

**PATTERNS AND PROCESSES OF ADAPTATION IN LACERTID
LIZARDS TO ENVIRONMENTS IN SOUTHERN AFRICA**

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DECLARATION

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ABSTRACT

The phenotype of an individual has often been used as the discriminating factor in distinguishing species. However, with the advent of more precise molecular techniques, the genotype of species is increasingly being used as the preferred method in taxonomic classifications. Many taxa have recently been demonstrated to be incongruent in terms of their genetic and morphological groupings, and this may be due to the influence that the environment may have on the morphological and functional aspects of a species. Selective pressures often act upon the performance of a species within a particular habitat first, and then selection for the morphological characters that allow for optimal performance occurs. Should genetically disparate species inhabit a particular environment, convergence in morphologies and performance may evolve. Historically, lizard species descriptions were based primarily on external morphologies, and thus misclassification of species may have occurred due to mistakenly grouping species with convergent morphologies together. In the current dissertation, the links between morphology, performance capacities, diet and behaviour is explored in comparison to the environment and genetic relationships of southern African lacertid lizards. The performance capacities and associated morphological traits were expected to be more closely linked with the environment, and not closely linked with genetic relationships. To investigate these expectations, a multidisciplinary approach was taken, and genetic, morphological and performance analyses were done and compared with dietary behavioural and environmental analyses. In the first chapter, the link between habitat openness and the lizard bauplan is investigated and the presence of convergent morphologies within this group of lizards is uncovered. These convergences are shown to have resulted in misclassification of two lacertid species, and taxonomic revisions within the family are discussed. The second chapter explores the link between performance and associated morphological traits, and the dietary composition of the members of the *Nucras* genus. The third chapter identifies the link between the predator escape strategies employed by the members of the *Meroles* genus, and their morphologies and performance capacities. The fourth chapter explores the intraspecific, inter-population differences in morphologies and investigates the link between the morphological groupings and the population genetic groupings within *Pedioplanis lineocellata*. The final chapter identifies whether adaptation to a novel habitat can occur over a relatively short period of time, and the morphological traits, functional aspects, and population genetic structure is investigated in conjunction with environmental analyses of vegetation and substrate between the populations of *Meroles knoxii*. It was concluded that the morphological and functional aspects of the southern African lacertid lizards are more closely related to the environment, particularly the microhabitat structure, than to their genetic relationships, and that future work using this group of lizards should involve a multidisciplinary approach as different selective pressures are playing a role in shaping the morphologies and performance capacities of these lizards, compared to those that are acting upon the genotypes of the lizards.

OPSOMMING

Die fenotipe van 'n individu is dikwels gebruik as die diskriminerende faktor in kenmerkende spesies. Maar, met die ontwikkeling van meer akkurate molekulêre tegnieke, word die genotipe van spesies toenemend gebruik as die voorkeur-metode in taksonomiese klassifikasie. Die onversoenbaarheid van genetiese en morfologiese eienskappe kom voor in 'n verskeidenheid taksas, dit kan wees as gevolg van die invloed wat die omgewing het op die morfologiese en funksionele aspekte van 'n spesie. Selektiewe druk beïnvloed dikwels doeltreffende funksionaliteit van 'n spesie in 'n bepaalde habitat eerste, en gevolglik word morfologiese karakters wat voorsiening maak vir optimale funksionaliteit geselekteer. Indien geneties uiteenlopende spesies woon in 'n bepaalde omgewing, kan konvergensie in morfologie en soortgelyke werksverrigtinge ontwikkel. Histories, is akkedis spesiesbeskrywings hoofsaaklik gebaseer op eksterne morfologieë, en kan dus misklassifikasie tot gevolg hê wat kan lei tot foutiewe taksonomie van spesies met konvergente morfologieë. In die huidige verhandeling, is die verband tussen die morfologie, werksverrigtingsvermoë, dieët en gedrag ondersoek, in vergelyking met die omgewing en die genetiese verwantskappe van Suider-Afrikaanse sandakkedis. Die werksverrigtingsvermoë en gepaardgaande morfologiese eienskappe word verwag om te meer verband te hou met die omgewing, en dus nie in noue verband te wees met die genetiese verwantskappe nie. Om hierdie verwagtinge te ondersoek, is 'n multi-dissiplinêre benadering geneem, en genetiese, morfologiese en werksverrigting-ontledings is gedoen in vergelyking met dieët, gedrags- en omgewings-ontleding. In die eerste hoofstuk, is die skakel tussen die habitat openheid en die akkedis bauplans ondersoek en die teenwoordigheid van konvergente morfologieë binne hierdie groep akkedisse word ten toon gestel. Hierdie konvergensies het gelei tot foutiewe klassifikasie van twee sandspesies, en taksonomiese hersiening binne die gesin word bespreek. Die tweede hoofstuk ondersoek die verband tussen werksverrigting en gepaardgaande morfologiese eienskappe, en die samestelling van die dieët van die lede van die *Nucras* genus. Die derde hoofstuk identifiseer die verband tussen die roofdier ontsnapping strategieë, morfologieë en werksverrigtingsvermoë van die *Meroles* genus. Die vierde hoofstuk ondersoek die intraspesifieke, inter-bevolkingsverskille in morfologieë en ondersoek die verband tussen die morfologiese groepe en die bevolking genetiese groepe binne die *Pedioplanis lineocellata* spesies kompleks. Die finale hoofstuk identifiseer hoe die aanpassings na 'n nuwe habitat kan plaasvind oor 'n relatief kort tydperk, en die morfologiese eienskappe, funksionele aspekte en die bevolking genetiese struktuur word ondersoek in vergelyking met die omgewingsanalise van plantegroei en substraat tussen die bevolkings van *Meroles knoxii*. Die gevolgtrekking is dat die morfologiese en funksionele aspekte van die Suider-Afrikaanse sandakkedis nader verwant is aan die omgewing, veral die mikrohabitat struktuur, as aan hul genetiese verwantskappe. Toekomstige werk op hierdie groep akkedisse moet 'n multidissiplinêre benadering behels siende dat verskillende selektiewe drukke 'n rol speel in die vorming van die morfologie en werksverrigtingsvermoë van hierdie akkedisse, in vergelyking met selektiewe drukke wat die genotipes van die akkedisse beïnvloed.

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General Introduction

GENERAL INTRODUCTION

Since the publication of Charles Darwin's "On the Origin of Species" (1859), the field of evolutionary biology has grown and diversified, and one of the more interesting, and hotly debated, questions in evolutionary biology is how a species originates. Speciation processes may occur due to the adaptation of living organisms to their surrounding environments, the exploitation of new niches or dispersal into more suitable habitats. Should a particular phenotypic variant result in a higher fitness in a given environment, the phenotype could become fixed, which may lead to species level diversification. Although genomic divergence takes place over millions of years, species may be produced at almost any time as a result of morphological, physiological and behavioural changes (Hewitt, 2000). Depending on the degree of the diversification, the species could diverge into two new species, populations, or perhaps into ecotypes.

Discordance may exist between phenotypic traits and genetics, possibly due to differences in molecular rates and morphological rates of change (Bromham *et al.*, 2002). These differences can come about as a result of rapid molecular lineage diversification (Wiens *et al.*, 2006), phenotypic plasticity (reviewed in Pfennig *et al.*, 2010) or rapid morphological divergence (Darwin, 1871; Endler, 1980, Schluter & Grant, 1984). Species' taxonomic classifications might be confounded by these incongruencies, if descriptions are based solely on morphological or functional traits (*e.g.* Miralles & Vences, 2013). For example, rapid morphological diversification due to strong directional selection on a fitness-enhancing phenotype in response to local environmental conditions may confound species' descriptions. Some morphological and functional characters can be plastic during an individual's lifetime (*e.g.*, Scheiner & Callahan, 1999; Van Buskirk & Saxer, 2001; Schmitt *et al.*, 2003; Pfennig *et al.*, 2010), and this plasticity can also lead to an over-estimation of species number. Conversely, taxa that were previously thought to be separate species have been grouped as a single species as a result of molecular studies using the phylogenetic species concept. New cryptic species have been discovered (*e.g.* Oliver *et al.*, 2009; Barata *et al.*, 2012), and thus the addition of molecular analyses has greatly aided taxonomists in delimiting species. With the incongruencies between morphology, performance, ecological associations and genetics of many taxa, a multidisciplinary approach to species designations is required to accurately identify taxonomic levels of various taxa (Pfennig *et al.*, 2010; Miralles & Vences, 2013).

Studies that have examined natural selection in performance traits (*e.g.* Irschick & Meyers, 2007) have added another dimension to understanding evolutionary processes in speciation, especially as to how variation in functional traits (*e.g.* bite force), and the linked morphological characters (*e.g.* head dimensions), may further confound taxonomic descriptions based on morphological traits. Natural selection may in fact operate first on functional traits, and secondarily on morphological characters (Arnold, 1983; Hertz *et al.*, 1988; Jayne & Bennett, 1990; Bonine & Garland, 1999). Interactions with

an individual's environment through its functional capacities may lead to directional selection on a particular functional trait (*e.g.* faster sprinting speed), and consequently selection on the related morphological trait (*e.g.* limb morphology) (Irschick & Meyers, 2007). Conversely, functional capacities have been shown not to be constrained by environmental factors, and are not linked with their morphologies, in some species (Kaliontzopoulou *et al.*, 2012*b*). Therefore, the inclusion of performance data in studies of evolutionary radiations may more fully describe the historical processes underlying speciation within the taxon in question.

Reptiles have been successively used in studies of evolutionary change (*e.g.* Huey & Pianka, 1981; Irschick & Meyers, 2007; Irschick *et al.*, 2008; Townsend *et al.*, 2008). Many studies have used the members of the family Lacertidae as test species in studies of adaptation and evolution (Herrel *et al.*, 1996; Vanhooydonck *et al.*, 2001*a*; Vanhooydonck & Van Damme, 2001*b*; Carretero *et al.*, 2006; Verwajen & Van Damme, 2007*a,b*; Huyghe *et al.*, 2007; 2009; 2010; Gabirot *et al.*, 2010; Kaliontzopoulou *et al.*, 2011, 2012*a*). The members of this family are distributed throughout the Old World, inhabiting many different environments, and exhibiting differing foraging behaviours, morphological traits and reproductive strategies (Branch, 1998; Arnold, 2002*a*; Spawls *et al.*, 2006; Kwet, 2009). The members of the family inhabiting the Middle-East and Europe have been more extensively studied than those in Africa, resulting in comparatively little knowledge about African species. However, current knowledge shows that a high diversity and endemism of reptiles are found in the sub-Saharan region of Africa (Branch *et al.*, 2006) resulting in the third richest reptile fauna in the world; the most diverse of which are the lizards (Bauer, 1993). The estimated time of the southern African lacertid genera radiation varies between 40 Mya (mitochondrial and nuclear data; Hipsley *et al.*, 2009), 16 Mya (mitochondrial data; Salvi, Bombi & Vignoli, 2011) and 13 Mya (mitochondrial and nuclear data; Mayer & Pavlicev, 2007), and such variation indicates that further work is needed in the group to identify speciation processes, both at a molecular and a morphological level. The species' ranges extend across differing biomes and environmental habitats throughout southern Africa (Branch, 1998). Morphologically many species differ markedly from one another, whilst others exhibit convergent morphological traits. Behavioural characteristics (*e.g.* reproductive strategies, foraging modes), as well as diet, differ between species (Branch, 1998). In short, the large diversity of lacertid species, inhabiting a diverse and changeable landscape, exhibit large phenotypic and genetic diversities, making them an excellent group for examining the environmental effects on phenotype, behaviour and genetic structuring.

Species variation

Selective pressures within certain environments may influence genetic and phenotypic variation within a species, and a convergence of phenotypes between genetically unrelated taxa may result if directional

selection pressures within a particular environment lead to a particular phenotype being expressed, confounding taxonomic classifications (e.g. Wainwright & Reilly, 1994; Schluter, 2000; Revell *et al.*, 2007; Wiens *et al.*, 2010). In some cases, only partial morphological convergence of species that occupy similar environments may take place (e.g. Vanhooydonck & Van Damme, 1999). In other instances, convergence between sister species may lead to similar morphotypes in genetically-distinct sister-species and species number would then be under-estimated (e.g. Renoult *et al.*, 2009). Rapid morphological divergence, on the other hand, may lead taxonomists to classify genetically-indistinct morphotypes as full species (e.g. in *Bradypodion pumilum* Measey *et al.*, 2009) or may under-estimate species number due to convergence of morphologies (e.g. in *Liolaemus monticola*; Torres-Pérez *et al.*, 2009). Thus, information about morphological convergence or divergence will provide insights into those processes involved in phenotypic diversity.

Cryptic species

Historically, species were described on morphological grounds, with limited use of molecular analyses, employing the biological species concept primarily to delimit species. However with the increasing ease of obtaining DNA sequences, many species have recently been described by incorporating the phylogenetic species concept. As such, many morphologically indistinguishable species that were historically described as one species have been separated into distinct species on genetic bases. These are known as cryptic species, and since Ernst Mayr introduced the concept in 1942, research and discovery of cryptic species has increased, mainly by incorporating molecular sequencing (see Bickford *et al.*, 2007 for references). Once a cryptic species complex is discovered, various questions are raised, for example: what are the factors influencing the morphology to be similar between species, and does stabilising selection play a role in driving the morphologically static cladogenesis? To answer the first question, factors in the environment are typically proposed as mechanisms driving the similarities in morphology between species (e.g. in *Podarcis*; Kaliontzopoulou *et al.*, 2012a). When environmental conditions cause the reduction or elimination of morphological change associated with speciation through stabilising selection, morphological stasis is said to have occurred (Nevo, 2001). To answer the second question, allopatric speciation as a result of habitat fragmentation followed by drift is typically proposed as the mechanism (Rothschild & Mancinelli, 2001). Alternatively, other methods, such as the evolution of differences in non-visual mating signals (*i.e.* olfactory or auditory signals; Bickford *et al.*, 2007), are also proposed. With the collection of new molecular data, taxa may need to be revised due to the discovery of cryptic species (e.g. *Scinax ruber* and *Rhinella margaritifera* Fouquet *et al.*, 2007; *Podarcis* Kaliontzopoulou *et al.*, 2011; *Iphisa elegans* Nunes *et al.*, 2012; *Atlantolacerta andreanskyi* Barata *et al.*, 2012). As the African lacertid lizards are relatively understudied, in comparison to their relatives in Europe and Asia, it is likely that with the addition of molecular data and more extensive morphological examinations further subdivisions within species and genera (as in Greenbaum *et al.*,

2011 and Barata *et al.*, 2012) and possible hybridisations, and leading to taxonomic misclassifications, will be uncovered.

Phenotypic plasticity

Organisms with similar genotypes that show differences in phenotypic traits (behaviour, morphology and physiology) in response to environmental changes within their life-times, possibly cyclically, are said to exhibit phenotypic plasticity (West-Eberhart, 1989). This phenomenon has received much attention from evolutionary ecologists, from both theoretical and empirical perspectives (see reviews in Stearns, 1989; Travis, 1994; Via, 1994; de Jong, 1995; Gotthard & Nylin, 1995; Via *et al.*, 1995). Some examples within squamates include seasonal bite force and head width changes, possibly due to hormonal changes, in *Urosaurus ornatus* (Irschick & Meyers, 2007), and body shape and size changes in response to diet in *Bitis gabonica* (Bonnet *et al.*, 2001). Such plasticity of phenotypic traits may confer a higher fitness to those organisms possessing this ability, in contrast to those that do not (Price *et al.*, 2003). Some plastic phenotypic traits have been seen to be heritable (Rosenblum & Beaupre, 2005), and thus such plasticity may facilitate genetic evolution (Price *et al.*, 2003).

Rapid morphological diversification

Unlike phenotypic plasticity, which occurs as a result of an individual adapting to local environmental changes within its lifetime, rapid morphological diversification may occur as a result of strong divergent natural or sexual selection (Harvey & Pagel, 1991; Bickford *et al.*, 2007), possibly through interspecific competition for resources (Mayr, 1942; Simpson, 1953; Schluter, 2000; Funk *et al.*, 2006). This rapid diversification, with little or no accompanying genetic differentiation using traditional neutral markers, may allow individuals with phenotypes that have significantly higher survival or reproductive success than others to better exploit their environment (Mayr, 1942; Simpson, 1944, 1953).

Higher morphological rates of change in relation to molecular rates may influence speciation processes to such a degree that morphotypes do not mate randomly with one another. This phenomenon has been found in several lizard species (Losos, 1990; Vitt *et al.*, 1997; Brehm *et al.*, 2001; Glor *et al.*, 2003; Irschick *et al.*, 2005). For example, in lacertid lizards, the three colour morphs in the Dalmatian wall lizard (*Podarcis melisellensis*) were found to be genetically indistinct, but differed in phenotypic traits involved in male-male competition for mates, suggesting sexual selection within this species (Huyghe *et al.*, 2007; 2010). Differences in body size, prevalence of infection by parasites and infection intensity were found in the different colour morphs in *Podarcis muralis* (Calsbeek *et al.*, 2010). Additionally, colour assortative mating may be driving the maintenance of the discrete colour morphs in *P. muralis* (i de Lanuza, Font & Carazo, 2013). Another lacertid species, *Gallotia galloti*, on the Canary Islands also shows differences in phenotypic traits involved in male-male competition for mates (Thorpe & Malhotra, 1998). Other divergent phenotypic traits, such as chemical signals between *Podarcis atrata*

endemic to the Columbretes islands and introduced mainland *P. hispanica*, may prevent hybridisation between genetically similar species (Gabirot *et al.*, 2009).

Adaptive radiations vs. non-adaptive radiations

Adaptive radiations are those rapid lineage diversifications accompanied by adaptive phenotypic change, divergent ecological specialisation, and competition (Schluter, 2000). Classic examples of these radiations include cichlid fish of the African Rift Lakes (Fryer & Iles, 1972; Kornfield & Smith, 2000; Kocher, 2004; Seehausen, 2006; Clabaut *et al.*, 2007), Caribbean *Anolis* lizards (Losos & Miles, 2002; Pinto *et al.*, 2008) and Darwin's finches (Burns *et al.*, 2002). Non-adaptive radiations, on the other hand, occur when species are genetically distinct, but with little accompanying ecological variation (Gittenberger, 2002). Typically, those taxa that exhibit non-adaptive radiations are allopatric, as those species with the same or similar ecological niches cannot coexist together, according to Gause's Principle (Gause, 1932; Gittenberger, 1991). It is thought that species are differentiated one another through chemical cues (Wake, 2006). These two definitions both indicate a close relationship between the environment and morphologies of vertebrates, in that members of an adaptive radiation are phenotypically distinct and are associated with the particular environment that they have adapted to, whilst members of non-adaptive radiations occupy the same ecological niche, essentially similar environments, and are therefore phenotypically similar.

The processes involved in speciation are complex and therefore a multidisciplinary approach to discern the species status of assemblages can be advantageous. The rates of change of phenotypic traits and genetics between species may differ, causing taxonomists to award species status to genetically indistinct assemblages by using the morphological species concept. Differences in the diversity of genomes may also confuse phylogeographic interpretations (as in *Hemidactylus turcicus*; Rato, Carranza & Harris, 2011). On the other hand, the difference in the rates of change may also mask the existence of multiple species within an assemblage deemed to be a single species, and these cryptic species may only be considered to be separate species when using the phylogenetic species concept. Divergence in certain phenotypic traits (*e.g.* chemical signals) may lead to reproductive isolation of genetically similar populations. Many morphological differences between genetically similar assemblages are largely due to adaptations to local environmental conditions, and investigations into how a taxonomic group radiated in the past may lead to better predictions of how the taxon may fare in a future changing climate.

Background to lacertid lizards

Lacertid lizards (Sauria: Lacertidae) are diurnal, mostly heliothermic lizards that generally measure less than 120 mm from snout to vent, with tail lengths, often substantially, longer than the body. They are

found throughout the Old World, mainly in Africa and Europe, in a variety of habitats, such as high mountain tundra (*Tropidosaura*), heath lands (*Lacerta*), Mediterranean scrub (*Gallotia*), tropical forest (*Holaspis*), semi-desert and desert (*Meroles*) (FitzSimons, 1943; Arnold, 1989; Branch, 1998). These lizards inhabit a wide variety of microhabitats, differing in substrate, openness and inclination (Vanhooydonck & Van Damme, 1999). Even though these lizards utilize such varying habitats, their general body shape has remained relatively conserved (Arnold, 1989; Vanhooydonck & Van Damme, 1999), with no major alterations in, for example, locomotor apparatus (severe reduction/loss of limbs, development of toe pads etc.), or additional ornamentation (such as extensive body armour, thorny spikes, head ornamentation etc), in differing habitats. However, within the conserved body shapes, variation is present that has been linked to performance capacities (Vanhooydonck & Van Damme, 1999) and the environment (Vanhooydonck *et al.*, 2001a).

Their diet consists predominantly of arthropods and some gastropods and vertebrates, and many species supplement their diet with vegetation (*e.g.* some *Gallotia* species are herbivorous; Arnold, 1989; *M. anchietae* supplements its diet with seeds; Branch, 1998; *pers. obs.*). Foraging strategies range from actively hunting for food to ambushing prey that ventures close (Branch, 1998). Reproductive strategies also vary within the family, with the majority of species laying eggs, whilst some populations of *Zootoca vivipara* and some species in the genus *Eremias* bear live young (Arnold, 1989; Branch, 1998).

Systematic relationships within the family Lacertidae

The members of the family Lacertidae are squamate reptiles forming part of the Laterata (including teiids, gymnophthalmids, amphisbaenians and lacertids) within the Scleroglossa (Vidal & Hedges, 2005; Wiens *et al.*, 2010). The first extensive systematics of the Lacertidae family based on external morphological characters, published by Boulenger (1920, 1921), remained uncontested for almost half a century until Arnold (1973) separated the genera *Podarcis* and *Gallotia* based on osteological traits. Generic status was ascribed to Boulenger's 'sections' of *Eremias* just two years later (Szczerbak, 1975). Arnold (1989) then utilised morphological traits to construct a concise phylogeny for the family, which revealed two groups: 1) a 'primitive Palearctic and Oriental assemblage' and 2) an 'advanced Saharo-Eurasian and Ethiopian clade'.

Early molecular studies, namely albumin-immunological studies, produced better resolution between species and divided the family into two subfamilies: Gallotiinae and Lacertiinae. The studies first showed even greater divergence within *Gallotia* and *Psammmodromus* (Lutz & Mayer, 1985), and then confirmed the distinct positions of the two genera within the newly established subfamily Gallotiinae (Mayer & Benyr, 1994). With this study, Arnold's (1989) 'advanced Saharo-Eurasian and Ethiopian' clade was shown to be paraphyletic, with some members of the Saharo-Eurasian genera being more closely related to the European taxa.

Harris *et al.* (1998a) used a combination of Arnold's (1989) morphological data and mitochondrial DNA (mtDNA) sequence data, which resulted in a phylogeny similar to the results of the morphological analyses. Arnold's (1989) 'primitive Palearctic and Oriental' assemblage was renamed as the subfamily Lacertinae, and the 'advanced Saharo-Eurasian and Ethiopian' clade was assigned to the subfamily Eremiinae, with the retention of the subfamily Gallotinae. The position of the genus *Takydromus* remained unresolved, but was presumed to be related to the members of Lacertinae (particularly *Zootoca vivipara* – formerly *Lacerta vivipara*). The use of mtDNA by Fu (2000) supported the division of Lacertidae into two subfamilies, Gallotiinae and the more speciose Lacertiinae, rather than three. The latter subfamily was divided into the African and Arabian genera (*Tropidosaura*, *Meroles*, *Nucras*, *Heliobolus*, *Acanthodactylus*, *Ophisops*, *Adolfus*, *Pedioplanis*, *Mesalina*, *Latastia*) and the Eurasian genera (*Eremias*, *Lacerta*, *Podarcis*, *Takydromus*). Although the deep nodes within the family were supported, the relationships within the subfamilies were not, and Fu (2000) suggested that increased sampling at the lower taxonomic levels would possibly provide better support for these levels.

Recent phylogenetic analyses of the family, using nuclear genes (Mayer & Pavlicev, 2007) and a combined dataset (Kapli *et al.*, 2011), again recovered the three recognised subfamilies: Gallotinae, Eremiinae and Lacertinae, the latter two of which were reclassified as tribes (Eremiadini and Lacertini; Arnold *et al.*, 2007). The southern African genera (*Australolacerta*, *Heliobolus*, *Holaspis*, *Ichnotropis*, *Meroles*, *Nucras*, *Pedioplanis* and *Tropidosaura*) were grouped into the tribe Eremiadini. Of the 45 species comprising the eight genera, 28 are endemic to the southern African region (Branch, 1998). Two of these genera (mountain lizards *Tropidosaura* and southern rock lizards *Australolacerta*) are strictly endemic to South Africa, whilst the remaining genera are more widely distributed. The sandveld lizards *Nucras*, rough-scaled lizards *Ichnotropis*, Bushveld lizards *Heliobolus* and tree lizards *Holaspis* all extend into central and East Africa, whilst the desert lizards *Meroles* and the sand lizards *Pedioplanis* are found in the more westerly semi-arid or arid regions of Namibia, South Africa and Botswana (Branch, 1998). Despite the fact that this species richness may represent a diversity hotspot for this group, the species level phylogenetic relationships of only two genera has been investigated, namely *Meroles* (Harris *et al.*, 1998b; Lamb & Bauer, 2003) and *Pedioplanis* (Makokha *et al.*, 2007; Conradie *et al.*, 2012).

The genera used in this dissertation were chosen on the basis that they are relatively speciose (Branch, 1998; Lamb & Bauer, 2003; Makokha *et al.*, 2007), with members being widely distributed throughout the varying environments found in southern Africa, are morphologically variable in terms of size, colouration, and body dimensions, and differ in life-history traits (*e.g.* reproductive strategies vary) (Branch, 1998).

Motivation

Because reptiles are known to undergo adaptive radiations (for a review see Camargo *et al.*, 2010) and there is high diversity and endemism in southern Africa (Branch *et al.*, 2006), particularly of lizards (Bauer, 1993), it is possible that some of the diversity is due to adaptive radiations. Investigations of radiations in southern African reptile fauna could benefit from a multidisciplinary approach, involving morphological analyses, molecular phylogenies and population genetic analyses, as well as investigations of the performance traits, including measures of sprinting and bite force. This approach would also provide information about the environments in which adaptive radiations occur, as well as contribute toward understanding the processes underlying the phenomenon.

In 2003, a phylogeny of *Meroles* using mitochondrial DNA (mtDNA) was published (Lamb & Bauer, 2003), and more recently a phylogeny of *Pedioplanis* incorporating a geographic component using both mtDNA and nuclear genes (nDNA) was published (Makokha *et al.*, 2007). At the beginning of the current work for this dissertation, a phylogenetic analysis of the genus *Nucras* had not been published. Also, the two species of *Australolacerta* had not been included in a phylogenetic analysis together, and due to the large geographic distance between their ranges (>1700 km), they may not be as closely related as they are presently considered.

The concordance between hindlimb morphology and foraging mode has been investigated in only four species from three southern African genera (McBrayer & Wylie, 2009), however the relationship between morphology, performance and microhabitat structure have not been explored for other species in any of the southern African lacertid genera. Because the species to be investigated from the proposed genera occur in a diversity of habitats and experience a variety of environmental conditions across their ranges, the expectation is for a correspondence between limb morphology and substrate and vegetation structure (as in *Anolis* lizards; Williams, 1983; Glor *et al.*, 2003; Harmon *et al.*, 2005). This would mean that there is strong selection on the phenotype due to local extrinsic factors, and phenotypic differences between species would be more closely linked with the environment, irrespective of genetic relationships between the species.

Although there are studies using geometric morphometric techniques on a few European lacertid lizards (*e.g.* Kaliontzopoulou *et al.*, 2007; Costantini *et al.*, 2010; Moazen *et al.*, 2013), such studies are lacking on southern African lacertid species. Traditional morphometric analyses have been used for the investigations into cranial morphological variation due to environmental conditions (*e.g.* in chameleons: Measey, Hopkins & Tolley, 2009). Cranial shape may change in relation to the environment, for example the diet of the lizards may change with differing environments causing a need for a stronger bite force with increased hardness of prey (*e.g.* Herrel *et al.*, 2001a). Limited resources may cause intra-

and interspecific competition to increase, necessitating a stronger bite force in inter-sexual and intraspecific fights (e.g. Huyghe *et al.*, 2005). Bite force and sprinting speed have been used in analyses of performance capacities and therefore the addition of these analyses to this study may be key to understanding the adaptive nature of the lacertid radiations in southern Africa.

In the current doctoral study, a multidisciplinary approach was used to investigate the radiations within southern African lacertid lizards. In the first chapter, the phylogenetic relationships between the southern African lacertid lizards were investigated and compared to morphological aspects of each species. The analyses found convergence in morphologies and the taxonomic implications of such convergence is discussed. In the second chapter, the interspecific phylogenetic relationships of *Nucras* are examined and used in phylogenetic comparative analyses. Dietary data, published previously, is then utilised to investigate the link between the dietary niche breadth and the morphological dimensions of five *Nucras* species. Performance capacities, namely the bite force and sprinting speeds of the five species, are then compared to the morphologies and diet data to identify possible links between diet, morphology and performance in *Nucras*. The third chapter investigates the link between predator escape strategies and morphology, and the possible links with bite force capacities in *Meroles*. The phylogenetic relationships between the members of *Meroles* are investigated and used in phylogenetic comparative analyses to determine if differences in the morphological aspects and performance capacities of *Meroles* are phylogenetically independent. The fourth chapter investigates the population genetic structure and morphological differences between populations within a wide-ranging species *Pedioplanis lineoocellata*. The taxonomic status of the species is discussed and the possible reasons for the incongruence between morphological groups and phylogenetic clades within the complex is explored. For the final chapter, adaptation to a novel habitat is explored in a translocated population of *Meroles knoxii*. Genetic, morphological and performance aspects of the members of the translocated population are compared to the source population and to a control population. These aspects are then compared with environmental variables to identify potential factors influencing morphology and performance in the populations. On the whole, the overarching aim of the dissertation is to show that at various taxonomic levels the phenotypes of the southern African lacertid lizards are more closely linked with the environment than to phylogenetic ancestry. As historic species descriptions were based on external morphological characters, the incongruencies between the morphological and genetic aspects of the lizards may have taxonomic implications and the use of various species concepts in lizard classifications is explored in the conclusion chapter.

AIMS AND OBJECTIVES

The main aim of the study was to investigate the processes underlying radiations within the genera and within species of southern African lacertid lizards, by comparing molecular phylogenetic analyses with morphological and performance trait data, as well as diet and environmental variables.

The overarching hypotheses for this study were as follows:

Species radiations represent diversification with adaptive implications and are therefore considered adaptive, unique to each habitat.

- Morphological differences are linked to environmental factors, irrespective of genetic relationships.
- Genetically distinct, but morphologically similar, taxa occurring on different substrates/environments will exhibit similarities in performance traits.
- Morphological differences are strongly associated with performance differences exhibited.

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Chapter 1

**CONVERGENT MORPHOLOGIES ASSOCIATED WITH HABITAT
STRUCTURE HAVE LED TO THE MISCLASSIFICATION OF SPECIES**

CONVERGENT MORPHOLOGIES ASSOCIATED WITH HABITAT STRUCTURE HAVE LED TO THE MISCLASSIFICATION OF SPECIES *

Introduction

Local environmental conditions can exert pressure on an organism to express a particular phenotype in order to survive within that environment. The organism is said have adapted to that particular environment, and confers a greater fitness to its offspring through the selection of the fitness-enhancing phenotype (Losos, 2011). When genetically distantly related species adapt to a particular environment in similar ways (*i.e.* development of a similar phenotype), their phenotypes are said to have converged. Although phenotypic convergence is a common explanation for morphological similarity, such occurrences can be the result of chance and/or pre-existing constraints ('exaptation') rather than adaptation to similar environments (Losos, 2011). Natural selection favours traits that increase fitness, even if the trait did not evolve in response to those selective pressures. While experimental conditions simulating environments can convincingly demonstrate whether natural selection drives convergence in morphological traits (Templeton, 1996), it is more difficult to test convergence through adaptation to shared environments within a natural setting (Stayton, 2006). Yet repeated evolution of convergent phenotypes in divergent lineages inhabiting similar environments is often considered strong evidence of natural selection operating on morphological traits.

Convergent evolution has been found between numerous squamate reptiles (*e.g.* Williams, 1983; Glor *et al.*, 2003; Harmon *et al.*, 2005; Revell *et al.*, 2007; Wiens *et al.*, 2010; Mahler *et al.*, 2013), showing that similarities in environmental conditions and micro-habitat use may elicit similar adaptive evolutionary responses by directional selection regardless of ancestry. Similar morphologies are observed between distantly related rock-dwelling (Gifford *et al.*, 2003; Vitt *et al.*, 1997; Losos *et al.*, 2002; Revell *et al.*, 2007), burrowing (Kearney & Stuart, 2004; Lee, 1998; Whiting *et al.*, 2003), as well as arboreal lizards (Losos, 1990; Losos *et al.*, 1998; Vanhooydonck & Van Damme, 1999; Schluter, 2000). In each of these cases, adaptation is ascribed to selection on an animal's body plan in order to optimize performance in a given habitat. For example, rock-dwelling species typically have flat heads and bodies that allow them to fit into narrow cracks, with long forelimbs adapted for climbing (Revell

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Edwards S, Tolley KA, Vanhooydonck B, Herrel A, Measey GJ, Branch WR. 2013. A review of the systematics of the southern African lacertid lizards (Sauria: Lacertidae). *Zootaxa*. 3669 (2): 101–114

et al., 2007). In contrast, some arboreal species are specialized to move on narrow substrates and have short limbs and narrow, tall bodies (Herrel *et al.*, 2002, 2008, 2011*a*; Hopkins & Tolley, 2011).

