Molecular Phylogenetics and Evolution 59 (2011) 664–674

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Systematics and evolution of the Australian knob-tail geckos (*Nephrurus*, Carphodactylidae, Gekkota): Plesiomorphic grades and biome shifts through the Miocene

Paul M. Oliver[a,b,*], Aaron M. Bauer[c]

[a] Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide, Adelaide, SA 5005, Australia
[b] Herpetology Section, South Australian Museum, Adelaide, SA 5000, Australia
[c] Department of Biology, Villanova University, 800 Lancaster Avenue, Villanova, PA 19085, USA

ARTICLE INFO

Article history:
Received 1 October 2010
Revised 11 March 2011
Accepted 14 March 2011
Available online 21 March 2011

Keywords:
Adaptation
Arid biome
Australia
Bayesian Inference
Bayesian dating
Gecko
Maximum likelihood
Underwoodisaurus
Uvidicolus gen. nov

ABSTRACT

Clades that predate the origin of biomes that they inhabit provide unique opportunities to examine both when major environmental transitions occurred, and how lineages adapted to these changes. The isolated island continent Australia has undergone a profound environmental transition through the Miocene, from relatively mesic to predominantly arid; however, we have much to learn about both the timing of this change, and how organisms may have responded to it. The family Carphodactylidae is an ancient Gondwanan group of geckos that occurs across all major Australian biomes. 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) reveals that two of three morphological taxa historically recognized (the 'spiny knob-tails' and '*Underwoodisaurus*') are relatively species depauperate, pleisomorphic basal grades that diversified through the late Oligocene and early Miocene, and are now absent from most of the arid biome. Based on their deep divergence and morphological distinctiveness we recognize two lineages (*milii* and *sphyrurus*) as monotypic genera, the later of which is named herein (*Uvidicolus* nov. gen). In contrast, a third morphological group, the 'smooth knob-tails,' is a monophyletic group of five exclusively arid zone burrowing species that has radiated relatively recently (mid-Miocene). Our phylogeny indicates that successful colonization of this novel and challenging biome by *Nephrurus* correlates with an initial shift to terrestriality and adaptation to at least seasonally arid conditions around the early Miocene, and the eventual evolution and subsequent mid-Miocene radiation of a lineage specialized for burrowing.

1. Introduction

Over geological time major environmental changes are relatively common; in contrast, clades of organisms tend to retain their ancestral ecologies, and successful colonization 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, it became dominated by one of the largest continuous arid zones on the planet (Martin, 2006; Byrne et al., 2008). Based on a growing body of research, a number of recent papers have presented a temporal scale for this transition. These have indicated that it 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).

This growing body of research has also generated a number of predictions about the origin and pattern of diversification of arid zone lineages (Byrne et al., 2008; Fujita et al., 2010). For older components of the Australian arid zone fauna (either Gondwanan lineages or Asian lineages whose arrival dates back to the Miocene or earlier), four key predictions are: (1) that arid lineages are derived from ancestrally mesic lineages, (2) that moderately arid conditions and lineages adapted to them date back as far as the early Miocene, (3) that the late Miocene (around 10–6 Mya) was a time of major aridification and concomitant diversification among many lineages that now characterize the Australian arid zone, and (4) that successful adaptation to arid conditions was accompanied by specific adaptations to aridity (Byrne et al., 2008; Jordan et al., 2008). 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 these hypotheses about when, and how, elements of the Australian biota adapted to this newly emerging biome. Nonetheless, species-level dated phylogenies

---

* Corresponding author at: Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide, Adelaide, SA 5005, Australia. Fax: +61 8207 7222.

E-mail address: paul.oliver@adelaide.edu.au (P.M. Oliver).

1055-7903/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2011.03.018

demonstrating a clear temporal correspondence between ecological shifts and the early expansion of the developing arid biomes are few.

