylogenetics and Evolution* **26**, 243-261.

Vidal N, Hedges SB (2009) The molecular evolutionary tree of lizards, snakes, and amphisbaenians. *C.R.Biologies* **332**, 129-139.

APPENDIX 1

A new species of large *Cyrtodactylus* (Squamata: Gekkonidae) from Melanesia.

P.M Oliver, Tjaturadi, B.T.; Mumpuni; Krey, K.; Richards, S.J.

PAUL OLIVER [1,2,5], BURHAN TJATURADI[3], MUMPUNI[3], KELIOPAS KREY[4] AND STEPHEN RICHARDS[1]

1. Terrestrial Vertebrates, South Australian Museum, North Terrace, Adelaide, South Australia 5000.

2. Centre for Environmental and Evolutionary Biology, Adelaide University, Adelaide, South Australia 5005.

3. Conservation International – Papua Program. Current address: Komp Ariau Dunlop Sentani, Papua; Indonesia.

4. Herpetology Division, Museum Zoologicum Bogoriense, Research Centre for Biology, Indonesian Institute of Sciences (LIPI), Widyasatwaloka Building-LIPI, Jalan Raya, Cibonong 16911, West Java, Indonesia

5. Department of Biology, University of Papua, Manokwari, Papua, Indonesia.

6. Corresponding author. E-mail paul.oliver@adelaide.edu.au

***Zootaxa* (2008), 1894: 59-68.**

Oliver, P.M., Tjaturadi, B., Mumpuni, Krey, K. & Richards, S. (2008) A new species of large Cyrtodactylus (Squamata: Gekkonidae) from Melanesia.
Zootaxa, v. 1894, pp. 59-68

APPENDIX 2

A new species of bent-toed gecko (*Cyrtodactylus*: Gekkonidae) from Seram Island, Indonesia.

PAUL OLIVER [1,2,7], PAUL EDGAR[3], MUMPUNI [4], DJOKO T. ISKANDAR[5] & RON LILLEY[6].

[1]*Center for Environmental and Evolutionary Biology, Darling Building, University of Adelaide, Adelaide 5005.*

[2]*Terrestrial Vertebrates, South Australian Museum*
North Terrace, Adelaide, 5000.

[3]*21 Heath Lawns, Fareham, Hampshire, PO Box 15 5QB, UK*

[4]*Herpetology Division, Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Widyasatwaloka Building-LIPI, Jalan Raya Cibinong Km 46, Cibinong 16911, West Java, Indonesia.*

[5]*Department of Ecology and Biosystematics, School of Life Sciences and Technology, Institut Teknologi Bandung, 10, Jalan Ganesa, Bandung 40132, Indonesia*.

[6]*Yayasan Alam Indonesia Lestari (LINI), The Indonesian Nature Foundation Office, Jl. Tirtanadi 21, Kelurahan Sanur Kauh, Kecamatan Denpasar, Selatan Bali, 80227.*

[7]Corresponding author. E-mail: paul.oliver@adelaide.edu.au

***Zootaxa* (2009), 2115: 47-55.**

Oliver, P.M., Edgar, P., Mumpuni, Iskandar, D.T. & Lilley, R. (2009) A new species of bent-toed gecko (*Cyrtodactylus:* Gekkonidae) from Seram Island, Indonesia.
Zootaxa, v. 2115, pp. 47-55

APPENDIX 3

On the status and relationships of the gecko species *Gehyra barea* Kopstein, 1926, with description of new specimens and a range extension.

PAUL OLIVER [1,2,5], MARK SISTROM[1,2], BURHAN TJATURADI[3], KELIOPAS KREY[4] AND STEPHEN RICHARDS[1]

1. Terrestrial Vertebrates, South Australian Museum, North Terrace, Adelaide, South Australia 5000.

2. Centre for Environmental and Evolutionary Biology, Adelaide University, Adelaide, South Australia 5005.

3. Conservation International – Papua Program. Current address: Komp Ariau Dunlop Sentani, Papua; Indonesia.

4. Department of Biology, University of Papua, Manokwari, Papua, Indonesia.

5. Corresponding author. E-mail paul.oliver@adelaide.edu.au

Zootaxa (2010), in press

Oliver, P.M., Sistrom, M., Tjaturadi, B., Krey, K. & Richards, S. (2010) On the status and relationships of the gecko species *Gehyra barea* Kopstein, 1926, with description of new species and a range extension.
Zootaxa, v. 2354, pp. 45-55

APPENDIX 4

Systematics of stone geckos in the genus *Diplodactylus* (Reptilia: Diplodactylidae) from northwestern Australia, with a description of a new species from the Northwest Cape, Western Australia

Paul Doughty[1], Paul M. Oliver[2,3] and Mark Adams, M. [2,4,5]

[1]Department of Terrestrial Zoology, Western Australian Museum, 49 Kew Street, Welshpool Western Australia 6106, Australia. Email: Paul Doughty@museum.wa.gov.au

[2]South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia.
[3]Email: Paul.Oliver@adelaide.edu.au

[4]Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide, South Australia, 5005, Australia.
[5]Email: adams.mark@saugov.sa.gov.au

Records of the Western Australian Museum (2008), 24: 247-265.

Doughty, P., Oliver, P.M. & Adams, M. (2008) Systematics of stone geckos in the genus *Diplodactylus* (Reptilia: Diplodactylidae) from northwestern Australia, with a description of a new species from the Northwest Cape, Western Australia.
Records of the Western Australian Museum, v. 24, pp. 247-265

APPENDIX 5

Phylogenetic uncertainty and molecular clock calibrations: A case study of legless lizards (Pygopodidae, Gekkota).

M.S.Y. Lee *; P.M. Oliver, M.N. Hutchinson

Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide, Room G04F, Darling Building, South Australia 5005, Australia

Terrestrial Vertebrates, South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia

* Corresponding author. Fax: +61 8 8207 7222.
E-mail address: Michael.S.Lee@Adelaide.Edu.Au (M.S.Y.Lee)

Molecular Phylogenetics and Evolution (2009), 50: 661-666

Lee, M.S.Y., Oliver, P.M. & Hutchinson, M.N. (2009) Phylogenetic uncertainty and molecular clock calibrations: A case study of legless lizards (Pygopodidae, Gekkota).
Molecular Phylogenetics and Evolution, v. 50(3), pp. 661-666