Southern Africa has a diverse assemblage of macro-habitats, from tropical forest to desert, and ranges from sea level to more than 3000m. This complexity at the macro scale is interwoven with a diversity of micro-habitat structure that includes different substrates and vegetation organization, and the heterogeneity at both scales may be a strong factor in producing the high diversity and endemism of reptiles in the region (Bauer, 1993; Branch *et al.*, 2006). Indeed, many species are restricted and habitat specific at the micro scale (*e.g.* chameleons, cordylids), whilst others are apparent generalists (*e.g.* skinks). Morphological adaptation to this diversity in habitat structure should be reflected in phylogenies as lineages showing morphological convergence in species living in similar habitat structure, or divergence in species occupying different habitat structure.

Here, the convergence of ecologically relevant phenotypic traits was examined within micro-habitats differing in degree of habitat openness in a diverse group of lizards (Eremiadini) from sub-Saharan Africa. It was predicted that ecologically relevant traits would converge in association with habitat similarity, regardless of evolutionary history. To test this hypothesis, the evolutionary relationships in the Eremiadini was investigated using a multi-locus phylogenetic approach, in combination with principal components analysis and hierarchical clustering for morphological data on traits that are considered ecologically relevant to lizards (Revell *et al.*, 2007; Bauwens *et al.*, 1995). The clusters were then compared to *a priori* micro-habitat usage data to identify convergent morphological traits between species.

Materials and Methods

Sampling

Samples for the genetic analyses were obtained from either field trips or from samples, collected by various researchers, housed in the collection at the South African National Biodiversity Institute. Samples for the morphometric analyses included measurement of live lizards during field work, as well as voucher specimens housed at the Port Elizabeth Museum (PEM), the Ditsong Museum (TM) and the Ellerman Collection at Stellenbosch University.

Laboratory protocols

Genomic DNA was isolated from the tail or liver tissue preserved in 95–100% ethanol according to standard procedures involving a proteinase-K digestion followed by salt-extraction (Bruford *et al.*, 1992). Standard PCR procedures were utilized to amplify two mitochondrial (16S and ND4) and two nuclear genes (RAG1 and KIAA-2018). The nuclear genes were chosen because these genes have been

shown to evolve at a rate that may allow for obtaining high confidences in both the terminal and the deeper nodes (Townsend *et al.*, 2008; Portik *et al.*, 2011). Details of the primer pairs used for the analyses are listed in Table A1. Amplification of the four genes was carried out with ~25-50 ng/μl genomic DNA and a 25μl reaction containing a thermophilic buffer (50mM KCl, 10mM Tris-HCl, pH 9.0), 1.5mM MgCl₂, 0.2μM of each primer, 0.2mM dNTPs, and 0.025 U/l Taq polymerase. Cycling profile for 16S, ND4 and KIAA-2018 genes included an initial denaturing step at 94°C for 4 minutes, followed by 35 cycles of 94°C for 30s, 50-55°C for 30s, and 72°C for 45s, with a final extension at 72°C for 8 min. The amplification of the RAG1 gene region involved a step-down procedure (Groth & Barrowclough, 1999). The PCR products were sent to Macrogen Corp. (Seoul, Korea) for sequencing, using the forward primers in all cases. Some of the individuals sampled have been sequenced previously for the 16S and RAG1 genes, and accession numbers and references are provided in Table A2. Sequences were aligned in BioEdit Sequence Alignment Editor v. 7.0.5.2 (Hall, 1999). All sequences have been deposited in EMBL-Bank (see Table A2 for all voucher information, with corresponding EMBL-Bank accession numbers).

Genetic analyses

First, the mitochondrial (16S vs. ND4) and nuclear (RAG1 vs. KIAA-2018) datasets were analysed separately to ensure that there was no conflict in the markers within each genome, using a partition homogeneity test (Farris *et al.*, 1994, 1995) in PAUP* v4.0b10 (Swofford, 2002). The two mitochondrial and the two nuclear genes were not incongruent, so the partition homogeneity test was run again (nuclear vs. mitochondrial) to ensure that there was no conflict between the two genomes. Phylogenetic trees were constructed of the 1) mitochondrial gene dataset, 2) the nuclear gene dataset and 3) the combined total evidence dataset. The saturation of the codon positions was assessed (Dambe v.5.2.65; Xia *et al.*, 2003) and the third codon position of the ND4 gene was found to be saturated, so it was coded as a separate partition (ND4b) in the maximum likelihood and Bayesian analyses using nucleotide substitution models (thus five partitions in total: 16S, ND4a, ND4b, RAG1 and KIAA-2018). Individuals from two genera (*Nucras* and *Heliobolus*) were used as outgroup, as they are nested within the sister clade to the southern African lacertids within the Eremiadini (Mayer & Palicev, 2007; Kapli *et al.*, 2011). Sequence divergences were determined by estimating the uncorrected p-distances between and within species using the program MEGA v.4 (Tamura *et al.*, 2007).

Two different algorithms were utilized to obtain phylogenetic trees. Bayesian inference (BI; MrBayes v.3.1.0; Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) was performed using the best-fit models of nucleotide substitution for all five gene partitions (jModeltest v.2.1; Posada, 2008). The best-fit models of nucleotide substitution for all the mitochondrial gene partitions were GTR+I+G and all the nuclear gene partitions were GTR+G, and uniform priors were kept for all other parameters. A

second Bayesian inference was performed, using a codon substitution model for all three partitions of coding genes (ND4, RAG1 and KIAA-2018) and the best-fit model of nucleotide substitution (GTR+I+G) for the 16S gene fragment partition. The nucleotide substitution parameters within the codon models were of the 6-rate variety (inferring different rates for all nucleotide pairs, GTR-like), with empirical codon frequencies. The MCMC were run with 2 parallel runs for 20 million generations each, with trees sampled every 1000 generations. The number of generations to discard as burn-in was determined by examining the number of generations 1) at which the standard deviation of split frequencies stabilized (at less than 0.001), 2) at which the log-likelihood tree scores reached stationarity, and 3) the effective sample sizes (ESS) of all parameters which were >600 (using the program Tracer v.1.5; Rambaut & Drummond, 2007). A 50% majority rule tree was constructed, with the burn-in excluded, using the “sumt” command in MrBayes, and nodes with ≥ 0.95 posterior probability were considered supported. A partitioned maximum likelihood (ML) analysis was also run in RaXML v.7.2.8 (Stamatakis, 2006), at the CIPRES Science Gateway (www.phylo.org/sub_sections/portal/) using the same partitions as the Bayesian analysis, a GTR+I+G model of evolution, and automatic halting of bootstrapping (Stamatakis, 2006; Stamatakis *et al.*, 2008).

Competing phylogenetic hypotheses of monophyly for *Ichnotropis* and *Australolacerta* were investigated using a Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa 1999; Goldman *et al.*, 2000) and the approximately unbiased (AU) test (Shimodaira, 2002) generating maximum likelihood scores for the trees (1000 replicates) using PAUP* v.4.0b10 (Swofford, 2002) and bootstrapping *P*-values for the SH and AU tests in Consel (Shimodaira, 2002). The Bayesian consensus topology obtained was compared to a topology which constrained 1) *I. squamulosa* to be within *Ichnotropis*, and 2) *Australolacerta australis* and *A. rupicola* as monophyletic.

Characterization of habitat type

Two broad habitat types (open and cluttered) were defined for our analysis based on the general characteristics of vegetation structure associated with each species sampled. Open habitat lacks vegetation completely (*i.e.* dunes) or is sparsely vegetated, and mainly characterized by open sand, gravel or rock patches briefly interspersed with bushes or grass tufts. A cluttered habitat is densely vegetated (*i.e.* with low vegetation such as grasses, sedges and restios, with an abundance of bushes in various sizes), with intermittent open patches (Fig. 1.1). In general, the southern African lacertid species are associated with particular types of habitat, and the association with either open or cluttered habitat is easy to ascertain for each species as a whole. Habitat associations were ascertained from personal observation and from published field guides and literature (FitzSimons, 1943; Branch, 1998).



Figure 1.1: Photographs of cluttered (A – Phillippi, Western Cape Province, South Africa) and open habitat (B – dunes near Gobabeb, Namibia), as examples of the two habitat categories defined for this study (Photos by SE).

Morphometric analyses

Morphometric measurements were taken of specimens housed at the Port Elizabeth Museum and the Ditsong Museum in South Africa, as well as individuals captured for this study in South Africa and Namibia. Body length (snout-vent length; SVL) and biometric characters on the head, hindlimbs and forelimbs were measured using digital callipers for each individual (Fig. 1.2). Measurements taken on the head were: head length (HL) from snout-tip to the back of the parietal bone, head width (HW) measured as the widest part of the head, head height (HH) measured as the height from the top of the interparietal scale to the bottom of the lower jaw (including muscles), posterior edge of the quadrate bone to snout-tip (QT), apex of coronoid bone to snout-tip (CT), lower-jaw length (LJL). Measurements taken on the limbs were as follows: the femur length (FM), tibia length (TB), humerus length (HM) and radius length (RD). Other body dimensions measured were body height (BH) and body width (BW). Whilst sexual dimorphism of phenotypic traits have been found in other lacertids, the differences between species tend to exceed the extent of the differences between sexes, and therefore the species as a whole was investigated, and not separated by sex for the current analyses.

Hierarchical clustering of the species was performed in the program R Studio v.0.97.248 (R Core Team, 2012; R Studio, 2012), to identify morphological clusters. The mean value per species (17 species) of each size-regressed measurement (12 measurements) was calculated (package: 'base', function: 'mean'; R Studio, 2012) and the mean values per species for each measurement were regressed onto the mean snout-vent length (SVL) using a linear model to eliminate the effect of size (package: 'stats', functions: 'lm' and 'resid'; R Studio, 2012). Hierarchical clustering of the residual distances was performed (package: 'pvclust', function: 'pvclust'; R Studio, 2012) in which the distance matrix was calculated using the 'correlation' option, the clustering dendrogram was constructed using the 'complete' option, and support values for the nodes were estimated using 1000 bootstrap replicates.

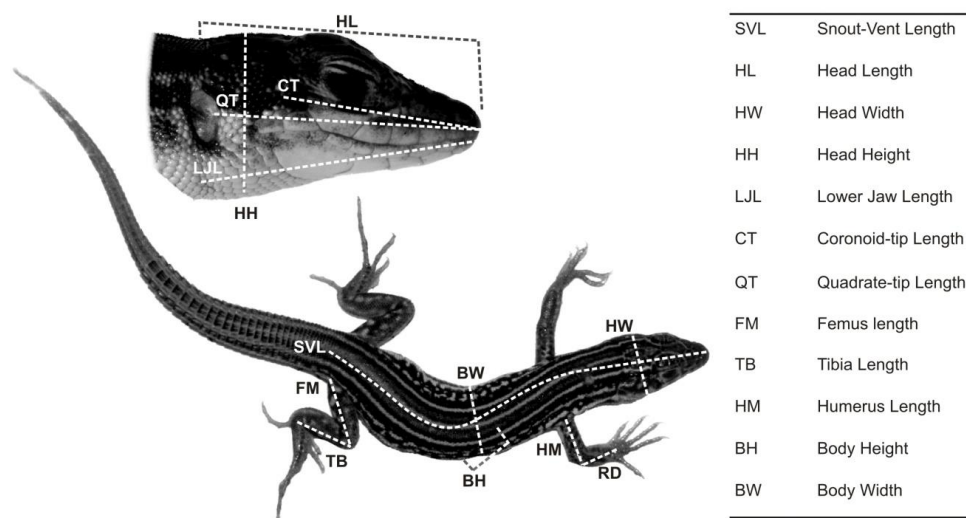


Figure 1.2: Photographs of a lacertid lizard indicating abbreviations used for the morphometric measurements taken. Inset table displays full names for the abbreviations indicated on the photographs.

To examine trait differences among the morphological groupings obtained in the hierarchical clustering, a principal components analyses (PCA) on the residuals was performed in the program SPSS v.15 (SPSS, Inc.). An exploratory factor analysis was performed, to investigate the proportion of variation explained by each relative measurement, and a correlation matrix was produced (the primary data used for the factor analysis) which was inspected for adequate determinant factor, sampling adequacy (Kaiser-Meyer-Olkin (KMO) test) and sphericity (Bartlett's test). Those factors that had eigenvalues ≥ 1.0 were extracted for further analyses using a PCA and rotated using a varimax rotation. The KMO test indicated sampling was adequate (*i.e.* KMO Measure of Sampling Adequacy was higher than 0.5), all communalities were high (*i.e.* in excess of 0.5) suggesting that all variables were reliable contributors to the analysis, there were sizeable correlations between all original variables, and low correlations in the residual correlation matrix (Tabachnick & Fidell, 1996). Three principal components (PC) were extracted, which accounted for *ca.* 74.45% of the total variance (Table 1.1) and boxplots were constructed using the PC scores for these same groups (package: 'stats', function: 'boxplot'; R Studio, 2012). Analysis of variance (ANOVA) was carried out on the three principal components extracted with the morphological cluster as the fixed factor (package: 'stats', function: 'anova'; R Studio, 2012).

Table 1.1: Principal components analysis of size-regressed measurements, with loadings of each measurement for the three axes that had eigenvalues ≥ 1.0 . Characters that loaded the highest within each PC axis are shown in bold. F-values of the analysis of variance between two main morphological clusters is shown (***: $P < 0.001$; ns: not significant).

Residuals	PC1	PC2	PC3
Body width (BW)	0.89	0.07	0.03
Head width (HW)	0.79	0.32	0.27
Body height (BH)	0.77	0.14	-0.02
Head height (HH)	0.60	0.53	0.21
Lower jaw length (LJL)	0.27	0.81	0.10
Quadrate-Tip length (QT)	0.29	0.78	0.29
Head length (HL)	0.40	0.76	0.23
Coronoid-Tip length (CT)	-0.07	0.70	0.27
Radius length (RD)	0.02	0.23	0.88
Humerus length (HM)	0.02	0.21	0.87
Tibia length (TB)	0.52	0.27	0.66
Femur length (FM)	0.58	0.30	0.59
% variance	50.74	14.27	9.47
F-value	430.19 (***)	2.60 (ns)	15.77 (***)

Results

The combined mitochondrial and the nuclear topologies (BI and ML) were congruent (Fig. 1.3) and largely consistent with previous work (Mayer & Pavlicev, 2007). The data however show two notable exceptions due to the inclusion of additional taxa (*Ichnotropis spp.* and *Australolacerta spp.*), both of which suggest that factors independent of ancestry are driving morphological evolution in the Ereimiadini. Firstly, the two species of *Australolacerta* are separate evolutionary lineages, and form part of a deep basal polytomy at the generic level (Fig. 1.3), despite the ecological and morphological similarities that were used to place them in the same genus (Arnold, 1989). Secondly, the phylogeny shows that *Ichnotropis squamulosa* shares its most recent ancestry with members of the genus *Meroles* (Fig. 1.3), rather than with species in the morphologically similar genus *Ichnotropis* (Fig. 1.4), leading to a misclassification at the generic level. *Ichnotropis squamulosa* grouped with *Meroles* with strong support, and inclusion of this species within a monophyletic *Ichnotropis* can be rejected by the SH and AU tests ($P < 0.01$, $P < 0.001$, respectively). In both cases, convergence in bauplan is coupled to traits associated with body/head width and limb dimensions (Fig. 1.4).

The phylogenetic analyses show that the two species, *A. australis* and *A. rupicola*, are separate evolutionary lineages, and form a polytomy with all other Ereimiadini genera except *Meroles*. Therefore, they have been incorrectly placed together in a single genus due to the similar body plan and this is also supported by the high sequence divergence between these lineages (16S: $9.55 \pm 2.08\%$, ND4: $22.69 \pm 1.60\%$, RAG1: $3.74 \pm 0.76\%$, KIAA: $1.90 \pm 0.47\%$), consistent with generic divisions in southern African Lacertidae (16S: $7.57 \pm 1.38\%$, ND4: $21.21 \pm 1.33\%$, RAG1: $4.07 \pm 0.54\%$, KIAA: $2.84 \pm 0.60\%$; this study) as well as others (combined RAG1 & C-MOS: 1.40% between *Archaeolacerta* and *Zootoca*; Mayer & Pavlicev, 2007). A monophyletic *Australolacerta*, however, could not be rejected using the SH and AU tests.

The adaptive nature of convergence in Ereimiadini is demonstrated by the significant association of ecologically relevant traits and habitat structure. Hierarchical clustering of morphological features resulted in two major clusters that correspond to A) cluttered and B) open habitats (Fig. 1.4). These morphological clusters do not correspond to the evolutionary history of these taxa (Fig. 1.4), but instead are significantly different with respect to sets of ecologically relevant characteristics related to habitat structure. Each cluster was further subdivided into either three (cluster A: A1, A2 and A3) or two (cluster B: B1 and B2) subclusters. Some of the subclusters can be linked to particular microhabitats within a cluttered or open habitat. For example, cluster B2 species are dune-dwelling, whilst species of cluster A2 and A3 are rupicolous.

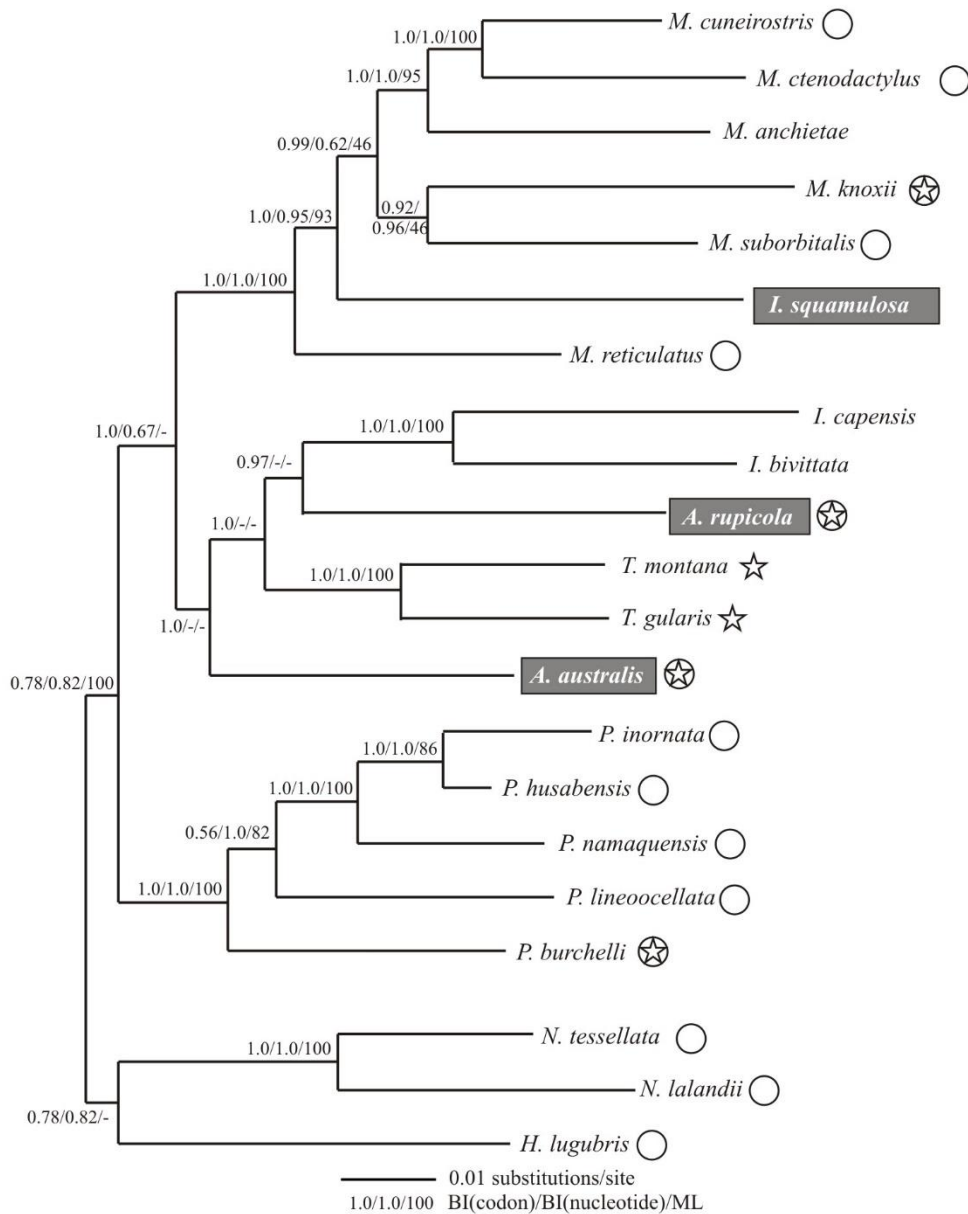


Figure 1.3: Phylogenetic tree of the southern African radiation of the lacertid subfamily Eremiadini based on the combined partial 16S, ND4, RAG1 and KIAA-2018 gene regions and inferred by BI and ML (Bayesian topology shown). Nodes that are supported using Bayesian inference (posterior probabilities > 0.95) using nucleotide and codon substitution models and maximum likelihood (bootstrap values >75%) using GTR+I+G nucleotide substitution model are shown at nodes (post. prob. using codon-substitution model/post. prob. using nucleotide-substitution model/bootstrap value for ML). A dash indicates that the node was not supported for the particular analysis. Stars next to species names indicate presence of gular fold; circles indicate presence of collar and a star within a circle indicate the presence of both a gular fold and a collar.

Patterns and processes of adaptation in lacertid lizards to environments in southern Africa

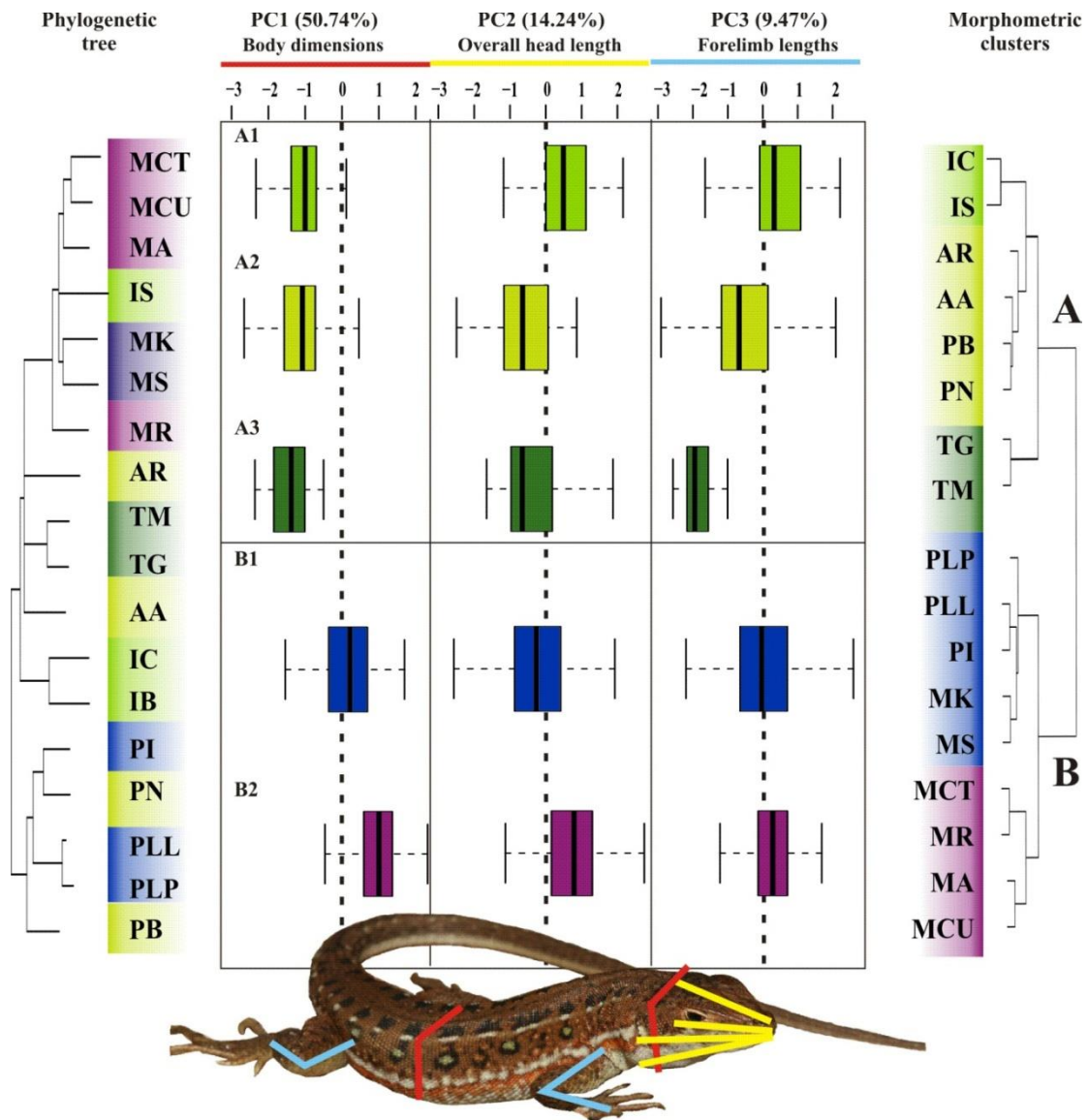


Figure 1.4: Boxplots of the first three principal component axes (centre) for each morphological group (A, B) retrieved by hierarchical clustering (shown right; well supported nodes are indicated by closed circles at the nodes). Positive values of the PC axes indicate larger body dimensions, whilst negative values indicate smaller body dimensions. Morphological groupings are shaded as follows: A1 = bright green, A2 = lime green, A3 = green, B1 = blue, B2 = purple. The phylogenetic tree (left) is colour coded by species according to their morphological group membership. Morphological measurements are shown on lizard schematic (*P. lineocellata*), and line colours correspond to sets of original variables that loaded onto each PC (PC1 = yellow, PC2 = light blue). Percentage of variation contributed to each PC axis is given. Key to the species abbreviations: AA = *Australolacerta australis*, AR = *A. rupicola*, IB = *Ichnotropis bivittata*, IC = *I. capensis*, IS = *I. squamulosa*, MA = *Meroles anchietae*, MCT = *M. ctenodactylus*, MCU = *M. cuneirostris*, MK = *M. knoxii*, MS = *M. suborbitalis*, PB = *Pedioplanis burchelli*, PI = *P. inornata*, PLL = *P. lineocellata lineocellata*, PLP = *P. l. pulchella*, PN = *P. namaquensis*, TG = *Tropidosaura gularis*, TMM = *T. montana montana*, TMR = *T. m. rangeri*.

Multivariate analyses (principal components analysis and analysis of variance) indicate that the two morphological clusters differ significantly in terms of body/head slenderness (PC1: $F = 430.19$, $P < 0.001$, 50.74% of the variation; Table 1.1), with species inhabiting cluttered habitats being slender and more elongate compared to those in more open habitats (Fig. 1.4). The two main morphological clusters did not differ significantly for the second principal component (PC2: $F = 2.60$, $P = 0.11$, 14.24% of the variation) that loaded positively with most head measurements, particularly lengths (Table 1.1). An exception is that dune-dwelling species (cluster B2) have significantly longer heads compared to clusters B1 ($F = 98.86$, $P < 0.0001$) and A2 ($F = 24.73$, $P < 0.0001$) (Table 1.2). The two clusters differed significantly for PC3 ($F = 15.77$, $P < 0.001$; 9.47% of the variation), however this may be due to the relatively shorter forelimbs of *Tropidosaura* (cluster A3).

Table 1.2: Analysis of variance (ANOVA) results for morphological clusters (A1, A2, A3, B1 and B2; as in Fig. 1.4). Significant differences ($P \leq 0.05$) are indicated in bold font.

PC1*				PC2				PC3						
	Df	F-value	P-value		Df	F-value	P-value		Df	F-value	P-value			
A1	A2	1	1.54	0.22	A1	A2	1	56.04	<0.001	A1	A2	1	34.28	<0.001
	A3	1	6.64	0.01		A3	1	17.71	<0.001		A3	1	108.44	<0.001
	B1	1	147.48	<0.001		B1	1	36.36	<0.001		B1	1	7.09	0.01
	B2	1	409.44	<0.001		B2	1	2.78	0.10		B2	1	2.41	0.12
A2	A3	1	1.30	0.26	A2	A3	1	0.49	0.48	A2	A3	1	24.28	<0.001
	B1	1	181.98	<0.001		B1	1	7.79	0.01		B1	1	17.15	<0.001
	B2	1	384.49	<0.001		B2	1	97.15	<0.001		B2	1	42.71	<0.001
A3	B1	1	77.89	<0.001	A3	B1	1	0.54	0.46	A3	B1	1	50.98	<0.001
	B2	1	197.33	<0.001		B2	1	24.73	<0.001		B2	1	156.77	<0.001
B1	B2	1	100.31	<0.001	B1	B2	1	98.86	<0.001	B1	B2	1	4.07	<0.001

* PC = principal component, Df = degrees of freedom, P-value = significance value.

Discussion

Whilst morphological characters are traditionally used to define species, descriptions that incorporate multidisciplinary approaches, including morphological, genetic, behavioural and ecological aspects, are typically better informed (e.g. Leaché *et al.*, 2009). The data show that among the external linear morphological measurements upon which taxonomic classifications for Eremiadini are partly based (Arnold, 1989), some are the result of convergence due to habitat structure and not shared ancestry. In the case of the southern African lacertid lizards, convergent evolution of morphological characters has led to genetically distant, but partially sympatric (*Ichnotropis* spp.) and parapatric (*Australolacerta* spp.) species being considered as sister taxa. Such examples of misclassification due to phenotypic similarities between species are increasingly familiar, suggesting that morphological adaptation in response to similar environments is pervasive, rather than exceptional. Even what might appear to be obvious cases of shared evolutionary history, based on morphology, have been revealed to be more closely genetically related to morphologically disparate species (e.g. geckos of the genera *Pachydactylus/Elasmodactylus*, Bauer & Lamb, 2005; chameleons of the genera *Archaius/Rieppeleon*, Townsend *et al.*, 2011).

Convergence in phenotype can be the result of random evolutionary change (Losos, 2011), however the observed morphological convergence in the southern African lacertids suggests adaptation to particular environments (as in other lizards; Revell *et al.*, 2007; Mahler *et al.*, 2013). The high genetic divergence between morphologically and ecologically similar species suggests that vegetation density (*i.e.* habitat clutter) is a major driving force in the evolution of phenotypic diversity in these lizards, irrespective of ancestry.

Within the lacertid lizards, the phylogenetic position of species inhabiting particular environments (*i.e.* xeric or mesic environments) was investigated previously (Mayer & Pavlicev, 2007), and a unique monophyletic trend from mesic to xeric species within the Lacertidae could not be demonstrated, despite previous morphological phylogenies that showed this trend (Arnold, 1989, 2004). With the comparison of the molecular tree to the broad environmental categories, it was suggested that there are multiple origins of xeric-adapted species within Eremiadini. However, in this study it was shown that the morphology of a lizard is likely to be driven by its microhabitat, with less association to broad scale biome features. In fact, for many reptiles, geographic proximity influences phylogenetic position (e.g. Tolley *et al.*, 2004b; Leaché *et al.*, 2009) making it unsurprising that a link exists between broad scale environmental classifications and phylogenetic position. For example, within *Meroles*, *M. anchietae* and *M. cuneirostris* are in the same clade, have a similar body plan and both inhabit a xeric environment. However, the lack of phylogenetic independence means that similarities due to a common ancestor which inhabited the xeric region prior to diversification cannot be ruled out. Conversely, *M. reticulatus* is not within the same clade as *M. anchietae* and *M. cuneirostris*, but the bauplans of all three species

are similar suggesting a separate origin of this morphology due to similarity in microhabitat (open habitat) within the xeric macrohabitat.

Whilst the morphological clusters were significantly different with respect to overall body slenderness (PC1) and linked to habitat openness, the lack of a significant difference for PC2 (Table 1.2) indicates that head shape is driven by factors other than habitat structure such as substrate usage, diet or sexual selection (e.g. Measey *et al.*, 2009; Herrel *et al.*, 2011b). Convergence in head shape within the dune-dwelling species (cluster B2) may be as a result of their preference to sand-dive or to utilize burrows, both of which are behavioural adaptations for predator avoidance and thermoregulation (Bauwens *et al.*, 1995; Arnold, 1995). The *Ichnotropis* (cluster A1) head dimensions are not significantly different from the dunes cluster (cluster B2) ($F = 2.78$, $P = 0.10$), and this could be due to a propensity for digging burrows for shelter and reproduction (Branch, 1998), thereby evolving the same relative head morphology (Branch, 1998). Another possibility is that *Ichnotropis* may have a similar diet to the sand-dwelling species, which may be driving the similarity in head shape (Branch, 1998). Convergence in body shapes have been found between genetically disparate saxicolous species, and thus substrate usage, and not habitat openness, may be driving head shape in lacertid lizards.