Squamates (lizards and snakes) 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, 2010). Of the four deeply divergent gecko lineages present, all of which are currently ranked as families under rank-based nomenclature, only the family 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). Their long history within Australia suggests that they should reveal historical environmental change, as they have persisted through these events, and patterns of diversification are less likely to be confounded by radiation following colonization. These ancient lineages have also all successfully adapted to the changing environment, and a significant proportion of diversity in all three families now occurs in arid Australia.

Relative to other Australian gekkotans, the Carphodactylidae have a relatively unusual distribution. Over half the species diversity, and most of the generic diversity (*Carphodactylus*, *Orraya*, *Phyllurus* and *Saltuarius*) within this family is concentrated within a relatively small area of temperate and mesic Australia (the aseasonal wet biome: Crisp et al., 2004), where other major gekkotan clades are relatively depauperate. In striking contrast, the 11 described species of *Nephrurus* (*sensu* Bauer, 1990), the only other recognized genus of carphodactylid geckos, occur across most of Australia and inhabit all other major Australian terrestrial biomes (temperate, monsoonal and arid).

Nephrurus are morphologically highly aberrant geckos; the tail is variably reduced with autotomy 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 specialized subdigital scalation to assist burrowing (Bauer and Russell, 1988, 1991). However, the most distinctive feature of all but two species is the caudal knob (see below), which is characterized by a thickened dermis, hypervascularisation, and an aggregation of sensory organs. The function of the knob is uncertain, but it might have a role in mechanoreceptive monitoring of the environment (Russell and Bauer, 1988) or in pheromonal transfer (Annable, 2004).

Three major groups of *Nephrurus* have been recognized based on morphological similarity (Greer, 1989): (1) the 'smooth' knob-tails, which can be further divided into small-tailed (*Nephrurus deleani*, *Nephrurus laevissimus*, *Nephrurus stellatus*) and the big-tailed groups (*Nephrurus levis* (with three subspecies) and *Nephrurus vertebralis*), (2) the 'spiny' knob-tails (*Nephrurus amyae*, *Nephrurus asper*, *Nephrurus sheai* and *Nephrurus wheeleri* (with two subspecies)), and (3) a pair of species lacking a caudal knob on the tail, frequently placed in the separate genus *Underwoodisaurus* (e.g., Cogger, 2000; Wilson and Swan, 2010), comprising *Nephrurus milii* and *Nephrurus 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 they 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 arid to seasonally arid and predominantly summer rainfall 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 with exposed granite in the New England tableland, while *N. milii* ranges from similar temperate areas, through semiarid and into arid areas spanning the southern third of the continent (Wilson and Swan, 2010).

The wide environmental distribution of lineages within this ancient Gondwanan clade of geckos provides a unique opportunity to examine hypotheses about the timing of aridification and the nature of biotic responses to it. In particular it has been suggested 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 (Couper and Hoskin, 2009; Crisp et al., 2009). In this study we examine phylogenetic relationships among the 11 described species of *Nephrurus* and other carphodactylid geckos using a combination of nuclear (*RAG1*, *c-mos*) and mitochondrial data (*ND2*), and use these data to examine the trajectory and temporal scale of evolution within carphodactylid geckos, with particular focus on (a) testing the monophyly and relationships of morphologically recognized groups of *Nephrurus* (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 that may have mediated the ecological success of this lineage across such a broad range of Australian environments.

2. Materials and methods

2.1. 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 I. We amplified portions of *ND2* (~1000 bp), *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 II. 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 the Pygopodoidea, and used data from Oliver and Sanders (2009). Data for outgroups outside Carphodactylidae is summarized in Appendix III.

PCR products were amplified using primers and protocols described elsewhere (Appendix III; Pepper et al., 2006; 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 to 63 °C for 34–38 cycles. PCR products were visualized 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.

2.2. Phylogenetic analysis

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 recognized *Nephrurus* species, five other carphodactylids including all recognized genera except *Orraya*, 24 other