In terms of limb lengths, the two morphological clusters were significantly different (PC2), in particular because of the short limbs in *Tropidosaura*. The shorter forelimbs in conjunction with their slender bodies may allow *Tropidosaura* to optimize manoeuvring performance while negotiating cluttered vegetation (e.g. Herrel *et al.*, 2002; Bauwens *et al.*, 1995), whereas the long limbs of the *Ichnotropis* spp. (cluster A1) and those inhabiting more open habitats (clusters B1 and B2) should increase sprint performance (e.g. Bauwens *et al.*, 1995; Bonine & Garland, 1999; Melville & Swain, 2000; Vanhooydonck *et al.*, 2001a; 2002). Relative forelimb and hindlimb dimensions, however, need to be investigated in conjunction with substrate type and structure, as opposed to habitat structure, in order to better understand the evolution of limb dimensions in Eremiadini.

Although sub-sets of taxa from *Meroles* and *Ichnotropis* were investigated as part of higher level lacertid phylogenies, the placement of *I. squamulosa* within *Meroles* was not identified previously due to the inclusion of only a single *Ichnotropis* (*I. squamulosa*) and various *Meroles* (*M. knoxii*, *M. suborbitalis* or *M. ctenodactylus*) in those analyses (Harris *et al.*, 1998a; Kapli *et al.*, 2011; Mayer & Pavlicev, 2007). Despite their placement in the phylogeny, *I. capensis* and *I. squamulosa* do not differ significantly morphologically, and cluster together when body dimensions, head measurements and limbs measurements are investigated. Both of these species possess more slender bodies relative to *Meroles*. In addition, they share characters not possessed by *Meroles* (rough scales and the absence of a nuchal collar). Because these two species have partially sympatric distributions, their overlapping niche might explain the observed morphological similarities. For example, limb dimensions could reflect adaptation

to substrate type, while head shape similarities could reflect adaptation to similar diets. Although neither have a nuchal collar, this is also absent in other *Meroles* (i.e. *M. anchietae*), as well as other lacertids (e.g. *Tropidosaura*). Thus, the presence/absence of the collar is unlikely to be a synapomorphy (Fig. 1.3). Similarly, the presence of a gular fold (similar to a nuchal collar, but does not extend all the way around the head) does not appear to be a character that can be used to indicate shared ancestry in southern African lacertids (Fig. 1.3). The other characteristic feature that has linked these species in the past is the presence of rough (strongly keeled) scales. However, this is also not a synapomorphy as other lizards and even lacertids (e.g. *Tropidosaura*) are known to have rough scales suggesting shared scale micro-ornamentation is not an indication of a shared ancestry in lacertid lizards but rather related to microhabitat use (Arnold, 2002b).

There are several interesting implications of the placement of *I. squamulosa* within *Meroles*, rather than *Ichnotropis*. Sympatry often leads to competition for resources particularly between closely related species. *Ichnotropis squamulosa* is sympatric with *I. capensis* in the northern regions of its distribution, but is allopatric with all *Meroles*. Whilst *Meroles* are primarily sand-dwellers, *Ichnotropis* are classified as terrestrial (Arnold, 1994), with a propensity for sandy habitats in mesic and arid savannah (Branch, 1998). The reproductive cycles of *I. squamulosa* and *I. capensis* are not discordant (Jacobsen, 1987; Branch, 1998; Goldberg, 2008), which is thought to prevent interspecific competition (Jacobsen, 1987; Goldberg, 2008). Both species are considered to be annual breeders, although the breeding times are staggered (Goldberg, 2008), and life-spans are unusually short for lacertid lizards. *Ichnotropis squamulosa* lives approximately eight to nine months, mating in late summer and hatchlings appear in spring (Branch, 1998; Goldberg, 2008). *Ichnotropis capensis* may live only marginally longer (13-14 months), mating in spring with hatchlings appearing in late summer (Broadley, 1967; Branch, 1998; Goldberg, 2008). It has been suggested that this staggered reproductive pattern arose to prevent interspecific competition between closely related species (Broadley, 1979). However, because these species are not closely related, this shared life-history trait cannot be associated with a reduction of competition between sister taxa, but rather suggests an independent evolution of a similar but temporally disjunct reproductive strategy. The reasons for this are not clear, particularly because *I. squamulosa* still exhibits the same reproductive strategy in regions where the two species are not sympatric (e.g. in Upington, South Africa; Goldberg, 2008) suggesting that the staggered reproduction of the two species is not driven by interspecific competition.

This study shows that convergence in morphology has led to a generic level misclassification of two lacertid species, previously described on external morphological characters alone. As a result, *Ichnotropis squamulosa* has been moved to *Meroles* and *Australolacerta rupicola* has been placed into a new, monotypic genus *Vhembelacerta*, causing *Australolacerta* to be a monotypic genus, consisting of *Australolacerta australis* (Edwards *et al.*, 2013; Appendix B). Recent work on relatively understudied

lacertid taxa, such as African species (Greenbaum *et al.*, 2011; Barata *et al.*, 2012) and Middle-Eastern species (Kapli *et al.*, 2012), have shown a greater diversity within Lacertidae, and further taxonomic revisions within Lacertidae are expected with more extensive sampling and use of molecular phylogenetic analyses to elucidate the relationships between the species.

Morphological adaptation to a particular microhabitat may confer a greater fitness to individuals through their performance (for a review see Irschick *et al.*, 2008). The results of this study show that habitat openness determines the morphological shape of southern African lacertid species and it is expected that these differences in morphology will, in turn, be associated to performance differences between the species. Those species adapted to open dunes may be better sprinters than those inhabiting cluttered rocky environments, whilst the rock-dwellers may be better climbers than sand dwellers. A closer investigation into associations between body and limb shape and performance in southern African lizards is needed to understand the functional implications of the morphological shape differences in southern African lacertid lizards.

Conclusions

The high genetic divergence between morphologically and ecologically similar species suggests that vegetation density is a major driving force in the evolution of phenotypic diversity in these lizards, irrespective of ancestry. Whilst convergence in phenotype in various taxa can be the result of random evolutionary change (Losos, 2011), the observed morphological convergence in the southern African lacertids suggests adaptation to particular environments. Although the link between morphological adaptation to a particular habitat and performance conferring a greater fitness to individuals has been documented previously (Irschick *et al.*, 2008), an investigation into how substrate characteristics affect performance in southern African lacertid lizards would greatly enhance the understanding of the mechanisms driving the morphological convergence between highly genetic divergent species.

Environment, namely how cluttered the microhabitat is, influences the shape of the morphology of southern African lacertids. However, the environment does not act alone on the selection of traits, but is in concert with sexual selective pressures, diet and other behavioural considerations. In the next chapter the effect of diet on the morphology of the *Nucras* lizards, and the influence on their performance, is investigated.

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Nucras holubi, Rooipoort Nature Reserve, South Africa.
Photo by: G. John Measey

Chapter 2

**DIETARY PREFERENCE DETERMINES CRANIAL SHAPE WITHIN THE
SANDVELD LIZARDS *NUCRAS***

DIETARY SPECIALIZATION DETERMINES CRANIAL SHAPE WITHIN THE SANDVELD LIZARDS *NUCRAS**

Introduction

Adaptations to particular habitats in lizards can be physiological, morphological or behavioural, and are often driven by a multitude of factors, such as habitat structure (*e.g.* Vitt, 1981; Vitt *et al.*, 1997; Revell *et al.*, 2007; Goodman & Isaac, 2008; Goodman, 2009; Measey *et al.*, 2009; Edwards *et al.*, 2012), prey composition (Herrel *et al.*, 2008) and seasonality (Huey *et al.*, 1977) amongst others. Variation in morphology may be driven by a number of factors, such as sexual selection (Braña, 1996), competition (Langkilde, 2009), foraging method (Huey & Pianka, 1981; Huey *et al.*, 1984; Verwaijen & Van Damme, 2007*a,b*, 2008; McBrayer & Wylie, 2009) and prey availability (Herrel *et al.*, 2001*a*; Verwaijen *et al.*, 2002). The dietary composition, particularly the type of prey taken, may influence the head morphology of lizards (*e.g.* Herrel *et al.*, 2001*a*; Verwaijen *et al.*, 2002). Lizard species that consume harder prey have been shown to have relatively wider, more robust heads (*e.g.* in lacertid lizards: Herrel *et al.*, 2001*a*), which are thought to allow more space for jaw adductor muscles (Herrel *et al.*, 1999*a*) or a more vertical orientation of the jaw adductors (Herrel *et al.*, 1998). Selective pressures on the functional aspects of the organism, *i.e.* organismal performance, may lead to the evolution of particular phenotypes, which may lead to greater fitness (Arnold, 1983). Functionally, a greater bite force may be advantageous for lizards in that they may be able to feed on harder and larger prey (Herrel *et al.*, 1999*a*). Relatively larger and more robust crania have been linked to greater bite forces in lizards (as in *Anolis*: Herrel *et al.*, 2007; and *Podarcis*: Herrel *et al.*, 2001*a*; Huyghe *et al.*, 2009), however in some lacertid lizards cranial morphology has been shown to linked with environment, but not with performance (Kaliontzopoulou *et al.*, 2012*b*). Other aspects of the crania, such as snout lengths, have been linked to the capture of evasive prey items: for example, in anoles, longer jaws are thought to facilitate easier capture of flying insects (Herrel *et al.*, 2007, 2011*a*). Other functional aspects of lizards, such as the sprint speed and endurance, have been linked to the capture of evasive prey (Vanhooydonck *et al.*, 2007).

Whilst the feeding on hard and/or evasive prey has been linked to head shape and functional aspects of head and limb morphology in lizards (Vanhooydonck *et al.*, 2007; Measey *et al.*, 2011), the relationship between dietary niche breadth (range of prey taken) and morphology has not been explicitly investigated. If a lizard species is specialized (low niche breadth value) to feed on a particular type of prey (*e.g.* hard or evasive prey), it will have particular phenotypic and behavioural traits that facilitate

* Edwards S, Tolley KA, Vanhooydonck B, Measey GJ, Herrel A. 2013. Is dietary niche breadth is linked to morphology and performance in Sandveld lizards *Nucras* (Sauria: Lacertidae)? *Biological Journal of the Linnean Society*. 110(3): 674-688

the capture of that prey. On the other hand, if the species is a generalist, feeding on a large range of prey items, its morphology would be versatile, to process a large range of prey types (e.g. hard or soft and/or evasive or sedentary prey). Investigations of the relationship between body size and niche breadth in lizards have been undertaken (Costa *et al.*, 2008), where a negative relationship was found between body size and niche breadth in 159 lizard species. This was contrary to positive body size-niche breadth relationships in birds (Brändle *et al.*, 2001; 2002b), butterflies and moths (Wasserman & Mitter, 1978; Brändle *et al.*, 2002a) and herbivorous insects (Novotny & Basset, 1999), but the negative relationship in lizards was attributed to the overall frequency distribution of body sizes in lizards. Little information, however, is available on the link between dietary niche breadth and morphology in lizards, and the associated variation in performance.

The southern African lacertid genus *Nucras* (Eremiadini, Lacertidae) was used to investigate the link between dietary niche breadth and morphology, as the species of this genus differ in dietary niche breadth (Van Der Meer *et al.*, 2010). *Nucras* are predominantly insectivorous, supplementing their diet with spiders, scorpions and centipedes, and each species preys upon arthropods of varying degrees of hardness and evasiveness (Branch, 1998; Spawls *et al.*, 2006; Van Der Meer *et al.*, 2010). All *Nucras* are described as active foragers (Branch, 1998), and thus morphological differences between species are likely not driven by foraging methods, but by other factors (such as diet). There are ten described species from East and southern Africa (Branch, 1998), however dietary data for only five species are available to date (Van Der Meer *et al.*, 2010).

In this study we hypothesized that cranial shape in lizards of the genus *Nucras* is related to dietary niche breadth, and that functional capacities are linked to dietary composition. Although all *Nucras* are described as active foragers (as opposed to sit-and-wait foragers), the type of prey that they are able to prey upon may be determined by their morphology. We predicted that species specializing on hard prey items would have more robust crania and higher bite forces, and that those species feeding on evasive prey would have longer limbs and better sprinting capacities. We constructed a phylogeny for the genus, using both mitochondrial and nuclear markers, to determine the evolutionary history of the genus and to investigate potential phylogenetic effects driving morphological similarity between species. We used linear morphometric techniques to identify morphologically similar groups of species. Using the five species for which dietary data are available, we first investigated the relationships between cranial morphology (using geometric morphometric techniques), dietary niche breadth, prey characteristics, and bite force. We then investigated the relationship between limb lengths and sprinting capacity, and the proportion of evasive prey taken.

Materials and Methods

DNA extraction and sequencing

For the phylogenetic comparative methods, we estimated the phylogeny of *Nucras* using 48 individuals from eight of the 10 described species (*N. scalaris* and *N. caesicaudata* were not included due to lack of samples; Table A2). Thirty individuals were collected in the field and tissue was stored in 95-100% ethanol. The dataset was supplemented with sequences from six individuals available on GenBank/EMBL. Individuals from seven related genera within the Eremiadini (*Australolacerta*, *Heliobolus*, *Ichnotropis*, *Latastia*, *Meroles*, *Philocortus*, and *Pseuderemias*) obtained from GenBank were used as outgroup taxa (Mayer & Pavlicev, 2007; Kapli *et al.*, 2011). For all newly sequenced individuals, genomic DNA was isolated from tail or liver tissue according to a standard salt-extraction protocol (Bruford *et al.*, 1992). Standard PCR procedures were utilized to sequence two mitochondrial (16S and ND4) and two nuclear (RAG1 and KIAA-2018) gene regions and are described in Chapter 1 using the same primers pairs as in Chapter 1 (Table A1). Sequences were aligned using ClustalOmega v.1.1.0 (Sievers *et al.*, 2011) and checked in BioEdit Sequence Alignment Editor v. 7.0.5.2 (Hall, 1999). A 168 base pair portion of the 16S marker that could not be unambiguously aligned was excluded from the analyses. Details of the samples and EMBL accession numbers are provided in the appendix (Table A2).

Phylogenetic tree estimations

A partition homogeneity test (Farris *et al.*, 1994, 1995) was implemented in PAUP* v4.0b10 (Swofford, 2002), and no conflict was found between markers within each genome, nor between genomes. Sequence divergences were determined by estimating the uncorrected p-distances between and within species using the program MEGA v.4 (Tamura *et al.*, 2007).

Phylogenetic trees were constructed from the combined total evidence dataset from all four markers. Bayesian inference (BI) was performed with uniform priors for all parameters (MrBayes v.3.1.0; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The third codon position of the ND4 gene was found to be saturated (Dambe v.5.2.65; Xia *et al.*, 2003), so it was partitioned separately from the other two codon positions of the ND4 gene (ND4a: the first and second codon positions, and ND4b: the third codon position). The remaining markers were partitioned separately resulting in 5 partitions in total. Evolutionary models best fitting the individual marker datasets were chosen (jModeltest v.2.1; Posada, 2008) and model priors were set accordingly (16S: GTR+G, ND4a & b: GTR+I+G, RAG1: HKY+G, KIAA-2018: HKY+G). Two parallel runs for 20×10^6 generations each were run for the MCMC, with trees sampled every 1000 generations. The number of generations to discard as burn-in (1×10^6 generations) was determined by examining the number of generations 1) at which the standard deviation of split frequencies stabilized (at less than 0.001), 2) at which the log-likelihood tree scores

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reached stationarity, and 3) the effective sample sizes (ESS) of all parameters were ≥ 400 (Tracer v.1.5; Rambaut & Drummond, 2007). A 50% majority rule tree was constructed with the burn-in excluded using the ‘sumt’ command in MrBayes, and nodes with ≥ 0.95 posterior probability were considered supported. A partitioned maximum likelihood (ML) analysis was also run (RAxML v.7.2.7 via the Cipres Portal; Stamatakis, 2006; Stamatakis *et al.*, 2008) using the same partitions as the Bayesian analysis, a GTR+I+G model of evolution, and automatic halting of bootstrapping (Stamatakis, 2006; Stamatakis *et al.*, 2008).

Linear morphometric analyses

For the linear morphometric analyses, 187 individuals of nine *Nucras* species from across the species localities were measured using digital callipers (~20 per species, *N. scalaris* was not included due to lack of specimens; Table A4). As in Chapter 1, individual sex was noted, but the data were not separated by sex, as variation between sexes has been shown often be smaller than between lacertid species. Measurements taken on the body and limbs were: body length from snout-tip to anal opening (SVL), femur length (FM), tibia length (TB), humerus length (HM), and radius length (RD). Head measurements taken were: Head length (HL), head width at the widest part of the temporal region (HW), head height of the posterior part of the cranium (HH), and lower jaw length (LJL) (see Fig. 1.1). Unless otherwise specified, all analyses were performed in the program R Studio v.0.97.248 (R Core Team, 2012; R Studio, 2012). To eliminate the effect of size in the traditional morphometric analyses, \log_{10} -transformed head and limb measurements were regressed onto the geometric means of the particular set of measurements using a linear model (package: ‘stats’, functions: ‘resid’ and ‘lm’; R Studio, 2012). The absolute values and the size-corrected residuals for each morphometric character were used in further analyses. In order to identify whether the morphology of the lizards was linked to their genetic relationships, hierarchical clustering of the means of the size-corrected residuals for each species (package: ‘stats’, function: ‘mean’; R Studio, 2012) was performed to identify the morphological clusters and support for the nodes was obtained using 1000 bootstrap replicates (package: ‘pvclust’, function: ‘pvclust’, method.hclust: ‘complete’, method.dist: ‘euclidean’, nboot: 1000; R Studio, 2012). If the morphological clusters do not correspond to genetic clusters then differences in morphology may be driven by environmental factors such as diet or substrate and not solely by phylogenetic relationships, and further investigations into these factors would be warranted.

Dietary analyses

Five species (*N. holubi*, *N. intertexta*, *N. lalandii*, *N. ornata*, and *N. tessellata*; hereafter referred to as the ‘dietary species’) were used to investigate the relationship between diet and head shape, as dietary information on these species was available (Table 2.1; adapted from Van Der Meer *et al.*, 2010). These species can be considered as characteristic for major patterns in the genus because they are distributed

across the southern African landscape (Branch, 1998), are representatives from each major genetic clade within the genus (see Results for phylogenetic analysis), as well as representatives of each major morphometric cluster (see Results for hierarchical cluster analysis). The percentage volume in the diet for each arthropod order was used in the analyses (adapted from Table 4 in Van Der Meer *et al.*, 2010). In the dietary analyses, sexes were combined as there were no significant differences in the percentage volume of the different prey eaten by the two sexes (Van Der Meer *et al.*, 2010). Whilst the diet of both sexually mature and sexually immature individuals were examined in the analyses by Van Der Meer *et al.* (2010), mean prey volume was significantly correlated with SVL for *N. intertexta* and *N. ornata*, but not for *N. holubi*, *N. lalandii* and *N. tessellata* (Van Der Meer *et al.*, 2010), indicating that perhaps ontogenetic effects are at play in terms of the percentage volume of prey consumed by each age class in *N. intertexta* and *N. ornata*. Because the differences in prey volume, number or type between age-classes were not explicitly examined by Van Der Meer *et al.* (2010), we cannot exclude ontogenetic effects on prey consumption.

Dietary niche breadth values (hereafter referred to as the niche breadth) for each species was estimated using the inverse of Simpson's diversity index (Simpson, 1949):

$$B = 1 / \sum_{i=1}^n p_i^2$$

where B = the niche breadth value, i = resource category, n = total number of categories, and p = proportion of resource category i . These niche breadth values, ranging from one to n , indicate whether the species preys upon a large range of arthropod orders (high value, close to n) or specializes on a limited range of arthropod orders (low value, close to one). Each arthropod order was categorized as either hard or soft, sedentary or evasive through the use of a force meter to measure hardness and tests of evasiveness from previously published studies (Herrel *et al.*, 1996, 1999a, b, 2001a, 2006; Andrews & Bertram, 1997; Verwaijen *et al.*, 2002; Aguirre *et al.*, 2003; Vanhooydonck *et al.*, 2007) and percentage volume of two prey categories were calculated for each studied species of *Nucras* (Table 2.1).

Geometric morphometric analyses

Geometric morphometric analyses of the crania were performed to investigate the cranial shape of the five species used in the dietary analyses (14-22 individuals per species, totalling 100 individuals; Table A4). The heads were photographed using digital cameras (Fuji Finepix S2000HD, resolution 10.0 MP and Canon 50D, resolution 10.0 MP and macro lens F18/100). The dorsal and lateral profiles were used as head width, head height and snout length have been shown to be important in species feeding on hard and/or evasive prey; dimensions that would not have been apparent from other views of the crania (such as the ventral view). Homologous landmarks on the dorsal and lateral views of the crania were chosen

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to appropriately describe the shape of half of the cranium, and landmarks on the cheek region were included and digitized (tpsUtil v.1.26; Rohlf, 2004; tpsDig2 v.2.05; Rohlf, 2005; Fig. 2.1).

Table 2.1: Percentage volume of prey consumed per species used in the dietary analyses (adapted from Van Der Meer *et al.*, 2010), as well as prey hardness and evasiveness categories (Vanhooydonck *et al.*, 2007), and niche breadth values (estimated in this study) for each species. Percentage volume of hard and evasive prey consumed per species used in the dietary analyses and performance values (maximal bite force and sprint speeds) for each species. Values for the performance means are given as the mean values \pm the standard deviation. No available data for the performance capacities is indicated by ‘ND’.

Categories	Prey hardness	Prey evasiveness	N. holubi	N. intertexta	N. lalandii	N. ornata	N. tessellata
Prey Categories							
Araneae	soft	sedentary	1.90	6.70	1.40	15.30	1.30
Blattaria	soft	evasive	2.50	13.00	0.00	0.50	0.00
Chilopoda	soft	evasive	3.50	10.10	1.30	17.60	0.30
Coleoptera	hard	evasive	15.60	11.50	18.20	1.10	16.40
Diplopoda	soft	sedentary	0.00	0.00	0.50	0.20	0.00
Diptera	soft	evasive	3.30	0.90	0.00	2.70	0.00
Hemiptera	hard	evasive	0.80	4.40	0.00	0.40	0.60
Hymenoptera (ants)	hard	sedentary	0.20	0.00	0.00	0.30	53.00
Hymenoptera (other)	hard	evasive	1.00	0.50	0.80	0.00	0.00
Isoptera	soft	sedentary	39.30	7.60	3.30	8.50	11.30
Lepidoptera	soft	evasive	2.50	11.70	0.00	1.30	1.60
Mantodea	soft	sedentary	1.30	0.30	0.00	0.00	0.00
Neuroptera	soft	evasive	0.00	0.00	0.00	0.00	1.80
Orthoptera	hard	evasive	24.60	15.40	63.30	49.90	13.70
Scorpiones	soft	evasive	1.60	2.70	10.50	1.40	0.00
Solifugae	hard	sedentary	1.20	4.90	0.00	0.00	0.00
Insect eggs	soft	sedentary	0.00	0.00	1.40	0.00	0.00
Niche Breadth			4.10	10.75	2.24	3.21	2.94
Proportions							
Hard prey percentage			0.44	0.41	0.82	0.52	0.84
Evasive prey percentage			0.56	0.78	0.93	0.76	0.34
Performance (absolute)							
Maximum bite forces (N)			8.20 \pm 2.01	24.23 \pm 7.19	ND	ND	13.85 \pm 4.93
Maximum sprint speeds (m/s)			2.88 \pm 0.43	3.03 \pm 0.61	4.17	ND	2.39 \pm 0.16
Performance (relative)							
Residual bite force			0.25	0.21	ND	ND	0.02
Residual sprint speeds			0.03	-0.003	0.30	ND	-0.19

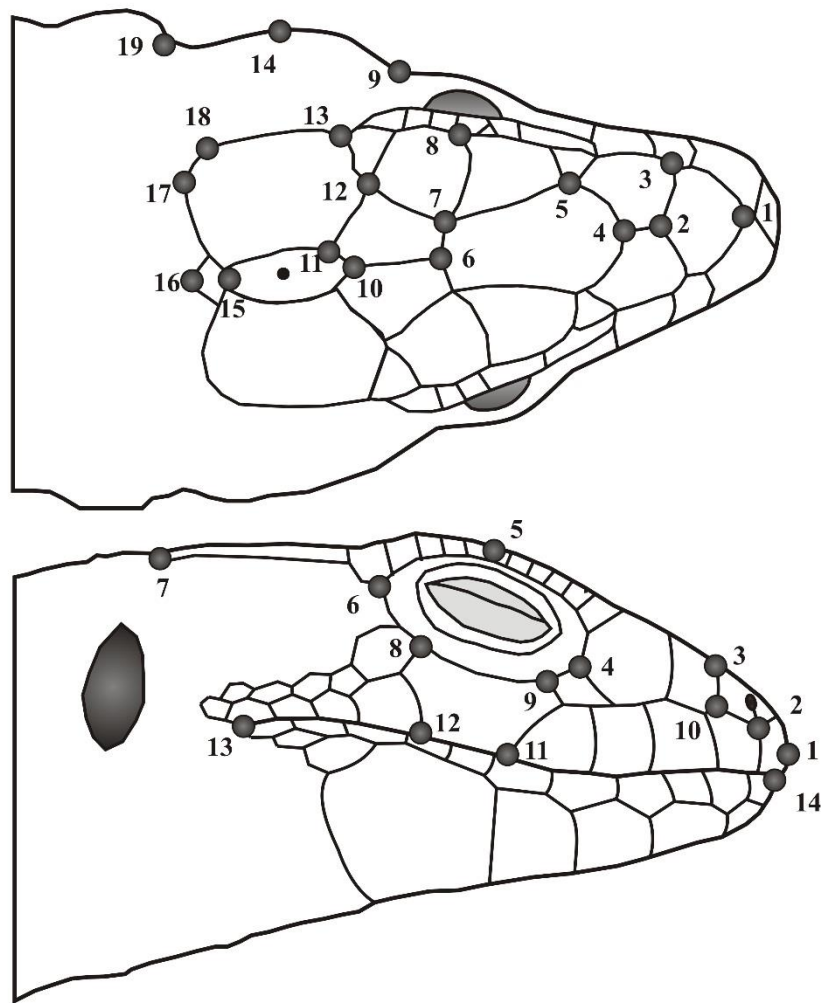


Figure 2.1: Diagram depicting the homologous landmarks that were digitized for the geometric morphometric analyses for the dorsal (top) and lateral (bottom) views of the *Nucras* crania.

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A Generalized Procrustes Analysis (GPA; Rohlf & Slice, 1990; Rohlf, 1999) was performed in which the sizes were standardized and the landmark configurations were translated and rotated. A relative warps analysis (similar to a principal components analysis) was performed on the residuals to identify which portions of the crania show the most variation between individuals and species (tpsRelw; Rohlf, 2003). Deformation grids (thin-plate splines) were used to visualize changes in cranial shape.

Performance analyses

The performance capacities of four *Nucras* species (*N. holubi*, *N. intertexta*, *N. lalandii* and *N. tessellata*; Table A5), caught and measured in the field, were used to identify the functional relationship between morphology and diet (sample sizes: *N. holubi* = 5, *N. intertexta* = 19, *N. lalandii* = 1 and *N. tessellata* = 2). The maximal bite force out of five trials was determined by having the lizard bite two metal plates connected to an isometric force transducer and a charge amplifier (see Herrel *et al.*, 1999a, 2001 for details on the experimental setup). For the bite force analyses, *N. lalandii* was not included due to the poor biting performance of the single individual obtained during field work. To eliminate the effect of size, the \log_{10} -transformed maximal bite force value of each individual were regressed onto the \log_{10} -transformed geometric means of the head measurements (*i.e.* the mean of the sum of HL, HW, HH and L JL) using a linear model (package: 'stats', functions: 'resid' and 'lm'; R Studio, 2012) and the mean residuals for each species were used in further analyses.

To determine the maximal sprint speed for each species, the lizards were allowed to rest in an incubator at 35°C for an hour before each trial to standardize body temperature. The temperature was chosen according to the preferred body temperatures for other lacertid lizards (Huey *et al.*, 1977; Bauwens *et al.*, 1995; Castilla *et al.*, 1999; Vanhooydonck *et al.*, 2001b), as optimal body temperature for performance trials have not been identified for all *Nucras* species (only *N. intertexta* and *N. tessellata*; Huey *et al.*, 1977). The sprint speeds were determined using a 2m long cork-covered racetrack with sensors placed at 25cm intervals along the track (see Vanhooydonck *et al.*, 2001a). Runs were repeated three times, and lizards were allowed to rest for at least one hour between each run, and the maximum of the sprint speeds for each individual were taken (measured in metres per second). The \log_{10} -transformed maximal sprint speed values were regressed onto the \log_{10} -transformed geometric means of the limb measurements to eliminate the effect of size (package: 'stats', function: 'resid' and 'lm'; R Studio, 2012) and the mean residuals for each species were used in further analyses.

Statistical analyses

Correlation analyses were performed between the mean morphometric variables for each species (both size-corrected linear morphometric residuals and geometric relative warp scores), dietary niche breadth

values, proportions of hard and evasive prey, and mean size-corrected performance residuals for each species (package: 'stats', functions: 'cor.test' and 'summary.lm'; R Studio, 2012).

Phylogenetic comparative analyses

A phylogenetic generalized least squares analysis (PGLS; Grafen, 1989; Hansen & Martins, 1996; Hansen, 1997; Martins & Hansen, 1997) was employed to identify the coevolution of morphological traits and dietary composition, and performance variables (package: 'nlme', function: 'gls', method: 'REML'; R Studio, 2012). The mean species values of the both absolute and relative \log_{10} -transformed morphometric and performance traits were used in the analyses. The PGLS method statistically accounts for the expected covariance of the measured variables between species resulting from phylogenetic relationship for regression-based or ANOVA analyses, whilst incorporating an explicit model of evolution. A significant result indicates that the relationship holds once phylogeny has been accounted for. The phylogenetic covariance matrix was estimated using the branch lengths from the phylogenetic tree and the expected pattern of phylogenetic covariance specified by the Brownian Motion (BM) model of evolution (package: 'ape', function: 'corBrownian'; R Studio, 2012). PGLS analyses were not performed for bite force values, as the low sample size (3 mean values) would give spurious results.

Results

Phylogenetic relationships and morphological clustering of all Nucras

Phylogenetic trees constructed using both methods (BI and ML) had the same topology with high support values for the clades recovered (Fig. 2.2 and 2.3A). All described species were recovered as monophyletic, with high sequence divergences (uncorrected p-distances) between them (16S: $5.80 \pm 2.47\%$, ND4: $13.31 \pm 1.12\%$, RAG1: $1.07 \pm 0.51\%$, KIAA: $0.58 \pm 0.29\%$). The separate clades are geographically proximate: The single sample of *N. boulengeri* (the only species from East Africa) is sister to the remaining *Nucras* species, which are themselves split into two well-supported main clades: Clade A (coastal and south-interior of southern Africa) and Clade B (savannah biome of southern Africa) (Figs. 2.2, 2.3A and 2.4). The sequence divergences between *N. boulengeri* and the other *Nucras* (16S: $5.98 \pm 1.44\%$, ND4: $16.95 \pm 1.03\%$, RAG1: $5.41 \pm 0.84\%$, KIAA: $1.25 \pm 0.41\%$) approximated the level of sequence divergence between other genera in this study (16S: $10.10 \pm 1.79\%$, ND4: $16.58 \pm 1.01\%$, RAG1: $5.59 \pm 0.80\%$, KIAA: $2.61 \pm 0.53\%$). Four morphological clusters were obtained using hierarchical clustering analyses (Fig. 2.3B), but with little support for the four clusters, whilst relationships between species within the clusters was highly supported. Morphological clusters did not correspond to genetic clades, indicating that morphology may not solely be driven by the shared ancestry, but by other factors, such as diet.

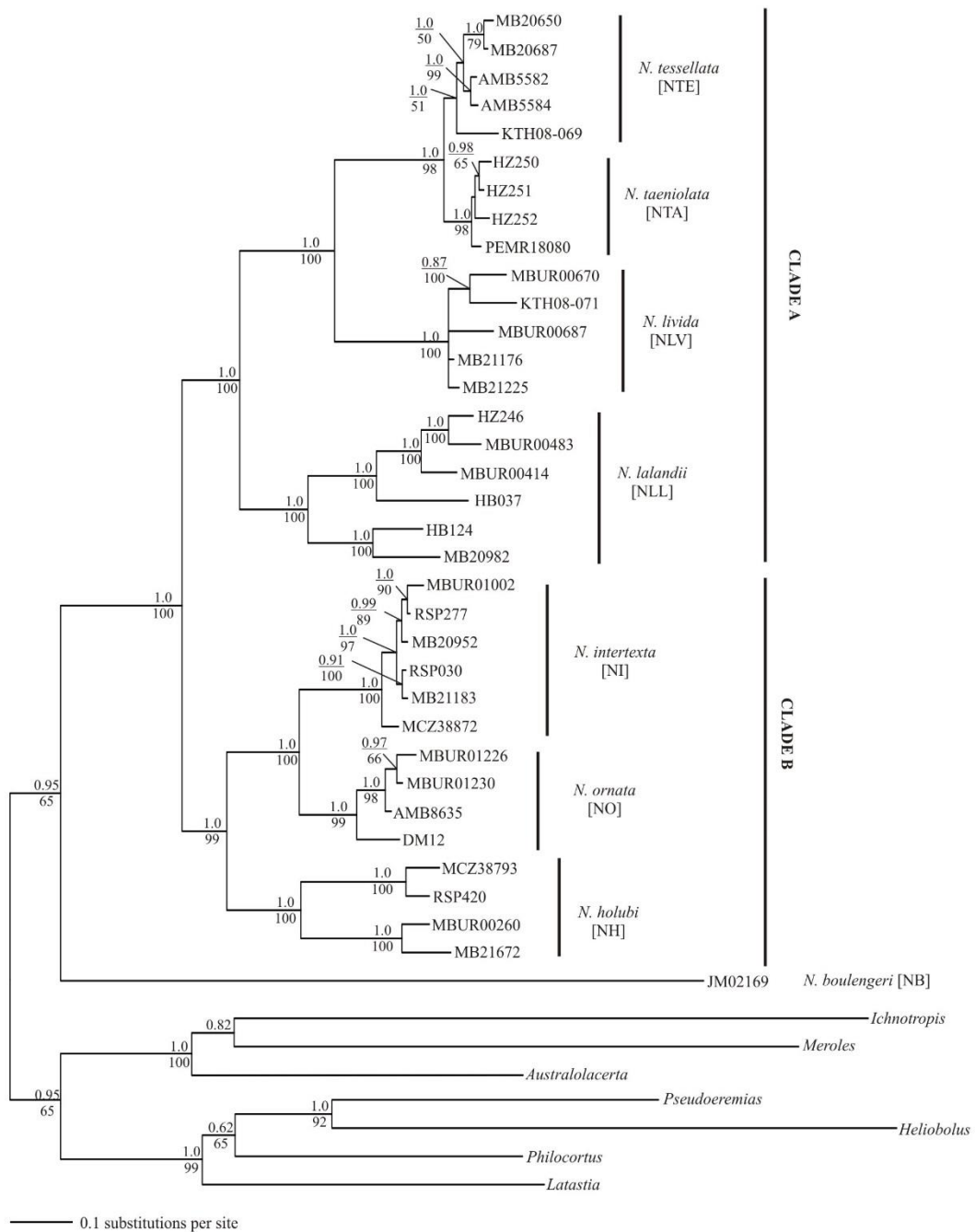


Figure 2.2: Phylogenetic tree of the genus *Nucras* based on the combined partial 16S, ND4, RAG1 and KIAA-2018 gene regions and inferred by BI and ML (Bayesian topology shown). Sample numbers are indicated at terminal tips, and species names are given. Nodes are considered supported if posterior probabilities > 0.95 (estimated using Bayesian inference) and/or bootstrap values > 75% (using maximum likelihood analyses).

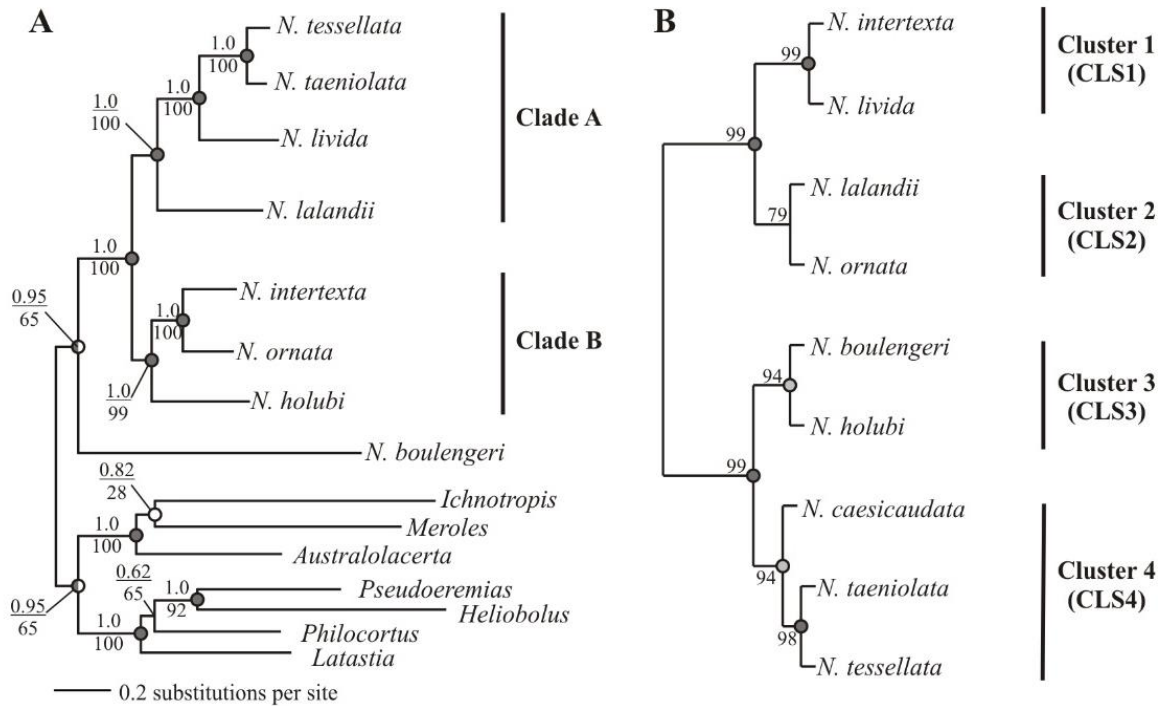


Figure 2.3: Phylogenetic tree shown (A) inferred from Bayesian analyses (BI) and likelihood methods (ML) using a combined dataset of mtDNA (16S, ND4) and nuclear DNA (RAG1, KIAA-2018) (topology from BI shown). Support values shown at the nodes and indicated by the circles at the nodes: Bayesian posterior probabilities >0.95 (above node; left fill of circle) and ML bootstrap values >75% (below node; right fill of circle). If node is supported using both algorithms, the circle at the node is filled completely. Hierarchical clustering dendrogram (B) of the morphometric measurements, showing the four morphological clusters (CLS1-4) obtained. Supported values (AU (approximately unbiased) *P*-values) shown at the nodes, and dark-grey filled circles indicate nodes with strong support (AU>95%), and light-grey filled circles indicate nodes with moderate support (95%>AU>90%).

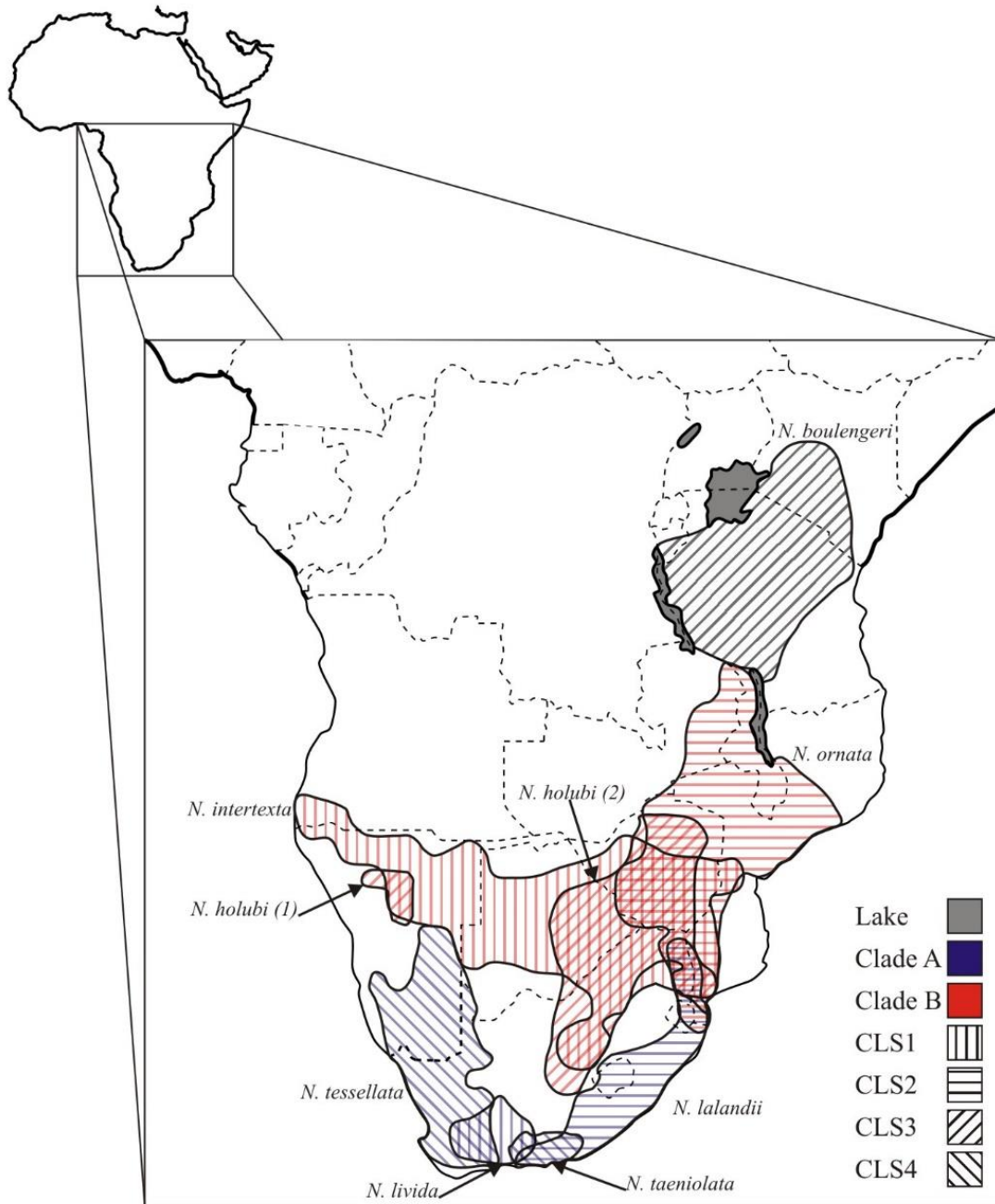


Figure 2.4: Map of the distributions of *Nucras* species used in the phylogenetic analyses. The key to the colouration (for genetic clades) and patterns (for morphological clusters) within each species distribution is shown. Countries are outlined and each species distribution is labelled. Distributions for the species were adapted from Branch (1998) and Spawls *et al.* (2006).

Dietary, morphological and performance analyses of five Nucras species

Two significant relationships were found between 1) niche breadth and the means of first dorsal cranial view relative warp scores (positive relationship; Table 2.2) and 2) between the proportion of hard prey eaten and absolute head width (positive relationship; Table 2.2 and Fig. 2.5). Bite force was significantly positively related to body size (SVL) and linear head measurements (HL, HW, HH and LJJ; Table 2.2). The proportion of evasive prey was not significantly related to either absolute or relative limb measurements, or sprint speeds (Table 2.3). Sprint speeds were positively related to absolute, but not relative, limb measurements, which was expected as larger individuals will have longer stride-lengths and therefore will be able to run faster than smaller individuals (Table 2.3).

The first three relative warps of the dorsal cranial view described the width and elongation of the cheek of the five *Nucras* species. The first dorsal view relative warp (DC-RW1) was positively related to niche breadth in the non-phylogenetic correlations (Table 2.2; Fig. 2.6), indicating that species that are more specialized, in this case specialist feeders on hard prey (*N. tessellata* and *N. lalandii*; Table 2.1), have cheek regions that are not as wide, and are more posteriorly elongated (landmarks 8, 9, 13, 14, 18 & 19; Fig. 2.5), compared to more generalist species (*N. intertexta*) (Fig. 2.5). The proportion of hard prey consumed was not related to any of the relative warps components, but it was significantly positively related to the absolute head width. There was no relationship between bite force and linear head measurements in the phylogenetic correlations, but this is likely due to the low sample size (3 data points = species means) used in the analyses. The lateral-view relative warp scores, describing the elongation of the snout (LC-RW1: landmarks 1-4, 10, 11, 14) and posterior cranial height (LC-RW2 and -RW3: landmarks 6-8, 11, 12) (Fig. 2.5), were not related to either niche breadth or proportion of hard prey taken, which was similar to results for absolute and relative linear measurements of head length and height (Table 2.2).

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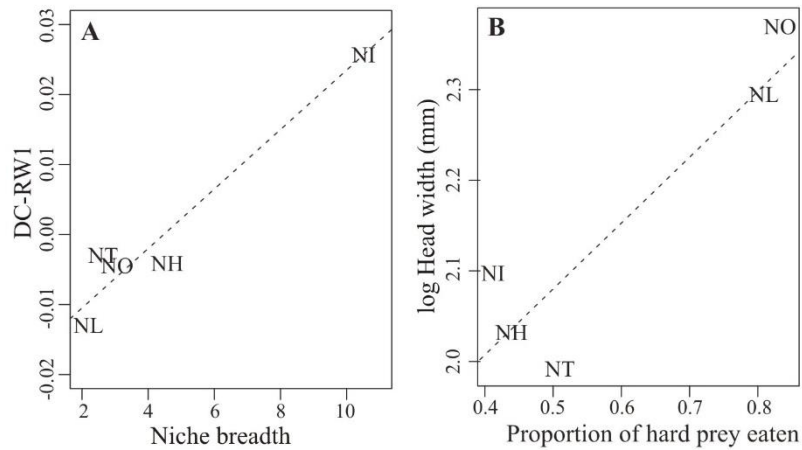


Figure 2.5: Scatterplots of the means of the significant correlations for the non-phylogenetic correlation analyses (see Tables 2.3 and 2.4), with the slope of a linear model regression shown with a dashed line within plots. Variables plotted are: (A) Niche breadth against the first dorsal relative warp component, (B) proportion of hard prey eaten against \log_{10} -transformed absolute head width. Key to species abbreviations in each plot: NH = *Nucras holubi*, NI = *N. intertexta*, NL = *N. lalandii*, NO = *N. ornata*, NT = *N. tessellata*.

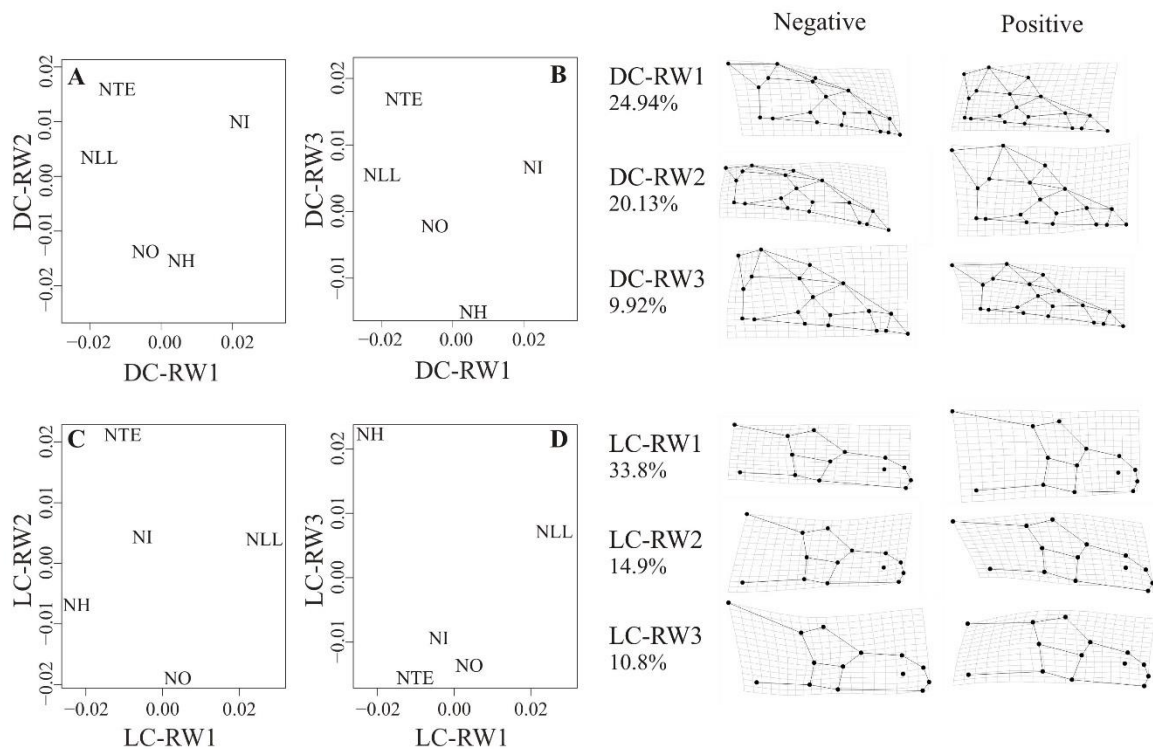


Figure 2.6: Scatterplots plotting the first three relative warps (RW) components for the dorsal (DC: A, B) and lateral (LC: C, D) views. Deformation grids indicate the cranial shape differences on either the negative or positive ends of the first three relative warp components for the dorsal and lateral views. Percentage of variation explained by each component shown. Key to the species abbreviations as in Fig. 2.4.

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Table 2.2: Non-phylogenetic and phylogenetic correlations between niche breadth, proportion of hard prey eaten, bite force capacity (absolute and relative) and cranial morphometrics (geometric morphometric scores, and relative and absolute linear morphometric measurements). Phylogeny was taken into account using the Brownian Motion (BM) model in a phylogenetic generalized least squares analysis (PGLS). Variances (R^2), slope of the correlation, and Pearson's correlation indices (r) shown of correlations between variables (without taking phylogeny into account). Significant correlations ($P \leq 0.05$) are indicated in bold font.

Independent	Dependent [#]	Non-phylogenetic				Phylogenetic			
		Variances (R^2)	Slope	Correlation (r)	P -value	Slope	Correlation (r)	P -value	
Niche breadth (prey-range)	Snout-vent length (SVL)	0.001	-0.002	-0.04	0.95	0.03	-0.69	0.33	
	LC-RW1	0.07	-0.002	-0.26	0.68	0.00	-0.69	0.09	
	LC-RW2	0.003	0.0003	0.06	0.93	0.00	-0.69	0.85	
	LC-RW3	0.03	-0.001	-0.18	0.77	0.00	-0.69	0.39	
	DC-RW1	0.84	0.004	0.91	0.03	0.004	-0.74	0.03	
	DC-RW2	0.08	0.001	0.28	0.65	0.002	-0.74	0.33	
	DC-RW3	0.01	0.0003	0.09	0.88	0.001	-0.74	0.66	
	Head Length (HL)	0.0004	0.001	0.02	0.98	0.01	-0.69	0.73	
	Head Width (HW)	0.08	-0.01	-0.28	0.65	0.02	-0.69	0.43	
	Head Height (HH)	0.02	-0.007	-0.15	0.80	0.02	-0.69	0.34	
	Lower Jaw Length (LJL)	0.005	-0.003	-0.07	0.91	0.01	-0.69	0.75	
	Relative HL	0.21	0.004	0.45	0.44	0.00	-0.69	0.51	
	Relative HW	0.35	-0.01	-0.59	0.29	0.01	-0.69	0.38	
	Relative HH	0.01	-0.002	-0.10	0.88	0.01	-0.69	0.32	
Relative LJL	0.02	0.001	0.16	0.80	0.00	-0.69	0.23		
Proportion hard prey	SVL	0.57	0.71	0.75	0.14	-0.03	-0.95	0.97	
	LC-RW1	0.60	0.08	0.78	0.12	-0.02	-0.95	0.76	
	LC-RW2	0.17	-0.03	-0.41	0.49	0.01	-0.95	0.86	
	LC-RW3	0.03	-0.01	-0.16	0.80	-0.07	-0.95	0.10	
	DC-RW1	0.43	-0.05	-0.65	0.24	0.02	-0.91	0.60	
	DC-RW2	0.09	-0.02	-0.31	0.62	0.03	-0.91	0.40	
	DC-RW3	0.01	0.005	0.10	0.88	0.02	-0.91	0.59	
	HL	0.54	0.48	0.74	0.16	0.20	-0.95	0.67	
	HW	0.82	0.73	0.91	0.03	-0.03	-0.95	0.96	
	HH	0.67	0.62	0.82	0.09	-0.21	-0.95	0.73	
	LJL	0.64	0.62	0.80	0.10	0.23	-0.95	0.69	
	Relative HL	0.08	-0.05	-0.28	0.65	0.11	-0.95	0.41	
	Relative HW	0.06	0.06	0.25	0.68	-0.15	-0.95	0.41	
	Relative HH	0.02	-0.04	-0.13	0.84	-0.33	-0.95	0.06	
Relative LJL	0.06	0.03	0.24	0.70	0.13	-0.95	0.08		
Bite force (N)	SVL	0.79	1.00	0.89	<0.0001	-	-	-	
	HL	0.89	0.21	0.94	<0.0001	-	-	-	
	HW	0.77	0.08	0.88	<0.0001	-	-	-	
	HH	0.74	0.11	0.86	<0.0001	-	-	-	
	LJL	0.88	0.20	0.94	<0.0001	-	-	-	
Relative bite force	Relative HL	0.03	0.03	0.19	0.38	-	-	-	
	Relative HW	0.09	-0.09	-0.30	0.16	-	-	-	
	Relative HH	0.001	0.01	0.02	0.91	-	-	-	
	Relative LJL	0.001	-0.005	-0.03	0.91	-	-	-	

[#] LC = Lateral cranial view, DC = Dorsal cranial view, RW = relative warp component

Phylogenetic comparative analyses

There were no significant relationships between the proportion of hard prey eaten and cranial morphology once phylogeny was taken into account (Table 2.2), indicating that the relationships between these variables in the non-phylogenetic correlations may be influenced by a shared ancestry. Interestingly, whilst there were no significant relationships between the proportion of evasive prey and limb morphology, once phylogeny was taken into account there were significant relationships between forelimb dimensions and the proportion of evasive prey taken (Table 2.3).

Table 2.3: Non-phylogenetic and phylogenetic correlations between proportion of evasive prey eaten, sprint speed capacity (absolute and relative) and limb measurements (relative and absolute). Phylogeny was taken into account using the Brownian Motion (BM) model in a phylogenetic generalized least squares analysis (PGLS). Variances (R^2), slope of the correlation, Pearson's correlation indices (r) and P-value shown of correlations between variables (without taking phylogeny into account). Significant correlations ($P \leq 0.05$) are indicated in bold font.

Independent	Dependent	Non-phylogenetic				Phylogenetic		
		Variances (R^2)	Slope	Correlation (r)	P-value	Slope	Correlation (r)	P-value
Proportion evasive prey	Snout-vent length (SVL)	0.66	0.69	0.81	0.09	0.64	-0.93	0.16
	Femur length (FM)	0.40	0.36	0.64	0.25	0.37	-0.93	0.22
	Tibia length (TB)	0.46	0.30	0.68	0.21	0.33	-0.93	0.14
	Humerus length (HM)	0.51	0.48	0.71	0.18	0.58	-0.93	0.05
	Radius length (RD)	0.60	0.46	0.77	0.13	0.54	-0.93	0.03
	Relative FM	0.03	-0.02	-0.16	0.79	-0.06	-0.93	0.38
	Relative TB	0.57	-0.07	-0.75	0.14	0.09	-0.93	0.24
	Relative HM	0.32	0.08	0.56	0.32	-0.09	-0.93	0.03
	Relative RD	0.18	0.06	0.42	0.48	0.13	-0.93	0.03
Sprint speed (m/s)	SVL	0.97	23.15	0.48	<0.0001	0.31	-0.97	0.07
	FM	0.97	3.86	0.26	<0.0001	0.23	-0.97	0.14
	TB	0.98	3.52	0.21	<0.0001	0.10	-0.97	0.47
	HM	0.97	2.46	0.49	<0.0001	0.07	-0.97	0.46
	RD	0.97	2.25	0.41	<0.0001	0.11	-0.97	0.37
Relative sprint speed	Relative FM	0.05	0.00	-0.23	0.27	0.11	-0.97	0.31
	Relative TB	0.07	-0.05	-0.26	0.23	-0.03	-0.15	0.78
	Relative HM	0.04	0.08	0.19	0.38	-0.06	-0.15	0.31
	Relative RD	0.04	0.06	0.19	0.37	0.06	-0.15	0.19

Discussion

In the genus *Nucras*, we show a link between head shape, diet and underlying functional performance at the whole-organism level, before phylogeny is taken into account. Clustering based on morphology did not correspond to the clades identified in the molecular phylogeny, indicating that not only ancestry is influencing the morphology of *Nucras*. The diet of selected species was compared to morphology and performance, to investigate the link between diet and phenotype in *Nucras*. Dietary niche breadth and the proportion of hard prey eaten were found to be correlated with cranial shape, but not when phylogeny was accounted for, suggesting that cranial shape in the five species investigated is somewhat constrained by evolutionary history. Absolute values of performance (bite force and sprint speeds) were significantly positively related to absolute head and limb measurements, respectively. When phylogeny was accounted for, the relationship between forelimbs and proportion of evasive prey was significant, indicating that forelimb lengths have co-evolved with the proportion of evasive prey taken, though this may be a by-product of co-evolution with other factors in the environment.

The phylogenetic relationships between the *Nucras* species reflected the current species designations that were described using external morphological characters. Two species, *N. tessellata* and *N. livida*, once considered subspecies of *N. tessellata* (FitzSimons, 1943), are morphologically and genetically distinct, which is consistent with the current species designations (Branch & Bauer, 1995). The phylogeny shows that *N. taeniolata*, *N. holubi* and *N. ornata*, once considered subspecies of *N. taeniolata* (Broadley, 1972) are separate lineages, as well as in separate morphological clusters, which is also consistent with the current species designations (Jacobsen, 1989; Branch, 1998). While related species are geographically proximate to each other, the morphological topology is incongruent with the phylogeny (Fig. 2.3). The phylogeny indicates the evolutionary patterns of radiations within the genus, whilst the morphology may be driven by other factors, such as diet, causing the topologies to differ.

Niche breadth (*i.e.* range of arthropod orders taken) was significantly correlated with cranial shape, indicating that species preying on a large number of arthropod orders have wider cheek regions (as in *N. intertexta*) and higher bite forces, whilst those species which specialize (low niche breadth values) on hard prey items have more robust crania (shorter snouts) but narrower cheek regions (as in *N. lalandii* and *N. tessellata*), and lower biting capacities. There was also a positive relationship between absolute head width and the proportion of hard prey consumed in *Nucras*. Previously it has been shown in other lacertid lizards that those species that consume harder prey have wider heads, due to the larger jaw adductor muscles (*e.g.* Herrel *et al.*, 2001a; Verwajen *et al.*, 2002; Huyghe *et al.*, 2009) facilitating a greater relative bite force. It was expected that those *Nucras* species specializing on hard prey would show harder bite forces, however this was not the case. In contrast, the dietary niche breadth (the variety of prey taken) was positively correlated with bite force. Although puzzling at first, variation in prey size

may explain this result. As hardness is known to increase with prey size (Herrel *et al.*, 2001a; Aguirre *et al.*, 2003), species eating only hard, yet small prey may not need very high bite forces. On the other hand generalist species may profit from high bite forces as this would allow them to consume a wide range of prey varying in size and hardness. Bite force has also been seen to be higher than necessary for the prey taken in other lizards (Herrel *et al.*, 1999a). With the small number of species included in the current study, however, the results involving bite force need to be treated as preliminary, and increasing sample sizes may clarify this relationship with more confidence. Thus further studies correlating individual prey hardness to bite force are needed to better understand the factors driving the evolution of head shape in *Nucras* lizards.

Sprint speed was related to body size and limb morphology in absolute terms, but neither of these was related to the proportion of evasive prey taken. This lack of a relationship was also found for other lacertid lizards (Vanhooydonck *et al.*, 2007). As was suggested previously (Vanhooydonck *et al.*, 2007), maximal sprint speed may not be as important as fast acceleration for the capture of evasive prey, due to the fact that once the prey takes flight it is essentially out of reach of the lizards and no amount of running at top speed will enable the lizard to capture the prey. Thus, the ability to immediately capture the evasive prey before it escapes would be crucial. In comparisons of dietary and functional capacities, measures of acceleration in addition to sprint speed and stamina may turn out to be more informative in understanding a lizard's ability to capture elusive prey.

The PGLS analyses retrieved significant relationships between niche breadth and the first relative warp score of the head in dorsal view, and between limb morphology and the proportion of evasive prey eaten. The proportion of hard prey taken did not show any relationship with head shape descriptors when phylogeny was accounted for, suggesting an important role of shared ancestry in the observed co-variation between head shape, diet and bite force. In contrast, the proportion of elusive prey eaten was shown to co-evolve with forelimb dimensions in the species included in our study.

Conclusions

As head shape was shown to be linked with the dietary niche breadth, I conclude that, within the five species investigated in the genus *Nucras*, diet is influential in shaping the morphologies and performance of lacertid lizards, as has been found in other lizards (e.g. Herrel *et al.*, 2001a; Verwajen *et al.*, 2002; McBrayer & Wylie, 2004; Verwajen & Van Damme, 2007a). Future analyses incorporating a larger number of species and incorporating data on both prey size as well as functional properties are needed to better understand the evolution of body proportions in relation to diet in this genus. Despite these limitations, the data do suggest interesting co-variation between morphology, niche breadth, prey type and performance that would be worth exploring further.

The life history of a lizard is complex, and as such many factors may be influencing their phenotypic expression. Firstly, it was shown that body shape is linked with habitat openness (Edwards *et al.*, 2012; Chapter 1). In *Nucras*, head shape is influenced by one aspect of the lizards' life history, namely their dietary range. But other factors may be influential in shaping lacertid crania and the next chapter explores how predator escape behaviour influences the cranial shapes of *Meroles*.





Meroles anchietae in Gobabeb, Namibia.
Photo by: G. John Measey

Chapter 3

**THE ADAPTIVE NATURE OF THE RADIATIONS WITHIN THE GENUS
MEROLES AND THE RESULTANT EFFECTS ON PERFORMANCE**

THE ADAPTIVE NATURE OF THE RADIATIONS WITHIN THE GENUS *MEROLES* AND THE RESULTANT EFFECTS ON PERFORMANCE.*

Introduction

Arnold (1983) suggested that selective pressures may act first upon the performance of the lizards, and then the morphologies are selected for that are best for optimal performance. As such, particular environmental characteristics may lead to convergence in morphologies in lizards inhabiting similar environments due to the similar behaviours and performance capacities required for optimal survival within that environment. Thus, investigations into the links between environment, performance and morphology are essential to understanding the selective pressures exerted by a particular environment. For example, various species within the Eremiadini radiation have similar morphologies if they occur in similar habitats (Edwards *et al.*, 2012). Species of *Nucras* were found to be morphologically similar if they consumed similar prey items (Edwards *et al.*, in press). In those cases, the morphological shapes were linked to habitat and/or behaviour, and were independent of ancestry. However, the ability to evade a predator is important for the survival of an individual, and reptiles have developed many different ways to avoid predation. Most commonly, terrestrial lizards will run away from predators, as complete evasion is preferential to confrontation. In addition to running away, other anti-predator mechanisms have developed in the morphology and functional capacities in lizards, such as scales that resemble thorns (*e.g.* thorny devils *Moloch horridus*, desert horned lizard *Phrynosoma platyrhinos*, armadillo girdled lizard *Ouroborus cataphractus*), camouflage (*e.g.* chameleons, the satanic leaf-tailed gecko *Uroplatus phantasticus*), tail autotomy (*e.g.* all lacertid lizards, most geckos), mimicry (*e.g.* juvenile Bushveld lizard *Heliobolus lugubris*), aposematic colouration (*e.g.* Gila monster *Heloderma suspectum*) and sand-diving (*e.g.* shovel-snouted lizard *Meroles anchietae*, *Acanthodactylus* species, *Uma* species), to name a few. Some species even have different morphotypes that utilize differing escape strategies (*e.g.* Carretero *et al.*, 2006). In different desert regions around the world, convergent behaviours (sand-diving) and morphologies have evolved in genetically disparate lizard families (Arnold, 1994; Robinson & Barrows, 2013). Such convergence in body shapes suggests that the desert environment, which is characterized as arid, sparsely vegetated and dominated by dune-sands, is driving selection for particular body shapes (Robinson & Barrows, 2013). If so, then selection for a particular behaviour (such as diving head-first into the substrate) may result in other performance capacities (such as bite force) being compromised, and a trade-off between better diving ability and harder biting capacity may be found in these lizards.

* In preparation: Edwards S, Tolley KA, Vanhooydonck B, Herrel A. The adaptive nature of the radiations within the genus *Meroles* and the resultant effects on performance.

In order to investigate the link between predator escape strategy and phenotype in lizards, we chose the genus *Meroles* as this genus consists of species that primarily run and hide in vegetation to escape predators (non-divers), and those that dive head-first into the sandy substrate (divers) (Branch, 1998). The desert lizards *Meroles* (Gray, 1838) are small-bodied, ground-dwelling lizards which inhabit the arid regions of southern Africa (Branch, 1998) and whilst all eight species range to some degree into Namibia, only four are Namib Desert endemics. *Meroles reticulatus* diverged from other *Meroles* around 13 Mya, and the split between *M. suborbitalis* and *M. squamulosus* occurred about 9 Mya and between *M. suborbitalis* and sand divers (*M. anchietae* and *M. cuneirostris*) about 12 Mya (Hipsley *et al.*, 2009; Hipsley, 2012). Molecular phylogenies for the genus (Lamb & Bauer, 2003) produced topologies with improved resolution over that of Harris *et al.* (1998b) by using two additional mtDNA genes, in which *Meroles knoxii*, a non-diver, was placed “basal” to all other species, and *M. anchietae* was placed firmly in a well-supported clade consisting of *M. cuneirostris*, *M. micropholidotus* and *M. ctenodactylus*; all four of which are sand-divers (Branch, 1998). *Meroles anchietae* was considered by Boulenger (1921) to be closely related to the *Saurites*, due to its ultra-psammophilic nature, a relationship which was found in molecular sequence analyses (Harris *et al.*, 1998b; Lamb & Bauer, 2003), but not electrophoretic data (Mayer & Berger-Dell’Mour, 1988). This “psammophilic” clade was more derived than *M. reticulatus*, which in turn was more derived than *M. suborbitalis* (a non-diver). *Meroles reticulatus* is an interested species as it primarily runs away when threatened, but if pursued long enough, will dive into the sand (Branch, 1998), thereby employing a dual escape strategy. This species may modify its behaviour according to the habitat, as southerly populations occur on dune habitats, where diving is possible, however many populations occur on gravel plains, where diving is not possible (A. Bauer, *pers. comm.*). *Meroles squamulosus* (previously in *Ichnotropis*) occurs in the Kalahari sands of the savannah biome (north-east South Africa, Botswana, Zimbabwe, north-east Namibia, Angola), parapatric to the other *Meroles* species, and environmental selective pressures have resulted in the morphological body plan of this species being convergent with the sympatric species *Ichnotropis capensis* (Edwards *et al.*, 2012). It lives on hard-packed soil, and does not sand-dive (Branch, 1998).

Predator escape strategies may influence lizard body shapes, and the balance between having a body plan optimal for foraging and obtaining a mate, and one that prevents the lizard from being predated upon, results in trade-offs between morphology and performance (e.g. Vanhooydonck *et al.*, 2001a, 2011). The environment may place a selective pressure on the lizard to utilize a particular predator escape strategy (sand-diving or primarily running away and hiding), leading to selection for a particular morphological dimension in the limb and cranial dimensions (e.g. Vanhooydonck & Van Damme, 1999). In *Meroles*, we expected that those species inhabiting open, sparsely vegetated, sandy habitats (desert dune fields) have cranial shapes that show associated morphological adaptations to diving (upper

labial scales form a lateral ridge, pointed snouts, nasal vestibule elongated, counter-sinking of lower jaws, and nasal valves present; Arnold, 1994). Functionally, the adaptation in cranial shape to sand-diving is expected to have affected the bite force capacity of the sand-diving species, due to the counter-sinking of the lower jaw and the effective change in the ratio between the in-lever and out-lever of the jaw mechanics. To facilitate easier entry into the loose sand when sand-diving, we expected that the sand-divers would have more dorsoventrally flattened heads with sharper anterior lips compared to non-divers, and these differences in head shapes may have an effect on the bite performance of the lizards. On the other hand, in those species that primarily run away from predators (non-divers) a sharp-edged anterior lip on the crania would not have evolved and the non-divers are expected to have rounder snouts and more robust crania. Thus, differences in head shape were investigated using geometric morphometric analyses of the crania, and compared with the species' biting capacities. In other lizards, those with short out-levers, large closing in-levers, and tall, round-snouted skulls have higher biting capacities (e.g. Herrel *et al.*, 2004). The length of the lever arms of the jaw opening and closing mechanisms were compared to the bite force values, compensating for the counter-sinking of the jaws. As similarities in phenotype between species could be largely due to shared ancestry, and not due to environmental influence, phylogenetic analyses of variance were performed, once a molecular phylogeny for the genus was constructed using both mitochondrial and nuclear markers.

Materials and Methods

Sampling

For the morphometric analyses, individuals of all eight species were obtained from field trips and from the wet collections housed at the Port Elizabeth Museum, the Ditsong (previously Transvaal) Museum and the Ellerman collection at Stellenbosch University. Tissue (tail or liver tissue) for the individuals utilized in the phylogenetic analyses was either obtained during field trips or from the Herpbank tissue collection housed at the South African National Biodiversity Institute (SANBI). Performance was measured for the same live specimens caught during field trips (for all species except *M. micropholidotus*, which was not found during field work). Although *M. micropholidotus* was included in the morphometric analyses, no tissue was available for genetic sequencing nor were we able to capture any live specimens and therefore this species was not included in the phylogenetic or the performance analyses. *Meroles squamulosus*, originally described as *Ichnotropis* but found to be part of *Meroles* (Edwards *et al.*, 2013), was included in all of the current analyses. The species were categorized according to their predator escape strategy (Branch, 1998), namely as diving (*M. anchietae*, *M. ctenodactylus*, *M. cuneirostris* and *M. micropholidotus*), non-diving (*M. knoxii*, *M. squamulosus* and *M. suborbitalis*) or dual-strategy (*M. reticulatus*) species. A complete list of the individuals sampled, including museum accession numbers and EmblBank accession numbers, is detailed in Appendix A.

Unless otherwise specified, all of the following analyses were performed in the program R Studio v.0.97.248 (R Core Team, 2012; R Studio, 2012).

Geometric morphometric analyses

Geometric morphometric analyses were used to investigate differences in cranial shapes in all *Meroles* species, using high resolution photographs taken with a digital camera (Fuji Finepix S2000HD: resolution 10.0 MP, and Canon 50D: resolution 10.0 MP and macro lens F18/100). Dorsal (282 individuals, ~ 35 per species) and lateral (216 individuals, ~ 27 per species) views of the crania were photographed. As in Chapter 1, individual sex was noted, but the data were not separated by sex, as variation between sexes has been shown often be smaller than between lacertid species. Homologous landmarks were digitized (Fig. 3.1), and a Generalized Procrustes Analysis (GPA, Rohlf & Slice, 1990; Rohlf, 1999) was performed in which the sizes were standardized and the landmark configurations were translated and rotated using programs from the TPS programs suite (tpsUtil v.1.53, Rohlf, 2004; tpsDig2 v.2.16, Rohlf, 2005; tpsRelW v.1.49, Rohlf, 2003). Relative warps analyses were performed to identify which portions of the crania showed the most variation, and deformation grids (thin-plate splines) were used to visualize the differences in the cranial shape. Scores from each relative warp component were imported into the program R and ANOVAs were used to identify whether the species in the three categories of predator escape strategies differed in each relative warp component (package: 'stats', function: 'anova'; R Studio, 2012). Fisher's least significant difference (Fisher's LSD test) post-hoc tests, with Bonferroni corrected *P*-values, were used to determine which category differed from one another (package: 'agricolae', function: 'LSD.test', p.adj: 'bonferroni'; R Studio, 2012).

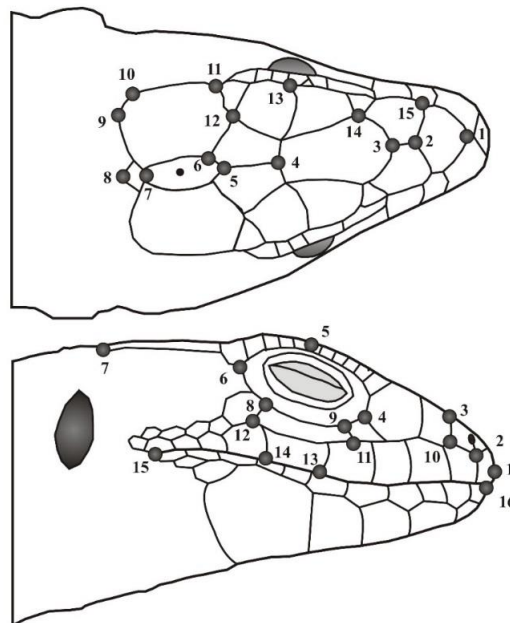


Figure 3.1: Homologous landmarks chosen for the geometric morphometric analyses on the dorsal (top) and lateral (bottom) views of the heads of *Meroles*.

Performance analyses

Bite force capacities (122 individuals, ~17 individuals per species; *M. micropholidotus* not included due to lack of samples) were determined through five trials of a lizard biting on two metal plates connected to an isometric force transducer and a charge amplifier (see Herrel *et al.*, 1999a for more details on the experimental setup) and the maximum bite force was used for each individual in further trials. Analyses of variance (ANOVAs) were used to identify whether there were significant differences between the absolute bite force values, using the predator escape strategy categories as the fixed factor in the analyses (package: ‘stats’, function: ‘anova’; R Studio, 2012). To eliminate the effect of size, various measurements on the head were taken and log₁₀-transformed: head length (HL), head width (HW), head height (HH), lower jaw length (LJL) (see Fig. 1.2 in Chapter 1). Two additional measurements were taken to investigate the jaw opening and closing mechanisms: coronoid-to-tip length (CT) and quadrate-to-tip length (QT; jaw out-lever). The geometric means of the head measurements (geometric mean = (HL+HW+HH+LJL)/4) were used as covariates in analyses of covariance (ANCOVA), again grouping the species according to their predator escape strategy (package: ‘stats’, function: ‘anova’; R Studio, 2012). As above, Fisher’s LSD post-hoc tests, with Bonferroni adjusted *P*-values, were used to determine which category differed from one another in their relative bite forces (package: ‘agricolae’, function: ‘LSD.test’, p.adj: ‘bonferroni’; R Studio, 2012).

Phylogenetic comparative methods

Genomic DNA was isolated according to standard procedures involving a proteinase-K digestion from the tail or liver tissue preserved in 95–100% ethanol, followed by salt-extraction procedures (Bruford *et al.*, 1992). Two mitochondrial (16S and ND4) and two nuclear genes (RAG1 and KIAA-2018) were amplified using standard PCR procedures as in Chapter 1 (primer pairs detailed in Table A1). Sequences were aligned using Clustal-Omega (Sievers *et al.*, 2011), and checked in BioEdit Sequence Alignment Editor v. 7.0.5.2 (Hall, 1999) (see the Table A2 for all voucher information and corresponding EMBL-Bank accession numbers). Outgroups chosen were species from sister genera to *Meroles* (*Ichnotropis*, *Pedioplanis*, *Vhembelacerta* and *Australolacerta*; Mayer & Pavlicev, 2007; Kapli *et al.*, 2011; Edwards *et al.*, 2012, 2013; Engleder *et al.*, 2013). A partition homogeneity test (Farris *et al.*, 1994, 1995), implemented in PAUP* v4.0b10 (Swofford, 2002), was used to analyse the mitochondrial (16S vs. ND4) and nuclear (RAG1 vs. KIAA-2018) datasets separately to ensure that there was no conflict in the markers within each genome. As the two mitochondrial and the two nuclear genes were not incongruent, the partition homogeneity test was rerun (nuclear vs. mitochondrial) to ensure that there was no conflict between the two genomes. Phylogenetic trees were constructed of the 1) mitochondrial gene dataset, 2) the nuclear gene dataset and 3) the combined total evidence dataset. The third codon position of the ND4 gene (found to be saturated in Dambe v.5.2.65; Xia *et al.*, 2003) was partitioned separate from the first two codon positions. Models of evolution for each gene separately were identified in jModelTest v.2.1 (Posada, 2008).

To account for phylogenetic relationships, phylogenetic analyses of variance (ANOVA) and phylogenetic analyses of covariance (ANCOVA) (collectively referred to hereon as PDAN(C)OVAs) were performed to test if there were differences in the morphometric data and performance data between the different strategies of predator escape. The \log_{10} -transformed means for each species of each morphometric measurement and performance value were used in the analyses (package: 'stats', functions: 'mean' and 'log'). An empirical null distribution of F-statistics taking into account the phylogeny was generated for each variable using PDSIMUL v.2.0 (Garland *et al.*, 1993), during which we ran 1000 simulations utilizing a Brownian Motion (BM) model of evolution using branch lengths obtained from the genetic phylogeny. The simulations were then used in the PDAN(C)OVAs in the program PDANOVA v.3.0 (Garland *et al.*, 1993). The F-statistics of the simulations were used to create a null distribution and the F-statistic from the traditional ANOVAs and ANCOVAs (using SVL as the covariate; calculated in PDSINGLE v.2.0; Garland *et al.*, 1993) were compared to the new null distribution. Significance of the PDAN(C)OVAs was determined if the actual F-value exceeded the upper 95th percentile of the empirical F-distribution. The geometric means of the head dimensions were used as the covariate in all PDANCOVAs. The PDAN(C)OVAs were performed on the first three relative warps' components of the geometric morphometric analyses for both the dorsal and lateral views of the crania, for all species. PDAN(C)OVAs were also performed on the bite force values for all species measured.

Results

Morphometric and performance analyses

Morphologically, the species differed significantly in all of the first three relative warps components of both the dorsal and lateral views of the crania (Table 3.1 and 3.2). Diving species had more compressed parietal regions (landmarks 6-12; negative DC-RW1 scores), longer snouts (landmarks 1, 2, 15; positive DC-RW 3; and landmarks 1-3, 10, 16; positive LC-RW1 scores) with pointed anterior lip edges (landmarks 1, 2, 16; positive LC-RW1 scores), and larger occipital scales with smaller interparietal scales (landmarks 5-8; positive DC-RW3 scores), compared to non-divers (Fig. 3.2 and 3.3). The snout landmarks (landmarks 1, 2, 16; positive LC-RW1 scores) also indicated that the diving and dual-strategy species had lower jaws which are not flush with the upper jaws, indicating counter-sinking of the lower jaw in these species. The most highly psammophilic species *M. anchietae* differed markedly from all other species in both the dorsal and lateral cranial views. It had more compact parietal regions (landmarks 6-12; negative DC-RW1 scores), a higher posterior cranium (landmarks 1, 2, 16; negative LC-RW2 scores) with a pointed anterior lip edge (landmarks 1, 2, 16; negative LC-RW2 scores) (Figs. 3.2 and 3.3, Table 3.1). *Meroles reticulatus* had the most pronounced elongation of the snout region (most positive LC-RW1 scores), resulting in a significant difference between this dual-strategy species

and all other species (Table 3.2). *Meroles knoxii* had a rounded snout (landmarks 1, 2, 16; negative LC-RW1 scores) and a more elongated posterior cranial scales (landmarks 6-12; positive DC-RW1 scores) - a cranial morphology that was similar to the other non-divers.

There were significant differences in both the absolute and relative bite force values between diving and non-diving species (divers had significantly lesser bite force capacities), but there were no significant differences in bite force between the dual-strategy species and the other two categories. *Meroles anchietae* had the lowest relative bite force capacity (Fig. 3.4). The dual-strategy species *M. reticulatus* had a relative bite force capacity that was intermediate to that of divers and non-divers (Fig. 3.4), though it differed significantly morphologically from both categories of species (Table 3.2).

Table 3.1: Results of traditional (trad) and phylogenetic (phyl) analyses of variance of relative warps components of the geometric morphometric cranial shapes and absolute and relative bite force for *Meroles* categorized according to their predator escape strategies. Significance ($P \leq 0.05$) is indicated in bold font.

Components [#]	% Variation	F (trad)*	P (trad)*	P (phyl)*
DC-RW1	44.88	109.7	< 0.0001	0.19
DC-RW2	18.40	5.14	0.006	0.89
DC-RW3	9.72	65.48	< 0.0001	0.29
LC-RW1	36.38	277.74	< 0.0001	0.002
LC-RW2	15.85	7.01	0.001	0.74
LC-RW3	12.01	21.07	< 0.0001	0.32
Bite force (absolute)	-	0.44	0.51	0.81
Bite force (relative)	-	7.73	0.006	0.73

* Key to headings: F (trad) = F-value of traditional AN(C)OVA; P (trad) = P-value of the traditional AN(C)OVA; P (phyl) = P-value of the phylogenetic AN(C)OVA

[#] Key to components: DC = dorsal view of crania; LC = lateral view of crania; RW = relative warp component

Table 3.2: Results of Fisher's least significant difference post-hoc tests relative warp components or bite force of *Meroles* with species grouped according to their predator escape strategy. Significant differences ($P \leq 0.05$) are shown in bold. Key to components as in Table 3.1.

Components	Dive vs. Dual-strategy	Dive vs. Non-dive	Non-dive vs. Dual-strategy
DC-RW1	0.04	< 0.0001	< 0.0001
DC-RW2	0.01	1.00	0.005
DC-RW3	< 0.0001	< 0.0001	< 0.0001
LC-RW1	< 0.0001	< 0.0001	< 0.0001
LC-RW2	0.02	0.002	1.00
LC-RW3	0.0002	< 0.0001	1.00
Bite force (absolute)	1.00	0.03	0.50
Bite force (relative)	1.00	< 0.0001	0.24

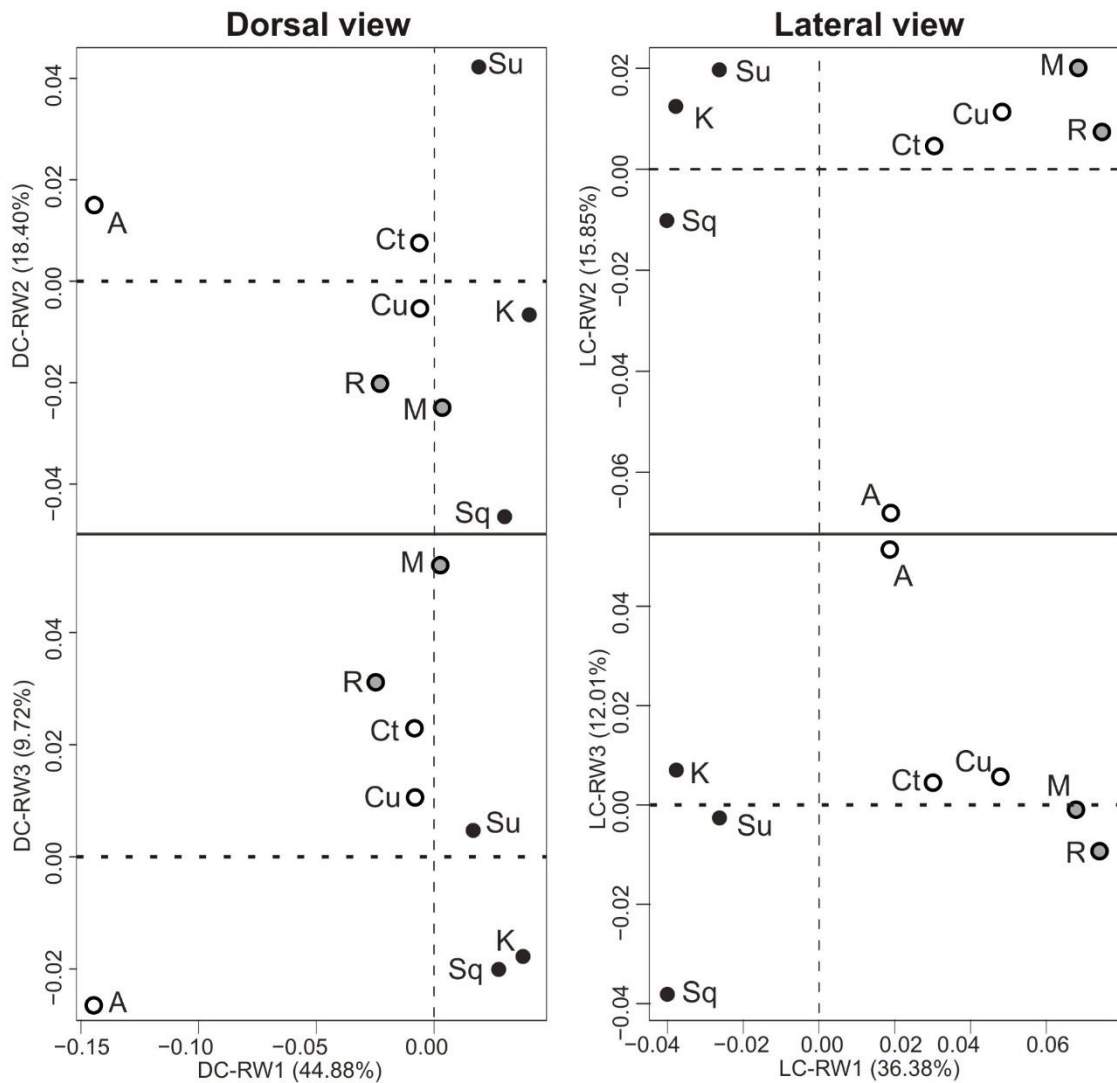


Figure 3.2: Species means of the first three relative warp (RW) components for the dorsal (DC; left) and lateral (LC; right) views of the crania. Colours denote the predator escape strategies: white = diving, black = non-diving, grey = dual-strategy. Percentage variation contributed by each axis to the whole shown. Key to species abbreviations: A = *Meroles anchietae*, Ct = *M. ctenodactylus*, Cu = *M. cuneirostris*, K = *M. knoxii*, M = *M. micropholidotus*, R = *M. reticulatus*, Sq = *M. squamulosus*, Su = *M. suborbitalis*.

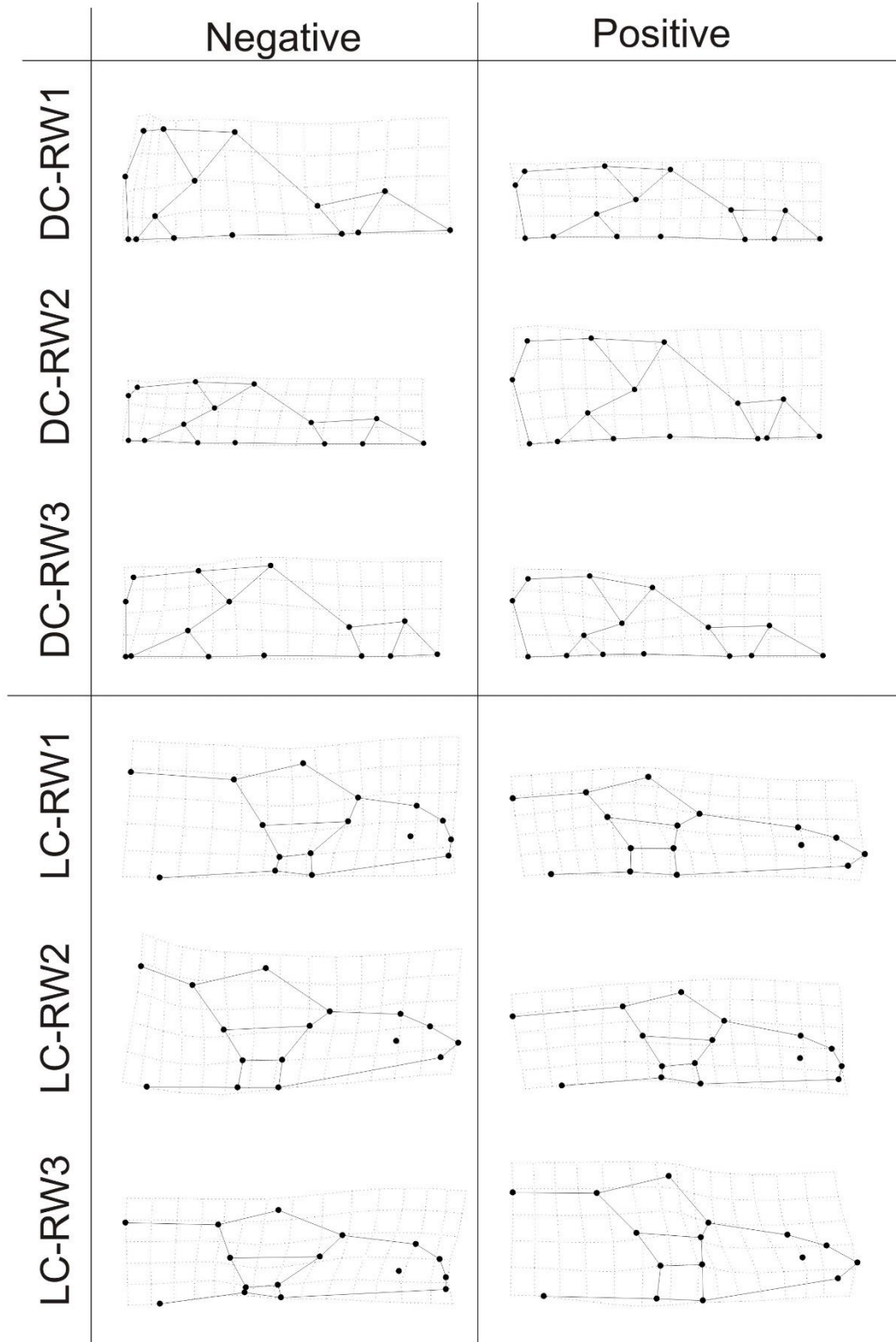


Figure 3.3: Deformed grids (thin-plate splines) indicate the shape of the crania of *Meroles* at each extreme (positive and negative) of the first three (RW1, RW2 and RW3) relative warps components for the dorsal (DC; top) and lateral (LC; bottom) views of the crania.

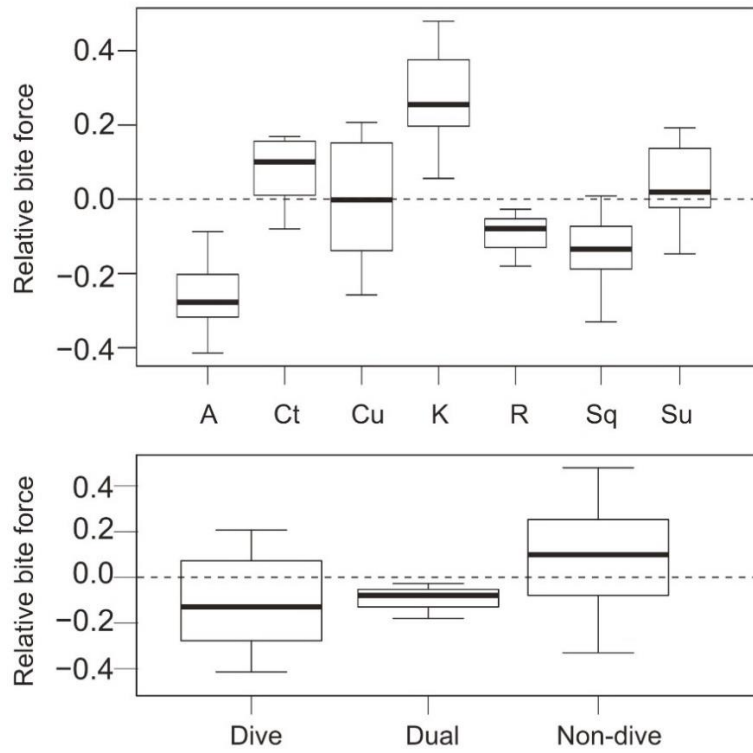


Figure 3.4: Boxplots of relative bite force (residuals obtained once size was removed), grouped according to species designations (top) and predator escape strategy (bottom). Species abbreviations as in Fig. 3.2.

Phylogenetic comparative analyses

In the phylogenetic trees constructed of the total combined dataset, each species was monophyletic and well-supported (bootstraps $\geq 90\%$ and posterior probabilities ≥ 0.95) (Fig. 3.5). *Meroles reticulatus*, a dual-strategy species, is sister to all other *Meroles*. The sand-divers formed a well-supported, with a non-diver *M. squamulosus* sister to it, though this relationship was not supported. *Meroles suborbitalis* and *M. knoxii*, both of which are non-divers, also formed a well-supported clade. Sequence divergences (uncorrected p-distance) between *Meroles* (16S: $5.07 \pm 1.36\%$, ND4: $15.90 \pm 1.43\%$, RAG1: $2.21 \pm 0.60\%$, KIAA: $1.76 \pm 0.44\%$) were comparable with those found between other lacertid species (Chapters 1 and 2; Podnar, Pinsker & Mayer, 2009). *Meroles knoxii* consisted of three lineages (MK1-3: West Coast, Karoo, Cape Peninsula), *Meroles squamulosus* consisted of two lineages (MSq1-2: Northern Cape Province and western Limpopo Province, eastern Limpopo Province), and *Meroles suborbitalis* consisted of three lineages (MSu1-3: Northern Cape Province, southern Namibia, and western Namibia). As the sequence divergences between the clades within the three non-diving species were lower than those between described species (Table 3.3), and as we are interested in the species-level differences in morphology and performance, we concentrated only on the described species, and not on the clades within the species.

There were significant differences between the LC-RW1 scores once phylogeny was taken into account, but not in any other relative warps, indicating that the differences in the snout regions (landmarks 1-3, 16; Fig. 3.5) between diving and non-diving species may be due to factors other than shared ancestry. There were no significant differences in performance between divers and non-divers when phylogeny was accounted for, indicating that while there is a significant difference in residual bite force capacities, this difference may be due to a shared ancestry of diving species and of non-diving species.

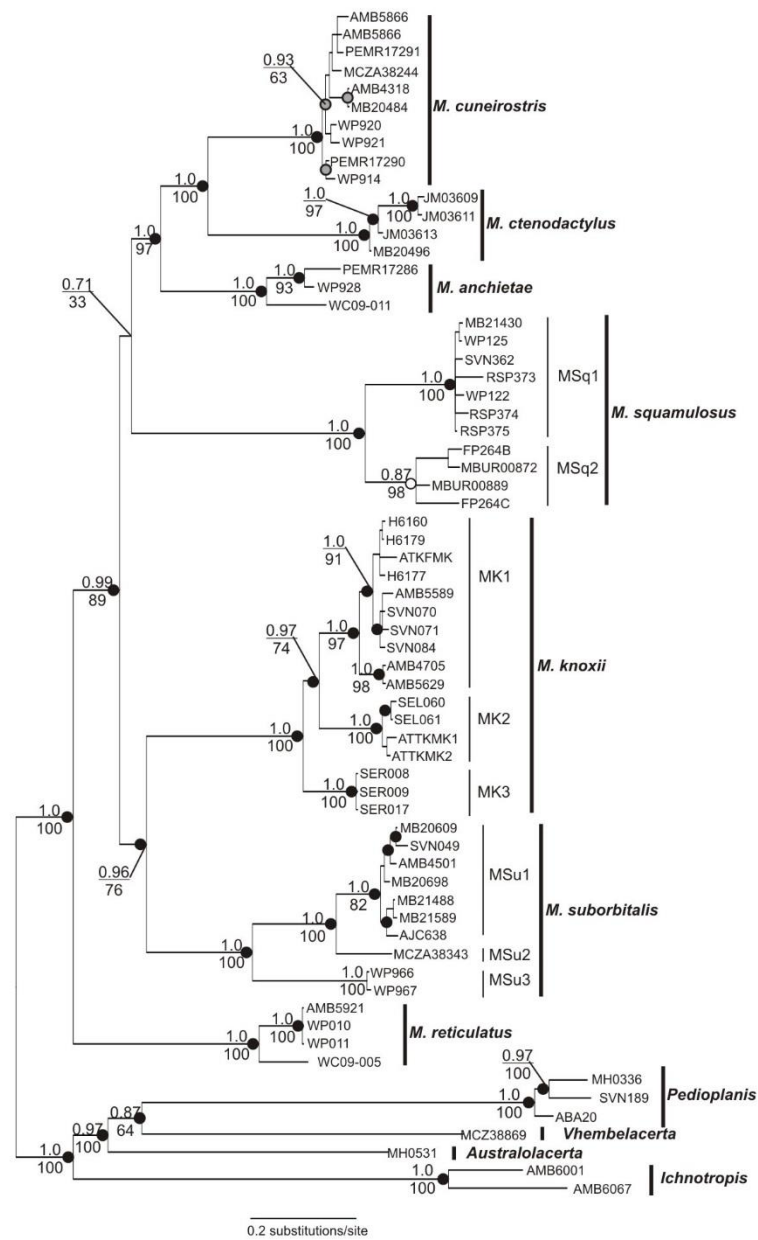


Figure 3.5: Phylogenetic tree of the genus *Meroles* based on the combined mitochondrial and nuclear datasets and inferred by Bayesian inference (BI) and maximum likelihood analyses (ML) (BI topology shown). Colour of the circle indicate support at the nodes: grey = BI only, white = ML only black = both methods (BI posterior probabilities > 0.95 and ML bootstrap support values >75%). Separate clades within *M. knoxii*, *M. suborbitalis* and *M. squamulosus* indicated. Outgroup taxa were *Ichnotropis*, *Vhembelacerta*, *Australolacerta*, and *Pedioplanis*. Details for individuals are listed in Appendix (Table A2).

Table 3.3: Sequence divergences (in percentages) between lineages within clades (light grey) and between clades of *M. knoxii* (MK), *M. squamulosus* (MSq) and *M. suborbitalis* (MSu) for the (A) 16S (below diagonal) and ND4 (above diagonal) mitochondrial gene regions, and the (B) RAG1 (below diagonal) and KIAA (above diagonal) nuclear gene regions.

A	MK1	MK2	MK3	MSq1	MSq2	MSu1	MSu2	MSu3
MK1		0.083±0.01	0.097±0.01	0.233±0.02	0.209±0.02	0.202±0.02	0.204±0.02	0.211±0.02
MK2	0.011±0.01		0.010±0.01	0.210±0.02	0.205±0.02	0.191±0.02	0.208±0.02	0.197±0.02
MK3	0.020±0.01	0.023±0.01		0.229±0.02	0.218±0.02	0.203±0.02	0.210±0.02	0.206±0.02
MSq1	0.051±0.02	0.060±0.02	0.06±0.02		0.134±0.02	0.225±0.02	0.232±0.02	0.226±0.02
MSq2	0.046±0.01	0.055±0.02	0.060±0.02	0.023±0.01		0.230±0.02	0.241±0.02	0.221±0.02
MSu1	0.058±0.02	0.066±0.02	0.060±0.02	0.094±0.02	0.089±0.02		0.086±0.01	0.152±0.02
MSu2	0.061±0.02	0.069±0.02	0.060±0.02	0.078±0.02	0.078±0.02	0.020±0.01		0.160±0.02
MSu3	0.052±0.02	0.060±0.02	0.050±0.01	0.069±0.02	0.064±0.02	0.034±0.01	0.028±0.01	
B	MK1	MK2	MK3	MSq1	MSq2	MSu1	MSu2	MSu3
MK1		0.001±0.000	0.005±0.002	0.022±0.004	0.022±0.005	0.010±0.002	0.012±0.003	0.012±0.003
MK2	0.003±0.002		0.004±0.002	0.029±0.006	0.028±0.006	0.021±0.005	0.023±0.005	0.022±0.005
MK3	0.004±0.003	0.007±0.004		0.030±0.006	0.029±0.006	0.025±0.005	0.027±0.006	0.027±0.006
MSq1	0.028±0.007	0.032±0.008	0.032±0.008		0.006±0.002	0.022±0.005	0.024±0.005	0.024±0.005
MSq2	0.023±0.007	0.026±0.007	0.027±0.007	0.000±0.000		0.024±0.005	0.025±0.006	0.025±0.005
MSu1	0.021±0.007	0.025±0.007	0.025±0.007	0.029±0.007	0.024±0.007		0.002±0.002	0.003±0.002
MSu2	0.030±0.008	0.034±0.008	0.034±0.008	0.042±0.009	0.036±0.008	0.009±0.004		0.005±0.002
MSu3	0.026±0.007	0.030±0.008	0.028±0.008	0.038±0.008	0.034±0.008	0.010±0.004	0.012±0.005	

Discussion

Effective predator escape strategies are crucial to the survival of an individual, and ultimately of a species. In *Meroles*, morphological features that maximise different escape strategies have evolved in response to environmental factors/features. Diving and non-diving species have significantly different morphologies, particularly with respect to snout length, due to upper labials forming sharp angles. The result however, is that bite force is reduced in diving species. Once phylogeny was accounted for, however, divers were not significantly different from non-divers, except in the snout region, indicating that the evolution of diving species is not independent and likely has a single origin.

The *Meroles* phylogeny estimated in this study differs from previous topologies (Harris *et al.*, 1998b; Lamb & Bauer, 2003) in the position of *Meroles reticulatus*, which was nested within *Meroles*. However, the topology could differ from previous studies due to the inclusion of *M. squamulosus* (Edwards *et al.*, 2012) but the exclusion of *M. micropholidotus*. As such, we found that the diving species and the non-diving species form separate well-supported clades, and a species (*M. reticulatus*) that is a dual-strategy species is sister to all other *Meroles*, a relationship that was found using electrophoretic data (Mayer & Berger-Dell'Mour, 1988). The diving and non-diving species are geographically separate, as the members of the diving clade occur in sparsely-vegetated, desert sands in the Namibian desert biome and north-western South Africa, whilst the members of the non-diving clade occur on more firm, sandy substrates in the western parts of South Africa and Namibia. The non-diving species which is not sympatric to any other *Meroles* occurs on firm, sandy substrates in the savannah biome of southern Africa (*M. squamulosus*). Arnold (1991) stated that the *Meroles* clade “shows steady progression from relatively firm substrates into very stringent habitats based on loose aeolian sand” and that this habitat shift “appears to have elicited many novel morphological features that are necessary for the survival in the extreme environments that the group has entered.” The phylogeny estimated in this study describes a different story, showing a split, and adaptive radiations into two different environments, with a dual-strategy species sister to all other species. These adaptive radiations may have occurred when the recent common ancestor of *Meroles*, which was likely a dual-strategy species like *M. reticulatus*, dispersed into the sparsely-vegetated, Namib Desert region. Sand-diving as the predominant predator escape strategy in *Meroles*, and diversification of the sand-diving clade, may be a relatively recent adaptation in comparison to the age of the Namib Desert, which is proposed to have developed around 35 Mya (van Zinderen-Bakker, 1975; Robinson & Barrows, 2013). Splits within the genus range from 9 Mya (between *M. suborbitalis* and *M. squamulosus*) to 12 Mya (between *M. suborbitalis* and sand divers *M. anchietae* and *M. cuneirostris*) and 13 Mya (between *Meroles reticulatus* and other *Meroles*) (Hipsley *et al.*, 2009; Hipsley, 2012). The sand-diving behaviour evolved as the primary predator escape strategy in the common ancestor of the *Meroles* species and was retained in the derived diving species. Open, sparsely-vegetated dune sand would be prerequisite for the sand-diving behaviour

to be necessitated, so perhaps an exaptation for burrowing and diving into holes, such as that already utilized by a dual-strategy species, led to the sand-diving into sands that do not hold their shape in a sparsely-vegetated landscape. One consideration is that although the Namib Desert was established millions of years before the diversification of the diving species, the Benguela up-welling system only developed around 10 Mya (Siesser, 1980) and the fog-bank developed due to the change in the seas off the west coast of Namibia. Fog provides a source of moisture to desert fauna and flora, as well as moderates the temperature in the coastal regions (Goudie, 1972; Seely, 1987), producing a more hospitable environment. These changes may have facilitated dispersal and radiation of the diving species within the region.

The morphological and performance results between divers and non-divers showed no significant differences when phylogeny was taken into account. This suggests whilst the morphology and performance are linked, and there is a link to habitat, it may be that the common ancestor of divers had a particular morphology, leading to a particular performance capacity, and this enabled it to thrive in a psammophilic environment. As the desert region in the western region of southern Africa increased in size, so the psammophilic species diversified and radiated within that particular habitat. Divers' head shapes (*i.e.* sharp-edged anterior lip, long snout) facilitate head-first diving into sand, this reduces the bite force capacities. Whilst the diving species have relatively longer, higher and wider heads, a higher head does not necessarily correlate to a higher bite force in all lizards (*e.g.* anolids; Herrel & Holanova, 2008; lacertids; Kaliontzopoulou *et al.*, 2012*b*). Also, diving species have counter-sunk lower jaws useful during diving, as it reduces the resistance during burrowing and also prevents the opening of the jaws during the diving process (Wake, 1993). As a result of the counter-sinking of the jaw, the out-lever of the jaw closing mechanism (the length of the lower jaw from the quadratum to the tip of the lower jaw) is shortened, a morphological proportion that is theorized to increase bite force (Herrel *et al.*, 2001*b*). Diving *Meroles*, however, have lower bite force capacities even though they have with shorter out-levers. A lower bite force, then, may be driven by other morphological features, such as the more compressed parietal and temporal regions, which may decrease the space for muscles involved in the jaw closing in-lever mechanism (measured as the distance from the quadratum to the coronoid process on the lower jaw). In contrast, non-diving species, such as *M. knoxii*, have much longer posterior crania and therefore they may have much larger jaw adductor muscles, facilitating a larger biting capacity. As to the reason why diving species have evolved more compressed posterior crania, perhaps a more robust cranial structure is necessary to withstand the forces exerted during diving, which led to the compression of the crania. Another consideration would be to investigate the shape of the crania in three-dimensions, as a wedge-shaped or arrow-shaped head would be more beneficial in sand-diving than a round head. Such speculation, however, requires evidential support, and investigations into forces experienced by sand-diving reptiles would clarify possible evolutionary changes in phenotypic expression directly facilitated by the arid environment.

Conclusion

In previous chapters, the bauplans of the southern African lacertid lizards were seen to be linked with the environment (habitat openness), and with the variety of prey items consumed in *Nucras*. In this chapter, it was shown that particular predator escape strategies are linked with cranial shapes as well as bite performance in *Meroles*. Adaptation to the sparsely vegetated, hot desert habitat in an ancestral species of the *Meroles* led to an adaptive radiation within the genus. Thus, the morphologies and performance capacities were linked with the environment, but were not phylogenetically independent. Whilst this genus provides another example of interspecific phenotypic differences being linked with the environment, the next chapter will explore the morphological differences between populations in a wide-ranging species.

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Pedioplanis lineocellata in Rooipoort Nature Reserve, South Africa
Photo by Krystal Tolley

Chapter 4

**DISPARITIES BETWEEN MORPHOLOGY AND PHYLOGENY IN A
WIDE-RANGING LACERTID SPECIES *PEDIOPLANIS*
LINEOCELLATA.**

DISPARITIES BETWEEN MORPHOLOGY AND PHYLOGENY IN A WIDE-RANGING LACERTID SPECIES *PEDIOPLANIS LINEOCELLATA*.

Introduction

The environment that a species inhabits places selective pressures on the species to express particular phenotypes, and the resultant morphological forms may not be reflective of the genetic relationships between taxa (*e.g.* convergent morphologies in rock-dwelling lizards; Revell *et al.*, 2007). Whilst the environment influences the bauplans of lizards at an interspecific level (*e.g.* Chapters 1, 2 and 3; Revell *et al.*, 2007; Hopkins & Tolley, 2011; Edwards *et al.*, 2012; Mahler *et al.*, 2013), phenotypic differences between populations within a species can also arise. In chameleons, ecotypes have been found in a species that occupies two different habitats, which exhibit markedly different external phenotypes, but are genetically indistinct (*Bradypodion pumilum*; Hopkins & Tolley, 2011). Indeed, the patterns of morphological adaptation found in the Greater Antillean anoles, one of the classic examples of adaptive radiation, has been shown to be mirrored within the brown anole (*Anolis sagrei*; Calsbeek *et al.*, 2007). The environment, therefore, may play a stronger role in shaping the phenotypes of lizards, than ancestry.

A wide-ranging, southern African lacertid species *Pedioplanis lineocellata* was chosen to examine the possible congruence between the intraspecific morphology and genetics, and macrohabitat. This species consists of three largely allopatric subspecies: (1) *P. l. lineocellata* from the savannah biome, grassland biome and Nama Karoo biome (South Africa, Botswana, Namibia and Zimbabwe), (2) *P. l. pulchella* from the western succulent Karoo biome, (South Africa), parapatric with *P. l. lineocellata* in the Nama Karoo biome (South Africa and Namibia) and north to the desert biome (Namibia), plus an isolated population in the savannah biome in Limpopo Province, (South Africa) and (3) *P. l. inoCELLATA* from a relatively smaller geographic area within the Luderitz region in the southern Namib desert (Fig. 4.1). The subspecies were distinguished from one another on morphological bases, including colour and pattern differences (FitzSimons, 1943; Mayer, 1989; Branch, 1998). The taxonomic relationships between the subspecies are, however, uncertain. Previous work on this group could not separate the subspecies morphologically, the authors concluded that *P. l. pulchella* should be synonymised with *P. l. lineocellata* (De Waal, 1978). Conversely, the three subspecies have been suggested to be raised to full species status on ecological and morphological bases (Jacobsen, 1989; Branch & Bauer, 1995; Bauer & Branch, 2001), however no published taxonomic review has been conducted to date. All three taxa were included in molecular phylogenetic analyses of the *Pedioplanis* genus (Makokha *et al.*, 2007; Conradie *et al.*, 2012), however as only a few individuals were included for each subspecies, the genetic relationships between the members of the *P. lineocellata* species complex, and the geographic limits of the ranges of each taxon, are unclear, because the taxa were paraphyletic in the phylogeny, suggesting that the species complex is not yet well understood.

Given the potential for taxonomic confusion, and that other studies show that directional selection on morphology as a result of habitat, resulting in diverse morphologies. The main aim of this study was to investigate whether morphology of the wide-ranging species *Pedioplanis lineoocellata* is linked to the macrohabitat (i.e. biome) and whether the morphological groups are congruent with the genetic groups and/or the current taxonomic designations. Because other lacertids from southern Africa show strong directional selection on morphology in relation to habitat, *Pedioplanis lineoocellata* occurring in different biomes were expected to differ morphologically, irrespective of genetic relationships. If directional selection is acting at the species or population level within this species complex, the expected open-habitat (i.e. Nama Karoo, succulent Karoo and desert biomes) morphotype should have longer limbs and more stocky bodies and heads (as was found between lacertid species in Edwards *et al.*, 2012). In contrast, morphotypes from densely vegetated habitats (i.e. fynbos and grassland) should have shorter limbs with more slender bodies and elongated heads. In addition, if these biomes correspond with barriers to gene flow, the morphology should reflect genetic patterns. To investigate these hypotheses, phylogenetic and phylogeographic analyses were used to examine the genetic patterns of the *P. lineoocellata* species complex from across the range of the complex using two mitochondrial markers. The phylogeny was used to examine whether current taxonomic designations are valid and to identify the phylogeographic patterns within the species complex. Morphometric characters relating to body, limb and head dimensions were investigated using linear morphometric analyses, and to examine if morphological groupings were more closely related to genetic patterns or to the environment (vegetation biome categories).

Materials and Methods

DNA extraction and sequencing

For the purposes of this study, the two species and one subspecies of the complex will be treated as three separate taxonomic groups. Tissue samples (tail or liver tissues) were collected from individuals in the field and stored in 95-100% ethanol. Genomic DNA was isolated from the stored tissue using a standard salt-extraction protocol (Bruford *et al.*, 1992).

Standard PCR procedures were utilized in order to amplify two partial mitochondrial gene fragments (16S and ND4), and details of the primer pairs used for the analyses are listed in Table A1. The two gene fragments were chosen as they are fast-evolving gene regions, with one being a coding region (ND4), and have been used successfully in population genetics studies previously (e.g. Pinho *et al.*, 2007). The 25µl PCR mixes contained approximately 50ng genomic DNA, thermophilic buffer (50mM KCl, 10mM Tris-HCl, pH 9.0), 1.5mM MgCl₂, 0.2µM of each primer, 0.2mM dNTPs, and 0.025 U/ll Taq polymerase (SuperTherm Taq; Southern Cross Biotechnologies). The PCR cycling profile was as follows: initial denaturing step at 94°C for 4 min, followed by 35 cycles of 94°C for 30s, 50-55°C for

Patterns and processes of adaptation in lacertid lizards to environments in southern Africa

30s, and 72°C for 45s, with a final extension at 72°C for 8 min. Sequencing of the PCR products was done by Macrogen (Seoul, Korea), using the forward primers in all cases. Sequences were aligned using Clustal Omega v.1.1.0 (Sievers *et al.*, 2011) and checked in BioEdit Sequence Alignment Editor v. 7.0.5.2 (Hall, 1999). Details of the samples and EMBL accession numbers are provided in Appendix A (Table A2).

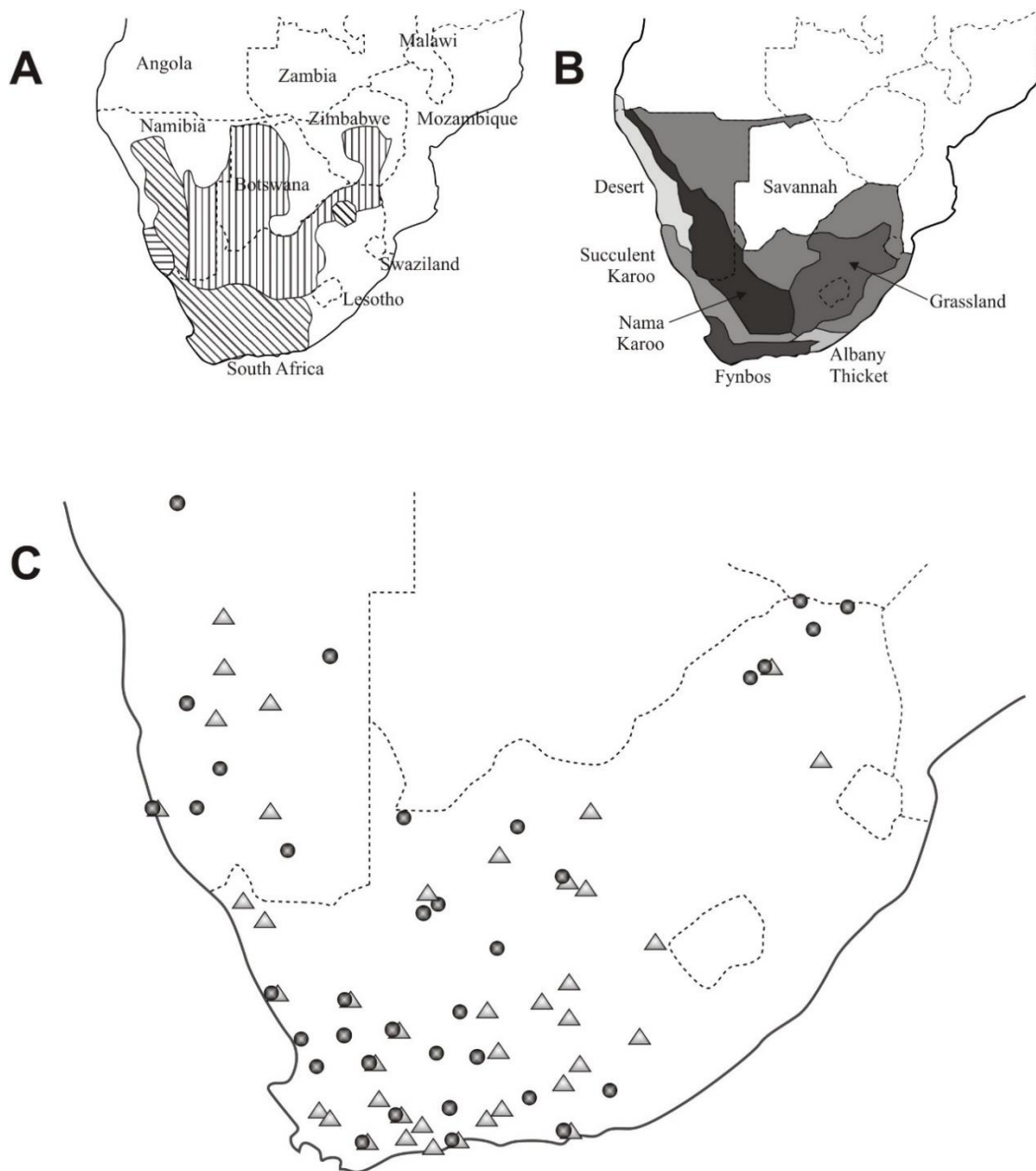


Figure 4.1 Maps of southern Africa showing (A) the country boundaries and the distributions of the currently described subspecies (vertical bars = *Pedioplanis lineoocellata lineoocellata*, diagonal bars = *P. l. pulchella*, horizontal bars = *P. l. inocellata*), (B) the biomes across the area sampled (South Africa and Namibia) and (C) the sampling localities for the molecular (circles) and morphological (triangles) analyses. Species distributions are adapted from Branch (1998) and biome boundaries adapted from Mucina & Rutherford (2006).

Phylogenetic analyses

To investigate whether the described species are monophyletic, phylogenetic analyses were carried out using 83 individuals of the three taxa complex (*P. lineocellata*, *P. inocellata*, and *P. pulchella*), from 35 localities across the distributions (Fig. 4.1), of which eight sequences were available on GenBank (<http://www.ncbi.nlm.nih.gov>). A partition homogeneity test (Farris *et al.*, 1994, 1995) was implemented in PAUP* v4.0b10 (Swofford, 2002), and no conflict was found between the two markers. Individuals from three related species (*P. breviceps*, *P. inornata*, and *P. namaquensis*) were used as outgroup taxa, as they are sister taxa to *P. lineocellata* species complex (Makokha *et al.*, 2007; Conradie *et al.*, 2012).

Phylogenetic trees were constructed of the combined total evidence molecular dataset. Bayesian inference (BI) was performed with uniform priors for all parameters (MrBayes v.3.1.0; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Evolutionary models best fitting the individual marker datasets (16S gene fragment and the ND4 gene region was GTR+I+G) and model priors were set accordingly (jModeltest v.2.1; Posada, 2008). Two parallel runs for 10 million generations each were run for the MCMC, with trees sampled every 1000 generations. The number of generations to discard as burn-in was determined by examining the number of generations (1) at which the standard deviation of split frequencies stabilized (at less than 0.001), (2) at which the log-likelihood tree scores reached stationarity, and (3) the effective sample sizes (ESS) of all parameters that were >200, though the ESSs of all parameters were above 1000 (Tracer v.1.5; Rambaut & Drummond, 2007). A 50% majority rule tree was constructed with the burn-in excluded using the “sumt” command in MrBayes, and nodes with ≥ 0.95 posterior probability were considered supported. A partitioned maximum likelihood (ML) analysis was also run (RAxML v.7.2.8; Stamatakis, 2006) using the same partitions as the Bayesian analysis, a GTR+I+G model of evolution, and automatic halting of bootstrapping, with 1000 bootstrap replicates (Stamatakis, 2006; Stamatakis *et al.*, 2008). To test the validity of current species designations, a Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) was performed to compare the consensus tree with a tree where the individuals were constrained according to their current species designations (package: ‘phangorn’, functions: ‘as.phyDat’, ‘optim.pml’, ‘pml’, and ‘SH.test’; R Studio, 2012). To investigate the level of genetic diversity between and within the clades obtained, sequence divergences were estimated using net uncorrected p-distances in MEGA v.5 (Tamura *et al.*, 2011).

Morphometric analyses

To investigate whether the morphological aspects of the *P. lineocellata* species complex are congruent with the genetic relationships between the species or correspond with the macrohabitat (defined using vegetation biome boundaries), linear morphometric analyses were performed on 163 individuals from 42 localities across the species' distributions (Table A8). Measurements taken using digital callipers on the body, head and limbs were: body length from snout-tip to anal opening (SVL), body width at the middle of the trunk (BW), body height at the same point as the body width (BH), femur length (FM), tibia length (TB), humerus length (HM), radius length (RD), head length (HL), head width at the widest part of the temporal region (HW), head height of the posterior part of the cranium (HH), and lower jaw length (LJL) (as in Fig. 2.1). Unless otherwise specified, all analyses were performed in the program R Studio v.0.97.248 (R Core Team, 2012; R Studio, 2012). To eliminate the effect of size, the \log_{10} -transformed absolute measurements were regressed onto the \log_{10} -transformed geometric means of the relevant subset of the body dimensions (head, limbs or body), using a linear model (package: 'stats', functions: 'lm' and 'resid'; RStudio, 2012).

To investigate the congruency between the genetic lineages and the morphological groups, the size-corrected residuals were used to estimate the number of morphological clusters by partitioning around κ -medoids (similar to κ -means clustering; package: 'cluster', functions: 'pam' and 'pamk'; R Studio, 2012). The size-corrected residuals were also used in an exploratory factor analysis (SPSS v.15; SPSS, Inc.), to investigate the proportion of variation explained by each relative measurement. After the correlation matrix, which was the primary data for the factor analysis, was generated, it was inspected for adequate determinant factor, sampling adequacy (Kaiser-Meyer-Olkin test) and sphericity (Bartlett's test). Those factors that had eigenvalues ≥ 1.0 were extracted for further analyses using a principal components analysis (PCA) and rotated using a varimax rotation. To ensure that the data is not biased due to intersexual differences, an analysis of variance (ANOVA) was done using the PC scores using the sexes a fixed factor (package: 'stats', functions: 'lm' and 'anova'; R Studio, 2012). If there were no significant differences in the PC scores between the sexes within each subspecies, three tests were conducted, using ANOVAs on the PC scores, with fixed factors 1) the currently described subspecies, 2) the genetic clades obtained in this study, and 3) the morphological clusters obtained by the clustering analysis.

Results

Phylogenetic analyses

The phylogenetic topology obtained showed that the *P. lineocellata* species complex consists of four well-supported clades (Fig. 4.2). The SH-test indicated that the obtained tree was the best fit tree ($P = 0.49$), and rejected the tree in which the individuals were constrained according to their current species

descriptions ($P < 0.001$). The clades were geographically separate: (1) Clade A was the most widespread, occurring in the savannah biome (Kalahari and the Namib desert, succulent Karoo and Nama-Karoo biomes), (2) Clade B occurs in the southern part of the Nama-Karoo biome and into the fynbos biome, (3) Clade C occurs in the Waterberg region of the Limpopo Province, and (4) Clade D occurs along the southern coast (Fig. 4.2). The sequence divergence between the clades for ND4 ranged between 3.8-14.0%, whilst those of 16S were around 1.5-2.6% (Table 4.1). The sequence divergences between Clade C and other clades were high relative to those between the other clades, and was similar to species level divergences found between other species in the genus (this study; Makokha *et al.*, 2007; Conradie *et al.*, 2012) and other southern African lacertid species (Chapters 2 and 3).

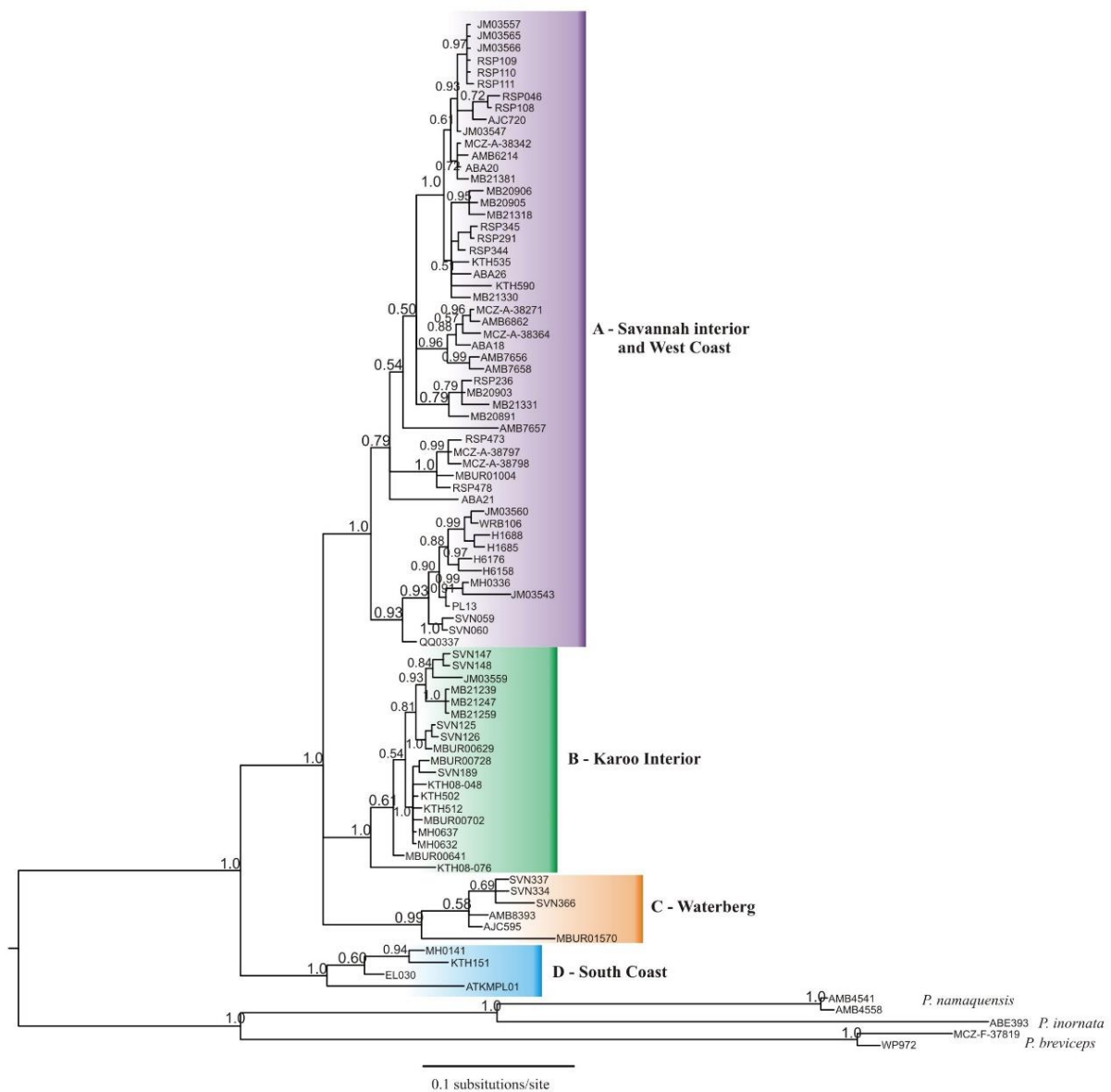


Figure 4.2: Phylogenetic tree of the *Pedioplanis lineocellata* species complex estimated using mitochondrial markers (16S and ND4) and inferred by Bayesian inference (BI) (left). Details for individuals listed in the Appendix. Bayesian posterior probabilities at the nodes are indicated.

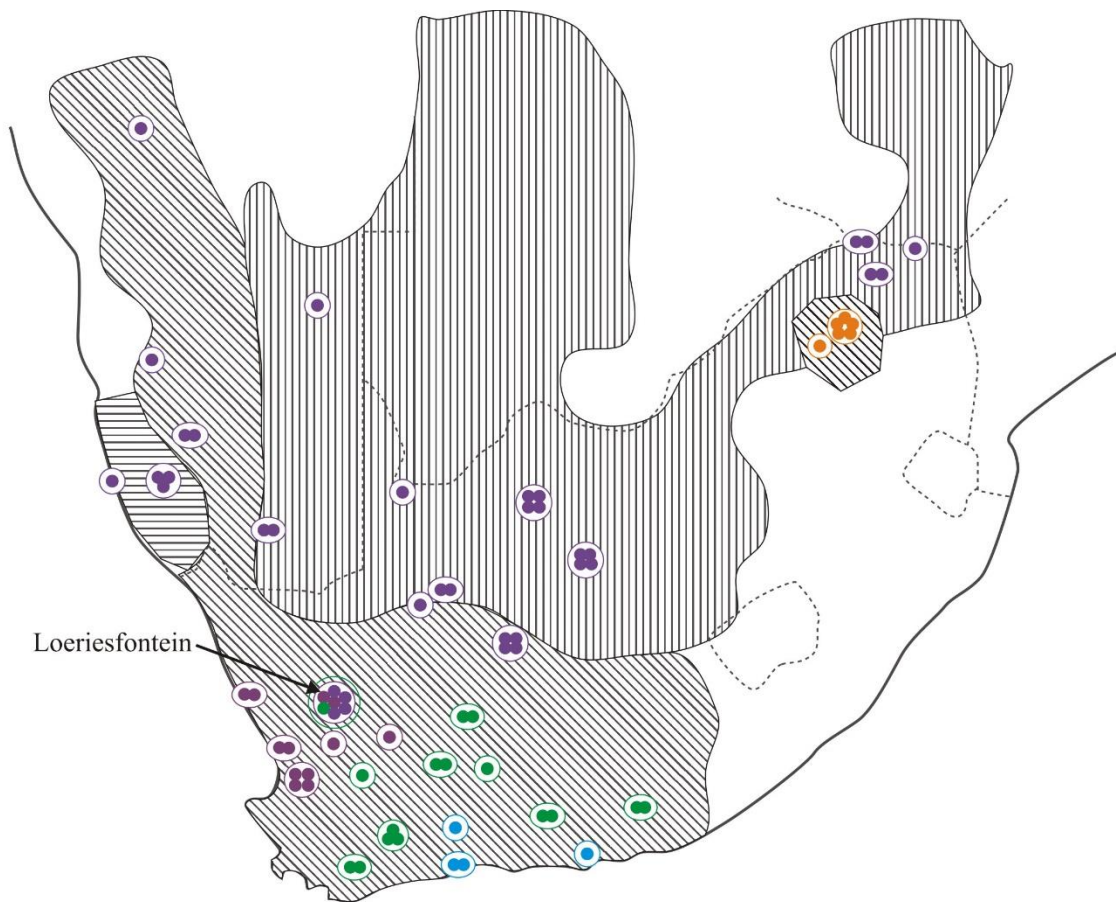


Figure 4.3: The individuals in the phylogenetic are plotted onto a map of southern Africa (right), coloured according to the clades obtained in the phylogenetic analyses (Fig. 4.2). Number of dots at each locality indicate the number of individuals sampled from that particular locality. Co-occurrence of individuals from two clades at Loeriesfontein is indicated. Distributions of the currently described subspecies (vertical bars = *P. l. lineocellata*, diagonal bars = *P. l. pulchella*, horizontal bars = *P. l. inocellata*).

Table 4.1: Net sequence divergences (p-distances) between-clades and three representative *Pedioplanis* species for the 16S (top) and ND4 (bottom) markers below the diagonal and standard errors above the diagonal, and within clade/species sequence divergences on the diagonal (shaded in grey).

16S	PB	PI	PN	Clade A	Clade B	Clade C	Clade D
PB	0.002	0.011	0.011	0.010	0.010	0.010	0.010
PI	0.066	-	0.009	0.011	0.012	0.011	0.011
PN	0.066	0.046	0.000	0.011	0.012	0.011	0.011
Clade A	0.069	0.075	0.071	0.007	0.006	0.005	0.004
Clade B	0.066	0.080	0.077	0.019	0.004	0.005	0.007
Clade C	0.062	0.076	0.072	0.015	0.016	0.006	0.006
Clade D	0.064	0.079	0.072	0.015	0.026	0.023	0.018
ND4	PB	PI	PN	Clade A	Clade B	Clade C	Clade D
PB	0.023	0.024	0.023	0.021	0.023	0.023	0.022
PI	0.216	-	0.021	0.024	0.025	0.026	0.026
PN	0.204	0.175	0.008	0.023	0.024	0.024	0.024
Clade A	0.185	0.211	0.184	0.032	0.009	0.014	0.013
Clade B	0.195	0.211	0.191	0.038	0.011	0.019	0.014
Clade C	0.232	0.253	0.230	0.079	0.105	-	0.020
Clade D	0.171	0.226	0.202	0.068	0.067	0.140	-

* Outgroup taxa: PB = *Pedioplanis breviceps*, PI = *Pedioplanis inornata*, PN = *Pedioplanis namaquensis*

Morphological analyses

The ANOVAs of the principal components scores showed that the sexes did not differ significantly in any of the first four principal components (Table 4.3), and therefore further analyses were performed on the dataset as whole. The κ -medoids partitioning analysis indicated that morphologically the *P. lineocellata* complex is divided into two clusters. In absolute terms, the clusters did not differ in body length (SVL; $F = 0.76$, $P = 0.38$). The principal components analysis extracted four PCs with eigenvalues ≥ 1.0 which explained 76.86% of the variation (Table 4.2). The first component (PC1) loaded highly with fore- and hindlimbs, the second (PC2) contrasted body width and height, the third (PC3) contrasted head width and lower jaw length, and the last (PC4) contrasted head height and length (Table 4.2). There was no significant difference for PC1, PC2 and PC4 between clades, nor for PC1 and PC2 for the subspecies. There were however significant differences in PC3 (head width and lower jaw length) for both clades and subspecies (Tables 4.2 and 4.3). The two morphological clusters differed significantly for PCs 1, 2 and 4 (Table 4.3), indicating that cluster 1 had relatively longer hindlimbs (negative PC1 scores), wider bodies (negative PC2 scores) and longer heads (positive PC4 scores), relative to cluster 2 (Fig. 4.4). The morphological clusters, therefore, do not reflect the same groupings as the clades or described subspecies distributions. The two morphological clusters were not geographically separate, with some geographically distant localities in Namibia, and south-west and east South Africa containing individuals from both clusters (Fig. 4.4). Because the two morphological clusters were sympatric, they did not reflect the biome boundaries (Mucina & Rutherford, 2006) either (Fig. 4.4).

Table 4.2: Loading values for the first four principal components analysis (PC1-4) of the relative linear measurements, with percentage of variation explained by each component shown below the loading values. Those measurements which loaded highly on each component are shown in bold font.

Relative measurements	PC1	PC2	PC3	PC4
Humerus length	0.85	-0.04	0.09	-0.25
Femur length	-0.78	0.03	-0.32	-0.04
Radius length	0.74	0.21	-0.09	0.29
Tibia length	-0.64	-0.17	0.33	0.08
Body width	-0.11	-0.96	-0.02	0.15
Body height	0.10	0.96	0.01	-0.14
Lower jaw length	0.11	-0.13	0.88	0.05
Head width	0.06	-0.16	-0.87	-0.12
Head length	-0.11	-0.03	0.17	0.88
Head height	-0.08	0.36	-0.02	-0.70
Percentage of variation	27.05	21.28	17.21	11.25

Table 4.3: Results of analyses of variance (ANOVAs) of the principal components, showing F-values and *P*-values (in brackets), using fixed factors (Treatment). Morphological clusters = clusters determined by the partitioning (clustering) of the data into 2 clusters around medoids. Significant results ($P \leq 0.05$) are shown in bold font. Degrees of freedom (Df) are indicated.

Treatment		PC1	PC2	PC3	PC4
Sex	Df=1	1.19 (0.28)	0.71 (0.40)	1.32 (0.25)	0.10 (0.75)
Subspecies	Df=2	1.51 (0.23)	2.53 (0.08)	10.22 (<0.001)	4.35 (0.01)
Clades	Df=4	1.96 (0.12)	2.58 (0.06)	10.54 (<0.001)	1.21 (0.31)
Morphological clusters	Df=1	9.93 (0.002)	167.31 (<0.001)	1.55 (0.22)	10.59 (0.001)

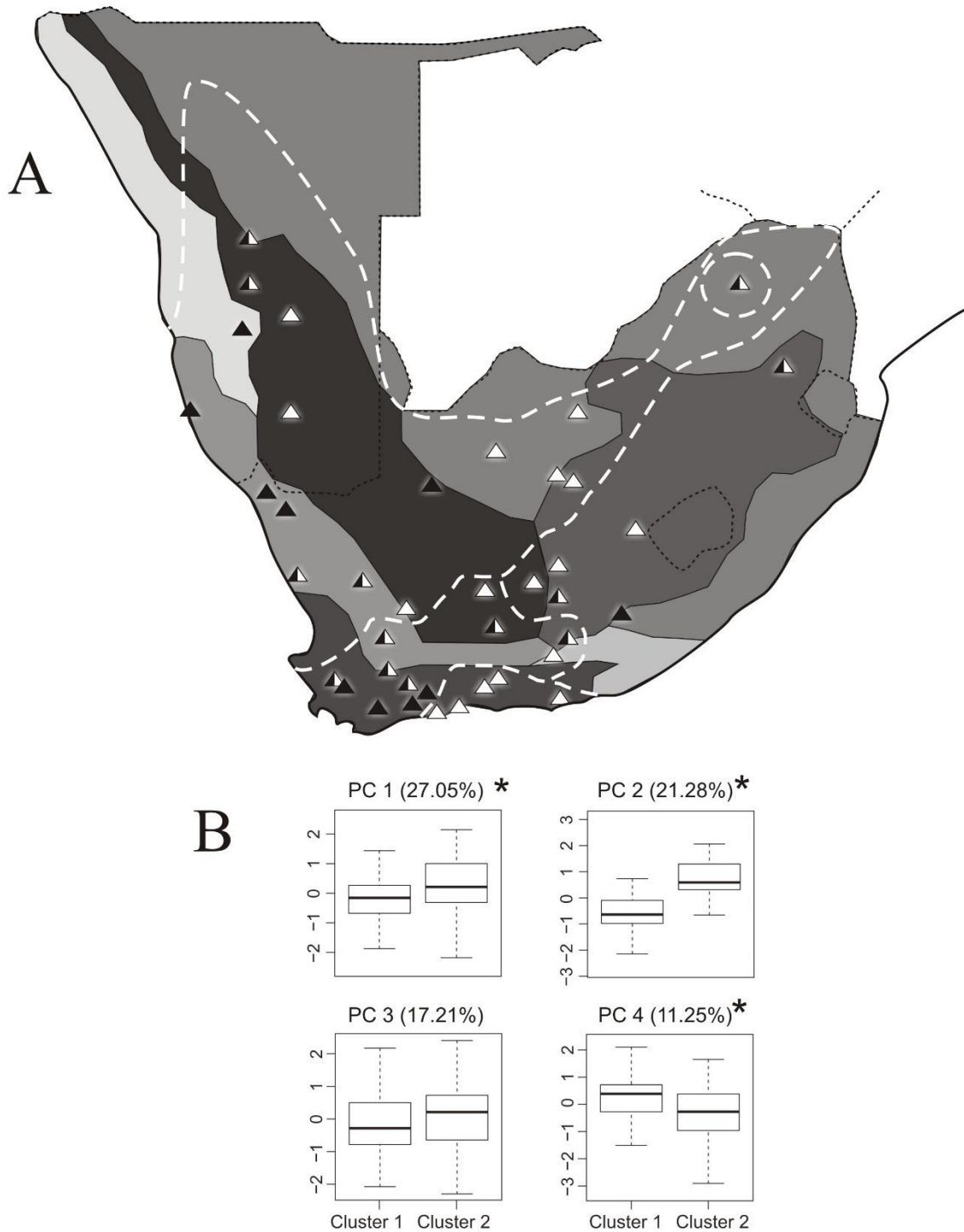


Figure 4.4: (A) Map of southern Africa indicating the localities sampled in the morphometric analyses, shaded according to the morphological clusters found using the κ -medoids (black = morphological cluster 1, white = morphological cluster 2, black & white = individuals from both clusters were found at that particular locality). Biome boundaries are indicated behind the localities, shaded as in Fig 4.1, and genetic clade boundaries are indicated by the white, dashed lines. (B) Boxplots of the first four principal components (PC), grouped according to the morphological clusters. Significant differences between the clusters are indicated by the star next to the headings for each boxplot (which also indicate the percentage of variation contributed by each PC).

Discussion

Morphological characteristics between genera in the tribe Eremiadini and between species within *Nucras* and *Meroles* were shown to be more closely linked to environmental variables (openness of habitat, dietary composition; Edwards *et al.*, 2012, 2013, in press) and behavioural aspects (predatory escape strategies; Chapter 3) than to genetic relationships. If environment is a predominant determinant of morphological shape, then the morphological aspects of wide-ranging species were expected to reflect the geographic distribution of the macrohabitats, in this case the vegetation biomes of the southern African region. However, contrary to expectations, the morphological clusters of the *P. lineoocellata* complex did not correspond to the vegetation biomes nor were they congruent with the genetic clades found. Furthermore, phylogenetic relationships between individuals sampled from across the range did not reflect the current taxonomic designations.

The topology of the phylogeny and the rejection of the tree constrained to the currently described species supports the conclusion that the currently described subspecies, based on morphology, do not have shared evolutionary histories. Although the species complex consists of four distinct genetic clades, sequence divergences between Clades A, B and D for both gene regions are somewhat lower (3.8-6.8% for ND4, 1.5-1.9% for 16S) than the values found among other species in the genus (cf. 7.2% for ND2, 1.8% for 16S), although divergences between Clade C and the other clades are similar to that found between *Pedioplanis* species (this study; Makokha *et al.*, 2007; Conradie *et al.*, 2012). Conclusions regarding clades A, B and D would be tenuous at this time, and the taxonomic level of the clades are still uncertain, as the sequence divergences are too low for species level divergence, and the phylogeny suggest that the clades are monophyletic, but with little resolution for the relationships between them. Further work that includes nuclear markers is needed to elucidate the taxonomic status of these clades. The phylogeny and the associated sequence divergences strongly suggest that Clade C constitutes a separate species, despite the lack of morphological features examined in this study. Other morphological features have distinguished individuals in the Waterberg from other *P. lineoocellata*, in that they have strongly keeled dorsal scales in comparison to the smooth or feebly keeled dorsal scales of the typical form (FitzSimons, 1943; Jacobsen, 1989). It has been found to occur on different substrates compared to the typical form, so further morphological and ecological analyses of this clade would be beneficial to elucidate whether this clade is indeed a separate species, based on characters not investigated in this study.

Whilst our analyses indicate that Clade A ranges from the western regions of Namibia and South Africa east to the Limpopo Province of South Africa, a large part of the northern distribution of *P. lineoocellata* (Botswana, Zimbabwe and eastern Namibia) was not represented in the dataset. Our sampling efforts were limited to South Africa and Namibia, and therefore further sampling is needed in Botswana and

Zimbabwe in order to identify whether populations within this region are in Clade A, or constitute a new lineage. There may be little genetic differentiation between Clade A and individuals from Botswana and Zimbabwe, as Clade A extends on both sides of Botswana and south of Zimbabwe. There is also a gap in our sampling distribution in the North-West and Free State Provinces of South Africa. Looking at historical sampling (data obtained from the South African Reptile Conservation Assessment - SARCA), very few individuals have been collected in this region of South Africa, and one reason may be that this region is very grassy, with little open gravel ground (the favoured microhabitat of *P. lineoocellata*), and so the species may be sparsely distributed in that type of microhabitat. On the other hand, they may be present within the region, but due to the thicker grass vegetation, are not easily seen and therefore not sampled as often as in other, more open, habitats. Addition of nuclear genes to these phylogenetic analyses may resolve the deeper relationships in the phylogeny, and I recommend that additional phylogenetic analyses, with increased sampling in Botswana and Zimbabwe, as well along other potential contact zone in addition to Loeriesfontein, would give a clearer picture of the clade boundaries and contact zones within this species.

The phylogeographic break at the southern regions of South Africa, separating Clade B from Clade D, has been found in a number of different taxa, including the rock hyrax *Procavia capensis* (Prinsloo & Robinson, 1992), the southern African scrub hare *Lepus saxatilis* (Kryger *et al.*, 2004), the rock elephant shrew *Elephantulus edwardii* (Smit *et al.*, 2007, 2008), the southern rock agama *Agama atra* (Swart *et al.*, 2009), Burchell's sand lizard *Pedioplanis burchelli* (Tolley *et al.*, 2009), the clicking stream frog *Strongylopus grayii* (Tolley *et al.*, 2010) and the Karoo bush rat *Myotomys unisulcatus* (Edwards *et al.*, 2011). A number of factors have been cited as the driving forces behind the limited gene flow between the south coast and the Karoo interior, including elevation (Edwards *et al.*, 2011), historic climatic shifts (Swart *et al.*, 2009; Tolley *et al.*, 2009) and the dispersal of a common haplotype from a refuge population in the south to the interior (Prinsloo & Robinson, 1992). Each explanation involves differing evolutionary selective forces acting upon the populations, ultimately resulting in limited gene flow between regions and divergences in the genetic makeup of the populations. In *P. lineoocellata*, a possible reason for the split may be that once the most recent common ancestor had differentiated from other *Pedioplanis*, the climate changed and favourable habitat contracted, leading to contraction of the populations and the formation of refuge areas. Populations underwent genetic differentiation within the refuge areas, and when the climate changed back and favourable habitat ranges expanded, the now genetically differentiated populations spread across the landscape, and came into contact again. Various areas within the southern African region have been identified as refugia during historical climatic changes (Hewitt, 2004; Lorenzen *et al.*, 2012; Barlow *et al.*, 2013). In a wide-ranging open-habitat snake species, the puff adder *Bitis arietans*, multiple regions that likely served as refugial areas through Plio-Pleistocene climatic oscillations were identified (Barlow *et al.*, 2013), but they did not reflect the refugia identified in savannah mammals (Hewitt, 2004; Lorenzen *et al.*, 2012). As was suggested by Barlow

and colleagues (2013), for a better understanding of the historical biogeography of Africa, the phylogeography of a variety of taxa from different vertebrate Orders need to be examined. Investigations using historical climate modelling and linking the dates of the habitat changes with the divergence times between lineages using molecular dating (such as those in Barlow *et al.*, 2013), may determine whether there existed refuge populations for *P. lineocellata* that expanded their distributions once the climate allowed for the expansion of favourable habitat between the refuge localities.

Neither the genetic clades nor the morphological clusters are associated with the vegetation biomes. This indicates that the morphological features examined are not linked to the macrohabitat, nor are they largely influenced by the factors driving the phylogeographic structuring of the populations. In fact, the morphological groupings are dispersed widely across the landscape and are not even geographically contiguous or coherent. One possibility is that micro-, not macro-habitat, is shaping the morphology and microhabitat can vary over the landscape in ways too subtle to be picked up by these analyses. Various factors present at the microhabitat level have been shown to influence body shape in lizards, such as local vegetation structure (Chapter 1; Edwards *et al.*, 2012) and prey composition (Chapter 2; Edwards *et al.*, in press), which can affect the body shapes and associated performance capacities. Wider heads in lacertid lizards have been linked with greater bite forces and the consumption of harder prey items (*e.g.* Herrel *et al.*, 2001a). Longer hindlimbs have been linked with higher sprinting and endurance capabilities in lizards (*e.g.* Losos & Sinervo, 1989; Losos & Irschick, 1996), and lizards that consume a higher percentage of evasive prey and employ 'sit-and-wait' foraging strategies have higher sprinting performances and longer hindlimbs (Huey & Pianka, 1981; Huey *et al.*, 1984; Garland, 1999). Thus, the differences in body width and height, limb lengths and head dimensions within populations may be due to a selective pressure exerted by a particular microhabitat to have better sprinting and biting capacities, however without interpopulation dietary, ecological and performance analyses such conclusions about the link between morphology, performance and dietary compositions within this species remain as hypotheses to be yet tested. This species would be ideal to further study the effects of microhabitat on the morphology and performance of a single, wide-ranging lizard species.

Conclusions

The *P. lineocellata* species complex consists of four genetic clades. As was previously found (Makokha *et al.*, 2007), the individuals from the Waterberg region in the Limpopo Province (Clade C) were sister to members of the clade. However, with the increased sampling within this study, an additional clade was identified along the south coastal region of South Africa. Whilst the clades are well-supported, the sequence divergences between the clades are not at a species level, and I suggest that all currently described subspecies within the complex (*P. l. lineocellata*, *P. l. pulchella* and *P. l. inocellata*) be synonymised (*P. lineocellata*) with recognition that there is phylogeographic structure, except for

Clade C, which may constitute a separate species. These genetic clades are not morphological ecotypes as morphological features do not correspond to genetic lineages or the macro-environment. Rather, the clades represent the phylogeographic structure of the species, whilst the morphological aspects investigated here are possibly influenced by factors other than shared ancestry or macrohabitat, and this has resulted in taxonomic confusion. Indeed, this effect is so pronounced that the different morphologies of the *P. lineocellata* lizards were taken to be an indication of species. Description of the *P. l. inocellata* subspecies was however predominantly done on the absence or presence of lateral blue spots (Mayer, 1989). As has been found in other lizards (e.g. *Atlantolacerta* Barata *et al.*, 2012; *Bradypodion*; Tolley *et al.*, 2004a, 2006; Measey *et al.*, 2009; Da Silva & Tolley, 2013), morphological divergence is not necessarily indicative of genetic divergence. So, I suggest that before taxonomic revision is conducted for this group, additional nuclear genes need to be included in the phylogenetic analyses, to try and resolve the polytomy within the species and to provide stronger evidence for the species level divergence of Clade C.

The southern coastal region appears to be an area of high genetic structure, with a number of taxa from different classes showing significant phylogeographic structure (Prinsloo & Robinson, 1992; Kryger *et al.*, 2004; Smit *et al.*, 2007, 2008; Swart *et al.*, 2009; Tolley *et al.*, 2009; Tolley *et al.*, 2010; Edwards *et al.*, 2011). Investigations into the phylogeographic structure within other Orders of vertebrates spanning the region would identify whether the forces that caused the divergence within lizards, amphibians and mammals have played a similar role in the phylogeographic structure of taxa with different life-history traits. Such investigations may possibly shed light on past vicariance events that may have influenced various taxonomic groups in a similar way.

If the environment places a selective pressure on individuals to express a particular phenotype in order to occupy a particular niche in that environment and to optimally survive within that niche, then a species would exhibit morphotypes. In the next chapter, the changes in morphology and performance through adaptation to a novel habitat over a short period of time will be explored.

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Meroles knoxii in Rietvlei Nature Reserve,
Photo by: S. Edwards

Chapter 5

**RAPID EVOLUTION OF MORPHOLOGY AND PERFORMANCE AFTER
AN INTRODUCTION INTO A NOVEL HABITAT IN KNOX'S
OCELLATED SAND LIZARD *MEROLES KNOXII*.**

RAPID EVOLUTION OF MORPHOLOGY AND PERFORMANCE AFTER AN INTRODUCTION INTO A NOVEL HABITAT IN KNOX'S OCELLATED SAND LIZARD *MEROLES KNOXII*.*

Introduction

The potential effects of habitat alternation on ecological systems and species has become one of the focal points in biological research, involving investigations into the possible effects on species due to anthropogenically-induced habitat alternation, climate change and associated global ecosystem shifts. Understanding how species can respond to changes should provide a basis for predicting species adaptive responses to future perturbations. One possible response to a changing environment is the movement of a species into novel habitats, as a consequence of the loss of favourable habitats (Parmesan *et al.*, 2000; Parmesan, 2006; Chown *et al.*, 2010) or through introductions (Phillips *et al.*, 2006). A novel habitat may present a species with different selective forces (*e.g.* Malhorta & Thorpe, 2000; Lehtonen & Lindström, 2004; Price *et al.*, 2008; Parker *et al.*, 2011), and in order to thrive in the new environment the species must adapt in a very short period of time. The different selective pressures may elicit new adaptive responses, including the development of different, and possibly even new, behaviours, performance capacities and morphologies (Phillips *et al.*, 2006; Yoder *et al.*, 2010). Although, phenotypic changes can develop in a very short time period in response to novel habitats, new morphological structures are not necessarily needed; merely adjustments to existing morphologies are required in order to optimally utilize a novel habitat. To date, a myriad of responses have been documented, such as changes in phenologies and thermoregulatory regimes in response to climate change, predator-evasion strategies in response to changes in predator compositions, and bauplans and signalling regimes in response to vegetation composition and density, to name a few examples (Irschick *et al.*, 2005; Visser & Both, 2005; Parmesan, 2006; Stuart-Fox *et al.*, 2007; Boyles *et al.*, 2011; Harris *et al.*, 2011; Da Silva & Tolley, 2013). Many responses are known to be coupled with changes in morphologies and performance. For example, the openness of habitat has been linked to body bauplan (Vanhooydonck *et al.*, 2002; Irschick *et al.*, 2005; Goodman, 2009; Measey *et al.*, 2009; Edwards *et al.*, 2012), as the differing densities of microhabitats in a novel environment may require a change in bauplan to optimally survive within that environment. Sprinting capacity has also been linked with limb length, with better sprinters possessing longer hindlimbs and shorter forelimbs (*e.g.* Vanhooydonck *et al.*, 2001a; Phillips *et al.*, 2006), and a novel habitat may provide a different prey availability, necessitating, for example, faster sprint speeds to capture more evasive prey. Thus limb morphologies may shift to allow for greater sprint speeds to capture the more evasive prey found in the novel habitat. Various

* In preparation: Edwards S, Tolley KA, Strauss P. Rapid evolution of morphology and performance after an introduction into a novel habitat in the lizard *Meroles knoxii* (Sauria: Lacertidae).

Patterns and processes of adaptation in lacertid lizards to environments in southern Africa

substrates have also been linked with limb lengths (*e.g.* Revell *et al.*, 2007), and a different substrate in the new environment may lead to associated changes in limb morphologies. In novel habitats, then, the new challenges presented to the organism, a new diet or new substrate type, may lead to selection for a differing performance capacity (faster sprinting speeds or better stamina capacities) and may then result in changes in morphology associated with the performance capacities.

To investigate adaptation to a novel environment in a lacertid lizard over a relatively short time period (ten years), the genetic and phenotypic aspects of an introduced population of a southern African lacertid lizard *Meroles knoxii* were compared to natural populations, including the population at the source of the introduction. This species is predominantly insectivorous and inhabits sandy substrates, including coastal dune sands, in south-western regions of southern Africa (Branch, 1998). In the year 2000, three individuals were transferred from a natural population (Phillipi, Western Cape Province, South Africa) to a man-made site (Zandvlei Nature Reserve (NR)) approximately 12km away (Fig. 5.1), where no individuals of this species were previously present. The man-made site at Zandvlei was created from dredge soils in the 1970s, and the environment was gradually established, until the year 2000, when the established habitat at Zandvlei NR was considered to be favourable for *M. knoxii*. The site was geographically close to the source population at Phillipi, and the man-made habitat appeared to be suitable (C. Dorse, *pers. comm.* 2010). Despite the small founding population size, in subsequent years the population grew and now appears to be a large and healthy population, although no population size estimates exist. This situation provides a good opportunity to investigate founder effects over a short period of time in lizards. Whilst this has been investigated previously in the brown anole *Anolis sagrei* (Kolbe *et al.*, 2012), the scenario at Zandvlei differs in that the translocated population was introduced into a completely artificial habitat, instead of an existing, rehabilitated habitat where there previously was a population. The Zandvlei site can be seen as a type of ‘ecological island’, as it is isolated from other *M. knoxii* populations by surrounding unsuitable wetland habitat, and it is unlikely that individuals from adjacent natural populations (approximately 10kms away) will have moved into the rehabilitated habitat. With the introduction of so few individuals into Zandvlei, founder effects may have led to genetic and morphological differences, compared to other populations. However, if there are differences in the habitat characteristics (such as vegetation or substrate characteristics) in the artificial habitat, directional selection of morphologies that would optimize survival in the new habitat may occur (as in *Anolis sagrei*; Kolbe *et al.*, 2012), with associated differences in performance.

To investigate whether the translocated population has adapted to the new habitat, we examined genetic composition, and both morphological and performance traits in the source and translocated populations, as well as at another natural site as a control (Rietvlei NR, Western Cape Province, South Africa). Due to the low founding population number, we expected that the translocated population at Zandvlei NR would have a low genetic diversity, compared to Phillipi and Rietvlei NR, and would be genetically

similar to individuals at Phillipi (the source). Despite this expectation, we hypothesized that if directional selection played a role due to habitat differences, we would detect differences in morphology and performance between the populations, and that running performance would be linked with these morphological differences. Whilst the sites experience similar climatic conditions, the vegetation composition and substrate potentially differs because Zandvlei site was created from dredge spoils, whilst Phillipi and Rietvlei are coastal dune sands. These differences in habitat may directly and indirectly affect the lizard morphologies due to differing vegetation structure and soil particle size.

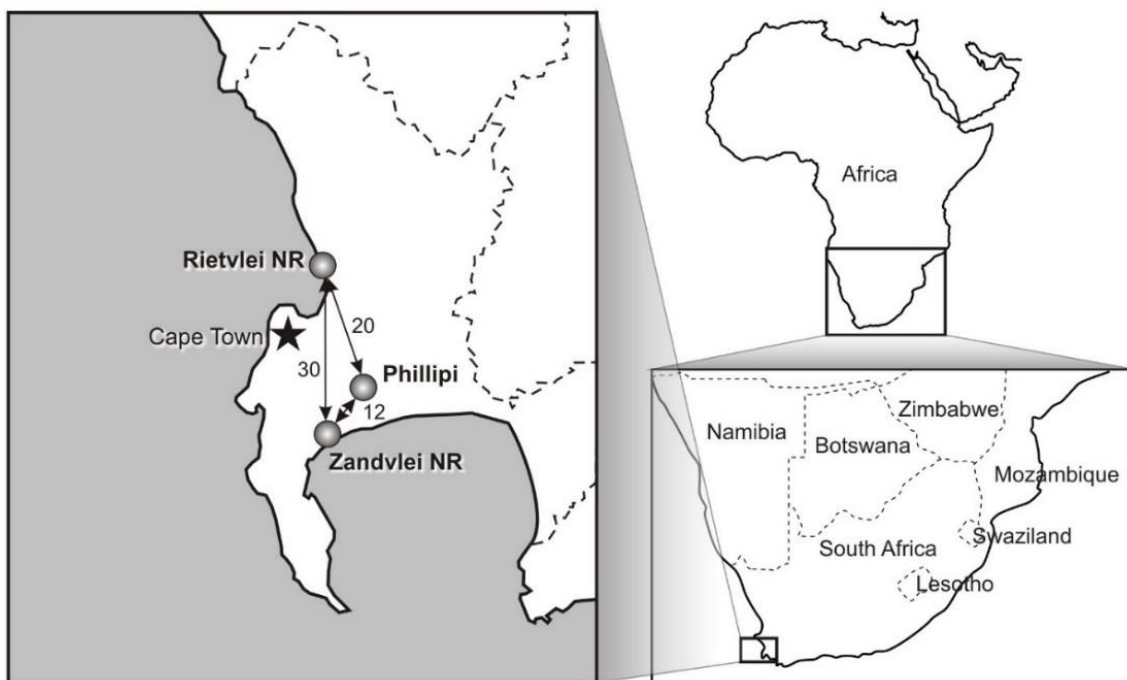


Figure 5.1: Map of the Cape Peninsula, showing the location of the three study sites: the translocated population (Zandvlei Nature Reserve (NR)), the source population (Phillipi) and the control population (Rietvlei NR). Numbers next to the arrows joining each site indicate the distance in kilometres between each site.

Materials and Methods

Sampling

The genetic and phenotypic variation between an introduced population of *Meroles knoxii* in an urban nature reserve (Zandvlei Nature Reserve; -34.092, 18.469; referred to herein as the translocated population) were compared to the original 'donor' population in Phillipi, Western Cape Province (also within the urban environment ca. 12km distant; -34.002, 18.532; referred to herein as the source population), and to another established population inhabiting a natural environment in Rietvlei Nature Reserve (ca. 30 km distant; -33.839, 18.491; referred to herein as the control population) (Fig. 5.1). Lizards were caught using pitfall traps and with noosing techniques, and after data collection they were released back at the site of capture. Tail tissue (approximately 1cm) was sampled and preserved in 95–100% ethanol for DNA analysis, after all measurements (morphological and performance) were done. All genetic DNA sampling and performance sampling matches a subset of the morphological sampling (Table A9).

Genomic DNA extraction, amplification and sequencing

Genomic DNA was isolated from tail tissue (136 individuals total; Zandvlei = 62, Phillipi = 35, Rietvlei = 39) according to standard procedures (proteinase-K digestion followed by salt-extraction; Bruford *et al.*, 1992). Standard PCR procedures were utilized to amplify the mitochondrial ND4 gene and details of the primer pairs used for the analyses are listed in Table A1. Amplification was carried out for the ND4 gene with ~25-50 ng/ μ l genomic DNA and a 25 μ l reaction containing a thermophilic buffer, 1.5mM MgCl₂, 0.2 μ M of each primer, 200 μ M dNTPs, and 0.025 U/ 1 Taq polymerase (Supertherm, Southern Cross Biotechnologies). The cycling profile began with a denaturing step at 94°C for 4 minutes, followed by 35 cycles of 94°C for 30s, 50-55°C for 30s, and 72°C for 45s, with a final extension at 72°C for 8 min. Sequencing was performed by Macrogen Corp. (Seoul, Korea), using the forward primer in all cases. The sequences were aligned in BioEdit Sequence Alignment Editor v. 7.0.5.2 (Hall, 1999). All sequences have been deposited in EMBL-Bank (Table A9)).

Population genetics analyses

A population genetics approach was used to examine the translocated population in comparison to the other two sites. Estimations of haplotype diversity (h) and nucleotide diversity (π) for each site were determined using the program Arlequin v.3.5.1.2 (Excoffier *et al.*, 2010). An analysis of molecular variance (AMOVA) was performed in Arlequin to identify whether the sites were significantly different using Φ_{ST} with the Tamura & Nei correction, using an alpha value obtained using the program jModeltest v.2.1 (Posada, 2008). Pairwise comparisons of the Φ_{ST} -values for each site were estimated. To identify whether there were haplotypes shared between the sites, a median joining haplotype network was

constructed in Network v.4.6.1.0 (www.fluxus-engineering.com; Bandelt *et al.*, 1999; Forster *et al.*, 2001; Polzin & Daneschmand, 2003).

Linear morphometric analyses

For the linear morphometrics, up to 40 adult individuals per site (~20 females and ~20 males; Table A9) were caught and linear measurements on the body were taken using digital callipers, namely the snout-vent length (SVL), the femur length (FM), the tibia length (TB), the humerus length (HM) and the radius length (RD); all measured on the right hindlimbs/forelimbs (Fig. 5.2). As the measurements on the limbs were on the external portions, and not on the internal skeletal bones, the lengths of, for example, femurs actually include part of the knee, and lengths of the tibia includes part of the metatarsals. Thus, although they are referred to herein as “femur” and “tibia” lengths, they include a portion of other skeletal features. However, as all individuals are measured in the same manner, the relative differences between individuals will still be evident. All statistical analyses were performed in R Studio v.0.97.248 (R Core Team, 2012; R Studio, 2012). The differences in the relative linear morphometric measurements between, first, sex and then sites were investigated using analyses of variance (ANOVAs) and analyses of covariance (ANCOVAs) (package: ‘stats’, function: ‘anova’; R Studio, 2012). The geometric means of the limb measurements ($\text{geom.l} = \text{FM} + \text{TB} + \text{HM} + \text{RD} / 4$) were used as covariates in the ANCOVAs (package: ‘stats’, function: ‘anova’; R Studio, 2012). ANOVAs were first performed between the sexes within the sites, which would provide an indication of whether the lizards’ body lengths (SVL) and limb dimensions differed between sexes. ANOVAs and ANCOVAs were then performed between the sites on the sexes separately.

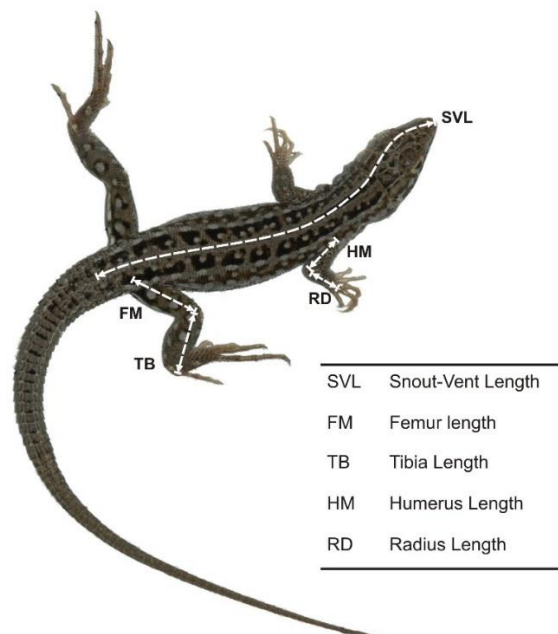


Figure 5.2: Morphometric measurements taken for lizards, shown on a photograph of *Meroles knoxii* (Zandvlei locality).

Performance analyses

Performance of the lizards from each site was measured through their sprint speed and stamina capacities in December 2011 (10 individuals per sex per site). For the sprint speed and stamina tests, the lizards were allowed to rest in an incubator at 35°C for an hour before each trial run to standardise the body temperature that the lizards ran at. The sprint speed was determined using a 2m long cork-covered racetrack with sensors placed at 25cm intervals along the track (see Vanhooydonck *et al.*, 2001 and Huyghe *et al.*, 2010 for methods). The process was repeated three times, allowing the lizards to rest for one hour between each run. The maximum of the sprint speeds were compared between sites, treating the sexes separately, using ANOVAs and ANCOVAs, using the geometric means of the all the limb measurements as covariates (package: ‘stats’, function: ‘anova’; R Studio, 2012). The maximum time spent running on the track and the maximum distances run of the three trials were compared between sites, treating the sexes separately, using ANOVAs and ANCOVAs, again using the limb geometric means as covariates (package: ‘stats’, function: ‘anova’; R Studio, 2012).

Habitat assessments

Soil samples of a 0.5m x 0.5m x 2cm sample of substrate from each chosen site was collected and weighed on a digital balance once dried out. The soil particle size was determined by sieving a 1kg sample from each site through progressively smaller sieves (sieve size range: 2mm to 63µm) and then each portion was weighed on a digital balance. The percentage retained for each portion and the cumulative percentages of the portions were calculated from the portion weights. The grain sizes for each site were plotted against the cumulative percentages in a semi-logarithmic scatterplot (producing what is known as a soil profile). The soil profiles were compared subjectively for similarity between sites. The 25th (Q1) and 75th (Q3) percentiles and the median (50th percentile; Md) for each site were determined from the plot. A sorting index (So) was obtained by the square-root of the quotient of the Q3 and Q1 ($So = \sqrt{Q3/Q1}$). A skewness index (Sk) was calculated from the product of Q1 and Q3 divided by the median ($Sk = (Q1*Q3)/Md$).

The vegetation density, percentage of cover and the amount of litter was quantified for each site, to obtain measures of habitat openness. Vegetation was assessed by randomly located 2m x 2m quadrat system (10 quadrats per site). Only plants that were rooted in the quadrats were included in the analyses, and the percentage cover and heights of the plants, as well as percentage of ground litter, were recorded. The values were compared between sites using ANOVAs (package: ‘stats’, function: ‘anova’; R Studio, 2012).

Results

Population genetic structure

The Phillipi population had the highest haplotype and nucleotide diversities ($h = 0.69 \pm 0.04$; $\pi = 1.48 \pm 0.92$; 5 haplotypes out of 35 samples), although 95% CIs for nucleotide diversities for Rietvlei overlapped with Phillipi ($h = 0.43 \pm 0.10$, $\pi = 1.28 \pm 0.84$; 8 haplotypes out of 39 samples). Zandvlei had significantly lower haplotype diversity ($h = 0.22 \pm 0.06$), with only two haplotypes out of 66 samples, but the 95% CIs for nucleotide diversity overlapped with the other two sites ($\pi = 0.43 \pm 0.40$). There was no overlap in the confidence intervals (CIs) of the haplotype diversity between any of the sites, indicating that Zandvlei had a low genetic diversity relative to the other two sites. There was a private haplotype for Zandvlei which was the most common in the population, comprising 87.5% of the sample (Fig. 5.3). The other Zandvlei haplotype was shared with Phillipi (Fig. 5.3), supporting the *a priori* knowledge that the three founding members of the Zandvlei population were taken from Phillipi.

Genetically all three populations were significantly different from each other, with AMOVA indicating that the most variation was found between the populations ($F_{CT} = 0.73$, $P = 0.01$), and approximately a third of the variation was found within the populations ($F_{ST} = 0.26$, $P < 0.0001$). Pairwise Φ_{ST} -values indicated differences are lowest between Zandvlei and Phillipi ($\Phi_{ST} = 0.66$, $P < 0.0001$), than Zandvlei and Rietvlei ($\Phi_{ST} = 0.90$, $P < 0.0001$), or Phillipi and Rietvlei ($\Phi_{ST} = 0.80$, $P < 0.0001$). Although Phillipi and Rietvlei share two haplotypes, their frequencies are largely different, which results in the significant difference detected by the AMOVA (Fig. 5.3).

Linear morphometric analyses

Within the sites, the sexes did not differ in absolute body size (SVL; Table 5.1). Within the Phillipi population, males had absolutely longer femur and radius lengths, and in Rietvlei, males had absolutely longer hindlimbs, compared to the females at each site. However, in relative terms the limb dimensions were relatively similar between the sexes at both Phillipi and Rietvlei (Table 5.1). The limb measurements within Zandvlei was contrary to the other two sites, as females had absolutely and relatively longer femur lengths (Table 5.1).

In absolute terms, Rietvlei individuals as a whole were significantly larger in body size (SVL) compared to Phillipi and Zandvlei individuals, due to the significantly larger absolute SVL of the females at Rietvlei (Table 5.1). Although different in absolute body size (SVL), females did not differ in absolute limb lengths between sites (Table 5.1; Fig. 5.4). In contrast, Rietvlei males had absolutely longer femur and tibia lengths, compared to Zandvlei males, and Phillipi males had absolutely the longest femurs.

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In relative terms, females did not differ in limb lengths between sites, whilst Zandvlei males had shorter femur lengths, compared to the other two sites, but longer tibias compared to Phillipi males and longer forelimbs compared to Rietvlei males.

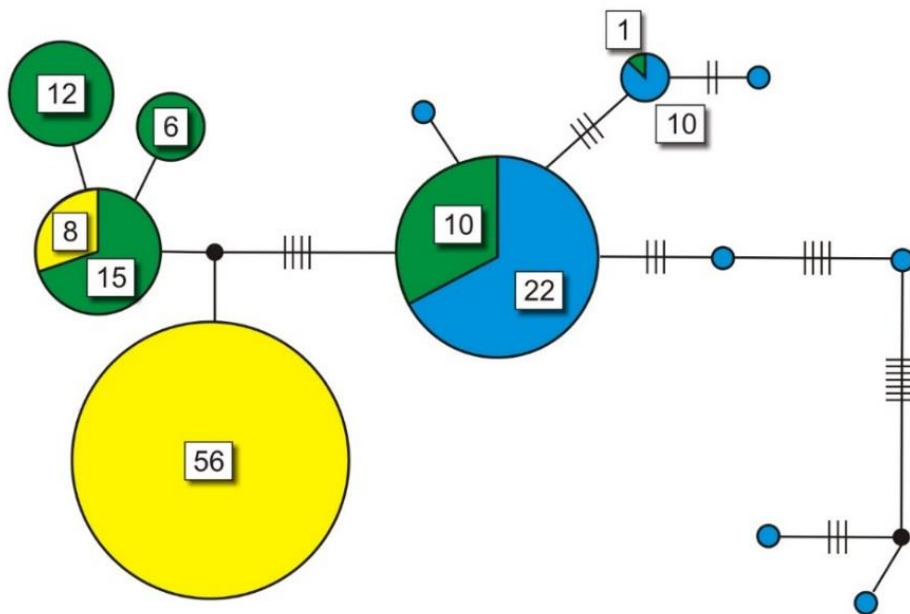


Figure 5.3: Haplotype network of the three populations of *Meroles knoxii*, with the sizes of the circles indicating the number of individuals and black-filled circles indicate unsampled haplotypes. Colours indicate locality: green = Phillipi (source), blue = Rietvlei (control), yellow = Zandvlei (introduced). Bars crossing the links indicate number of mutational steps between haplotypes.

Table 5.1: ANOVAs and ANCOVAs within populations used to test for sexual dimorphism for absolute size (SVL) and performance (bite force, sprint speed and stamina), as well as between sites to test for inter-site differences in SVL and performance, treating the sexes separately. Geometric means of limb measurements were used as covariates in the ANCOVAs. Significant values ($P \leq 0.05$) are indicated in bold font and highlighted in grey. When there was a significant difference between the sexes or sites, the particular sex/site with the larger value of the variable tested is indicated ('Larger?').

Variable	Treatment*	ANOVA			ANCOVA			
		F-value	P-value	Larger?	F-value	P-value	Larger?	
BETWEEN SEXES:								
Snout-vent length (SVL)	PH	1.24	0.27	-	-	-	-	-
	RV	1.43	0.24	-	-	-	-	-
	ZV	0.03	0.87	-	-	-	-	-
Femur lengths (FM)	PH	4.79	0.04	males	PH	0.20	0.66	-
	RV	10.53	0.002	males	RV	1.07	0.31	-
	ZV	8.90	0.005	females	ZV	7.46	0.01	females
Tibia lengths (TB)	PH	2.17	0.15	-	PH	0.55	0.46	-
	RV	5.29	0.03	males	RV	0.48	0.49	-
	ZV	0.62	0.44	-	ZV	2.87	0.10	-
Humerus lengths (HM)	PH	4.07	0.05	-	PH	0.14	0.71	-
	RV	0.72	0.40	-	RV	2.18	0.15	-
	ZV	0.15	0.71	-	ZV	1.69	0.20	-
Radius lengths (RD)	PH	5.27	0.03	males	PH	0.89	0.35	-
	RV	2.17	0.15	-	RV	0.25	0.62	-
	ZV	0.14	0.71	-	ZV	1.10	0.30	-
BETWEEN SITES (FEMALES):								
SVL	PH.RV	14.24	<0.001	RV	-	-	-	-
	PH.ZV	0.59	0.45	-	-	-	-	-
	RV.ZV	5.35	0.03	RV	-	-	-	-
FM	PH.RV	3.33	0.08	-	PH.RV	0.21	0.66	-
	PH.ZV	0.50	0.49	-	PH.ZV	0.20	0.66	-
	RV.ZV	1.27	0.27	-	RV.ZV	0.00	0.98	-
TB	PH.RV	2.99	0.10	-	PH.RV	0.02	0.90	-
	PH.ZV	0.52	0.48	-	PH.ZV	0.05	0.83	-
	RV.ZV	0.92	0.34	-	RV.ZV	0.01	0.91	-
HM	PH.RV	3.24	0.09	-	PH.RV	0.30	0.59	-
	PH.ZV	0.70	0.41	-	PH.ZV	0.08	0.78	-
	RV.ZV	0.84	0.37	-	RV.ZV	0.13	0.72	-
RD	PH.RV	1.98	0.17	-	PH.RV	0.01	0.94	-
	PH.ZV	1.03	0.32	-	PH.ZV	0.34	0.56	-
	RV.ZV	0.04	0.85	-	RV.ZV	0.27	0.61	-
BETWEEN SITES (MALES):								
SVL	PH.RV	1.01	0.32	-	-	-	-	-
	PH.ZV	0.02	0.89	-	-	-	-	-
	RV.ZV	1.38	0.25	-	-	-	-	-
FM	PH.RV	4.30	0.04	PH	PH.RV	1.09	0.30	-
	PH.ZV	16.26	<0.001	PH	PH.ZV	6.98	0.01	PH
	RV.ZV	37.50	<0.001	RV	RV.ZV	14.40	<0.001	RV
TB	PH.RV	3.39	0.07	-	PH.RV	2.22	0.14	-
	PH.ZV	2.68	0.11	-	PH.ZV	4.95	0.03	ZV
	RV.ZV	13.00	<0.001	RV	RV.ZV	0.47	0.50	-
HM	PH.RV	0.11	0.74	-	PH.RV	1.77	0.19	-
	PH.ZV	2.43	0.13	-	PH.ZV	1.42	0.24	-
	RV.ZV	3.78	0.06	-	RV.ZV	5.60	0.02	ZV
RD	PH.RV	0.04	0.85	-	PH.RV	2.64	0.11	-
	PH.ZV	2.72	0.11	-	PH.ZV	0.22	0.64	-
	RV.ZV	3.43	0.07	-	RV.ZV	4.83	0.03	ZV

* Site abbreviations: PH = Phillipi, RV = Rietvlei, ZV = Zandvlei.

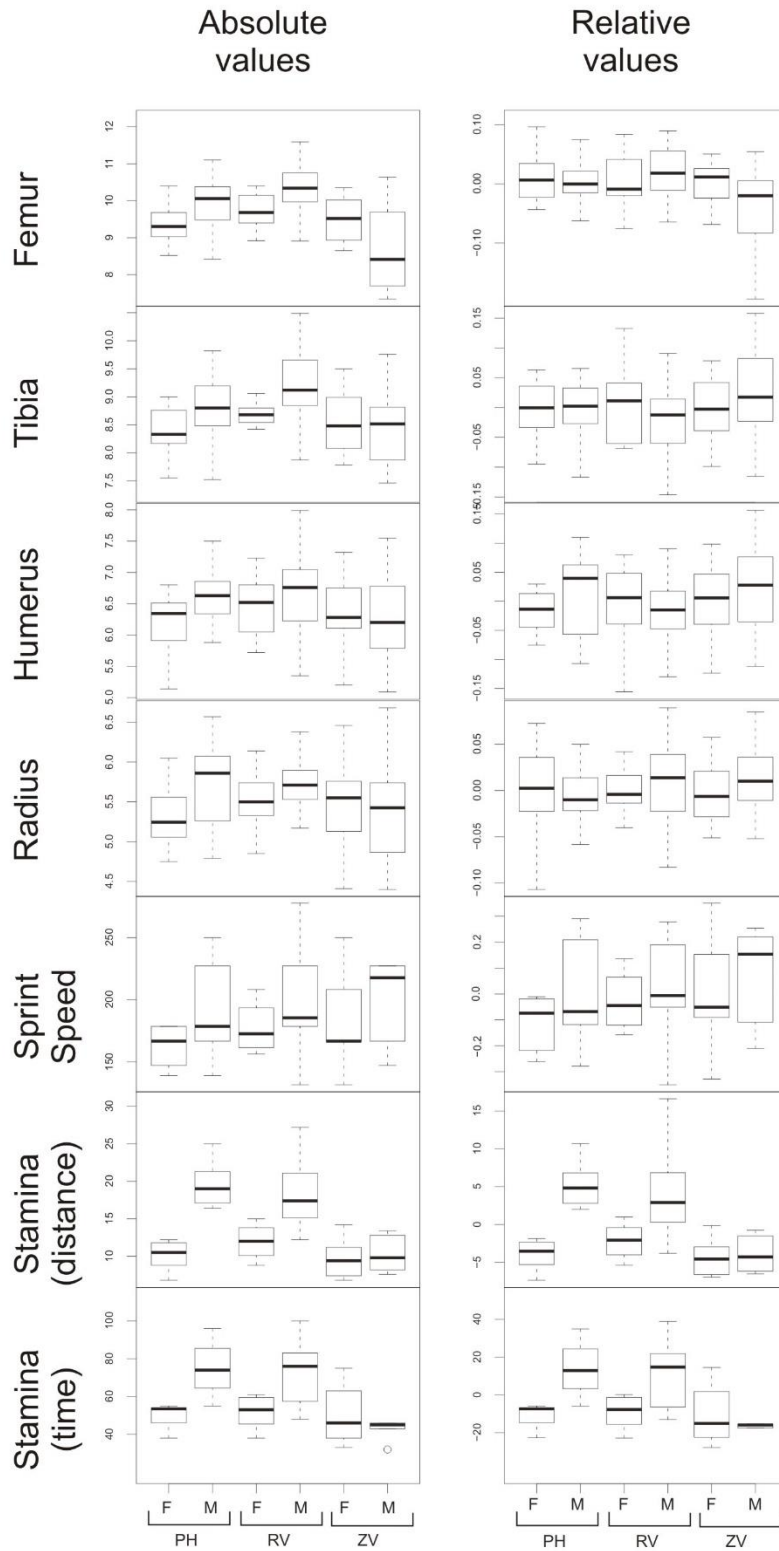


Figure 5.4: Boxplots of the absolute and relative values of linear morphometric limb measurements and the running performance capacities, categorised according to sites and sexes. Key to abbreviations: F = Females; M = Males; PH = Phillippi; RV = Rietvlei; ZV = Zandvlei.

Performance analyses

There were no significant differences in sprint speeds between the three sites for either absolute or relative values (Table 5.2). Nor were there significant differences between the sexes within in any of the sites in absolute or relative sprint speed values (Table 5.2). For stamina capacities, males within Phillipi and Rietvlei had absolutely and relatively better stamina than their female counterparts, however the sexes did not differ in their stamina capacities at Zandvlei (Table 5.2). This indicated that the males at Zandvlei had similar stamina capacities to the females at all three sites, whilst the Phillipi and Rietvlei males had similar, greater stamina capacities in comparison (Fig. 5.4).

Habitat analyses

The three sites all had soils that were within the medium soil particle size range (medians (Md): Phillipi = 0.43, Zandvlei = 0.31, Rietvlei = 0.30), were all poorly sorted (sorting index (So): Phillipi = 1.42, Zandvlei = 1.41, Rietvlei = 1.35) and were all skewed towards fine sand (skewness index (Sk): Phillipi = 0.43, Zandvlei = 0.28, Rietvlei = 0.27) (indices calculated from the quantiles in Fig. 5.5B). Phillipi, however, stood out from the other sites as being the most skewed the most towards coarser sand, due to a greater percentage of soils retained in the 425-710 μ m range (Fig. 5.5A). Rietvlei and Zandvlei were the sites with soils that were better sorted and equally skewed towards fine sand (Fig. 5.5A and 5.5B). Zandvlei, however, was the site with the least amount of large-sized soil particles (Fig. 5.5A), which is to be expected of dredge soils. On the whole, whilst there were a few differences, the soils at all sites are classified as “sand”, ranging from fine sand to coarse sand, as soils at all sites had >90% of grain sizes in the “sand” range according to the USDA (United States Department of Agriculture) soil texture classification (Rowell, 1994).

Vegetation structures at the three sites were not significantly different in terms of the percentage of litter, percentage of open sand, and the average height of the vegetation. Zandvlei did, however, have a significantly higher percentage cover of vegetation, compared to Phillipi ($F = 5.26$, $P = 0.04$) and Rietvlei ($F = 8.82$, $P = 0.01$), making the matrix through which the lizards move more dense at Zandvlei compared to the other two sites.

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Table 5.2: ANOVAs and ANCOVAs investigating differences in running performance (sprint speeds and stamina) between sexes within sites and inter-site differences of the sexes separately. Geometric means of limb measurements were used as covariates in the ANCOVAs. Significant F-values ($P \leq 0.05$) are shown in bold font and highlighted in grey, and the sex/site which significantly had the larger value is indicated (“Larger?”). Site abbreviations as in Table 5.1.

Variable	Treatment*	ANOVA			ANCOVA			
		F-value	P-value	Larger?	Treatment*	F-value	P-value	Larger?
BETWEEN SEXES:								
Sprint speed	PH	2.56	0.14	-	PH	1.58	0.24	-
	RV	1.15	0.31	-	RV	0.20	0.66	-
	ZV	0.85	0.37	-	ZV	0.63	0.44	-
Stamina (distance)	PH	37.65	<0.001	males	PH	35.78	<0.001	males
	RV	7.45	0.01	males	RV	5.55	0.03	males
	ZV	0.29	0.61	-	ZV	0.21	0.66	-
Stamina (time)	PH	14.63	0.003	males	PH	14.31	0.003	males
	RV	3.13	0.09	-	RV	2.79	0.11	-
	ZV	0.30	0.60	-	ZV	0.32	0.58	-
BETWEEN SITES (FEMALES):								
Sprint speed	PH.RV	1.31	0.29	-	PH.RV	1.22	0.31	-
	PH.ZV	1.24	0.29	-	PH.ZV	1.16	0.30	-
	RV.ZV	0.06	0.81	-	RV.ZV	0.03	0.86	-
Stamina (distance)	PH.RV	0.15	0.71	-	PH.RV	0.01	0.93	-
	PH.ZV	2.43	0.15	-	PH.ZV	1.92	0.19	-
	RV.ZV	0.93	0.43	-	RV.ZV	0.92	0.43	-
Stamina (time)	PH.RV	0.04	0.85	-	PH.RV	0.01	0.93	-
	PH.ZV	1.15	0.31	-	PH.ZV	1.01	0.34	-
	RV.ZV	0.49	0.63	-	RV.ZV	0.48	0.63	-
BETWEEN SITES (MALES):								
Sprint speed	PH.RV	0.17	0.69	-	PH.RV	0.00	0.99	-
	PH.ZV	0.11	0.75	-	PH.ZV	0.27	0.62	-
	RV.ZV	0.01	0.95	-	RV.ZV	0.39	0.54	-
Stamina (distance)	PH.RV	0.14	0.72	-	PH.RV	0.24	0.63	-
	PH.ZV	32.26	<0.001	PH	PH.ZV	31.64	<0.001	PH
	RV.ZV	13.64	0.002	RV	RV.ZV	10.61	0.004	RV
Stamina (time)	PH.RV	0.03	0.86	-	PH.RV	0.05	0.83	-
	PH.ZV	15.06	0.003	PH	PH.ZV	14.89	0.003	PH
	RV.ZV	8.30	0.01	RV	RV.ZV	7.65	0.01	RV

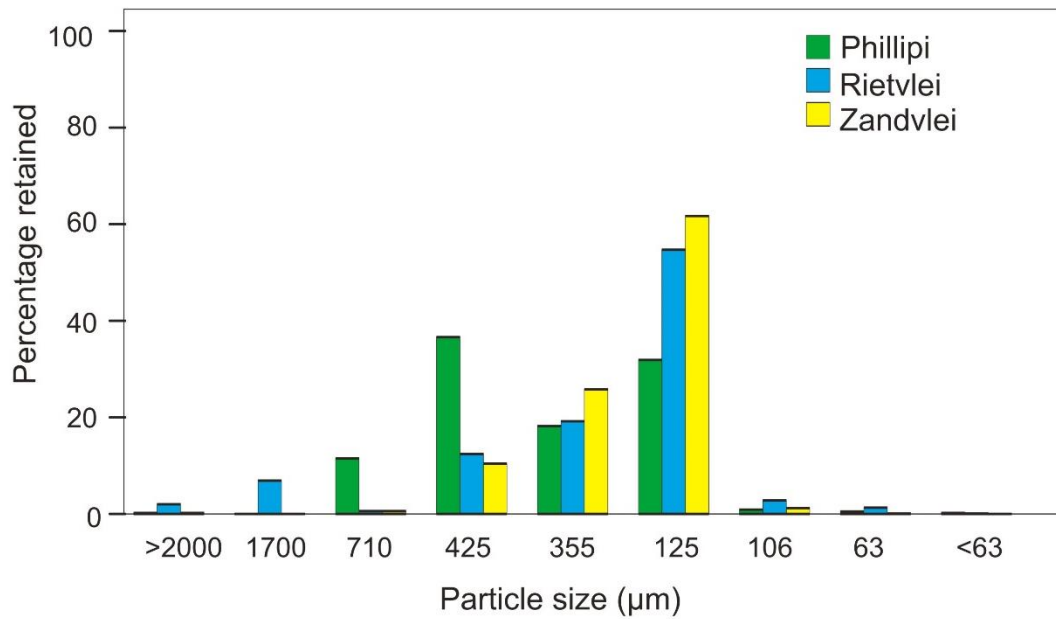
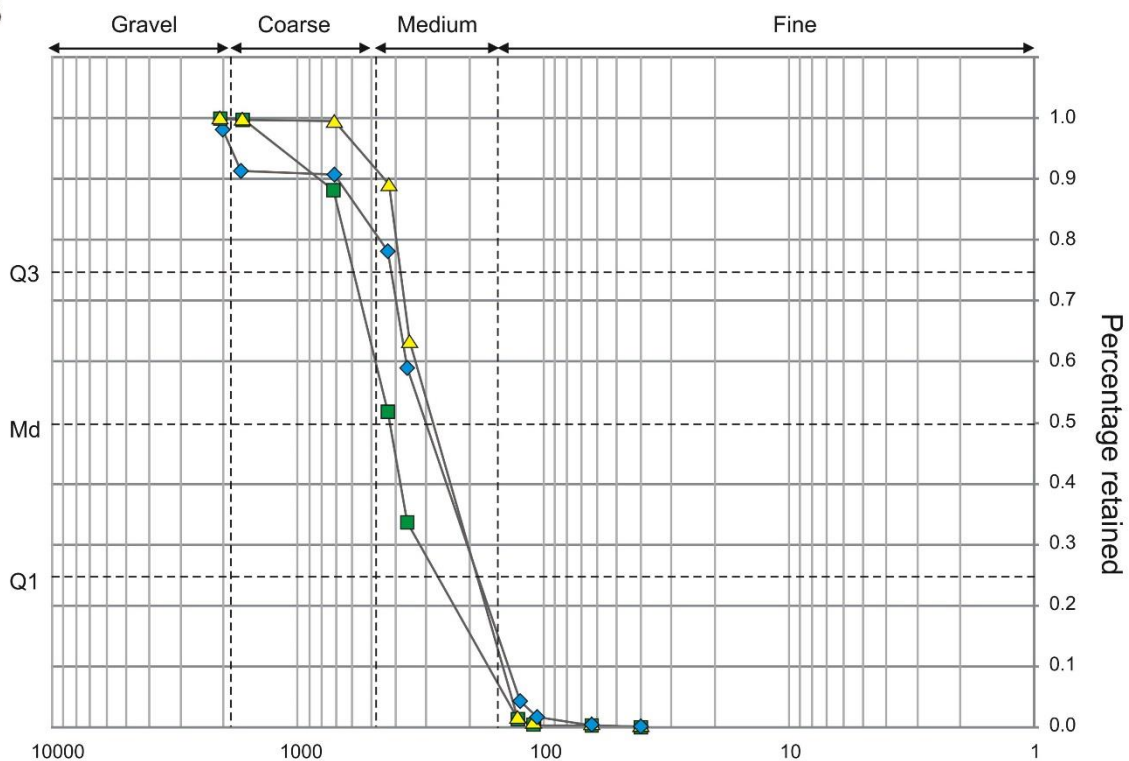
A**B**

Figure 5.5: (A) Barplots of the percentage retained for each soil particle size class for each site. Key to colours shown inset. (B) Semilogarithmic graph of the soil particle size against the cumulative percentage of each soil particle size class for the three study sites. The cut-off points for various soil particle classifications (Gravel, Coarse sand, Medium fine sand and Fine sand) is shown. Quantiles are shown (Q1 = 25%, Md = 50% (median), Q3 = 75%). Indices such as sortedness and skewness were calculated using the quantile values (see main text).

Discussion

Meroles knoxii has adapted phenotypically to the new environment at Zandvlei in a relatively short period of time after being introduced. Because there is presumably a healthy-sized population at Zandvlei, it appears that the small founding population number was not a factor in the successful colonisation of the site. This may be due to the fact that habitat at Zandvlei is not substantially different from the source population in terms of substrate structure (soil particle size) or climate (due to the small geographical distance between sites). However, the habitat at Zandvlei differs in vegetation density, which may have led to the differences in morphological dimensions and stamina capacities of the lizards at Zandvlei.

Zandvlei had the lowest genetic diversity, compared to the two other sites, likely due to the low founding population number. No information of the three translocated individuals was recorded at the time of introduction, however from photographs of the founder members they appear to be of juvenile size and colouration, and were probably not gravid females. Due to the existence of two mitochondrial haplotypes within Zandvlei, one of which is a mitochondrial haplotype shared with Phillipi (27% of the Zandvlei sample, 34% of the Phillipi sample), we can infer that the three founding individuals were most probably two females and one male. The original founding population could not have consisted of all females, nor could there have been two males and one female, as we found two maternally-inherited mitochondrial haplotypes. As the introduction site in Zandvlei is surrounded by wetlands (unfavourable habitat for *M. knoxii*), it is not surprising then that the population has a low genetic diversity, as without further translocations or immigrants from neighbouring populations the genetic pool is made up entirely from the original founding members.

The common haplotype at Zandvlei was not found in the Phillipi population with our sampling, which was unexpected. However, it could have been uncommon at Phillipi, and by chance was sampled as part of the founding population. Another possibility is that the frequency of that haplotype is now lower at the 'source' population than previously. The exact site in Phillipi where the three founding members were collected has since been transformed into agricultural lands and haplotypes could have been lost. For this study, our sampling was at an immediately adjacent site, but potentially this site may not ever have contained the haplotype that is common at Zandvlei.

Running performance involves applying force to the substrate (Lejeune *et al.*, 1998; Kerdok *et al.*, 2002) and has been shown to be influenced by substrate viscosity, rugosity (unevenness), and friction, as well as organismal traits related to locomotion. However, some lizards have sprint speeds that are not related to the substrate (Korff & McHenry, 2011), as was found for *M. knoxii*. Longer hindlimbs have been linked to better stamina capacity in other lizards (*e.g.* *Anolis sagrei* Calsbeek & Irschick, 2007), and the

relatively longer hindlimbs of Phillipi and Rietvlei males may be linked to their higher stamina capacities, compared to Zandvlei males. Thus, environmental factors other than the substrate (such as vegetation structure) may be driving the Zandvlei males to have lower stamina capacities, relative to the males at the other two sites (as in *Phrynosoma platyrhinos* Newbold, 2005). This may be due to directional selection of shorter limbs in the denser vegetation at Zandvlei, in order for the lizards to be able to manoeuvre through the denser microhabitat (as in *Niveoscincus* and *Pseudemoia*; Melville & Swain, 2000), which may in turn have led to the lower stamina in Zandvlei males.

The differences in morphology in the new habitat at Zandvlei could just be a result of founder effects, however evidence from some invasive species have also shown significant changes in morphology once in a new environment. Cane toads *Bufo marinus* are invasive in Australia, and the toads in the invasive front have evolved longer limbs, with associated increases in locomotor speed, over a short period of time (Phillips *et al.*, 2006). Thus, in *M. knoxii*, as shorter limbs are also paired with a decrease in stamina and a difference in vegetation, the differences in morphology at Zandvlei may be an adaptation to the new environment, and not just solely due to founder effects.

Conclusions

Translocations of populations to man-made habitats may be a solution to provide habitat for species (Griffith *et al.*, 1989). We show in the current study that a small founding population number led to a low genetic diversity, as is expected in colonising populations with few founders. The lizards at Zandvlei, despite the low genetic diversity of the population, have evolved morphologies and performance aspects that are linked to the environmental aspects. This suggests that adaptations to the environment in this population were not linked to the genetic relatedness of the lizards to the source population, but were driven by selective forces within the habitat at the translocation site. Questions of whether founder effects are the driving force behind changes in a newly established population, or whether natural selection plays a larger role in shaping the phenotypes of the new population, are important for conservation efforts, as understanding how animals will adapt to novel habitats will lead to better informed decisions in the future of conserving taxa that are endangered. As the lizards at Zandvlei have been shown to have adapted to the new habitat in their morphological and running performance capacities, further investigations into the cranial morphologies and dietary compositions of the lizards at each site would greatly elucidate whether the new habitat at Zandvlei has also led to differences in cranial dimensions and bite force capacities, and whether these difference can be attributed to the prey composition at the man-made site.

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Conclusions

CONCLUSIONS AND SUMMARY OF DISSERTATION

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This dissertation focussed on the effects that the environment can have on the phenotypes of the southern African lacertid lizards, and whether the differences in the phenotypes were linked with the genetic relationships between genera, between species and between populations within a species. In some cases, it was found that similar environments imposed selective pressures on the lizards to produce convergent morphologies, which has confounded historical species descriptions (as in *Meroles squamulosa* formerly *Ichnotropis* and *Vhembelacerta rupicola* formerly *Australolacerta*). Habitat openness was shown to be a deciding factor in shaping the bauplans of these lacertids (Chapter 1). Other factors have also shaped the heads of the lizards, namely the diet of the lizards, as seen in *Nucras* (Chapter 2). These shape changes were linked with performance capacities (bite force capacities). Differences in behaviour, namely the predator escape strategies, also played a role in shaping the morphologies and associated performance capacities of *Meroles* (Chapter 3). In the first two cases, morphological groups did not correspond with the genetic lineages found in phylogenetic analyses, and thus the morphologies of the different species can be thought of as adaptive. However, within a species, such as *Pedioplanis lineoocellata*, morphological aspects may be linked with local microhabitat, and not with macrohabitat or genetic relationships between populations. Factors that led to the population genetic structure within *P. lineoocellata* may not be playing as strong a role in shaping the bauplans of this species, as the two morphological groups found within this species were not geographically separate, nor were they linked with either the genetic lineage boundaries or macrohabitat (vegetation biomes) (Chapter 4). Local habitat conditions, therefore, may be playing a strong role in shaping the bauplans within *P. lineoocellata*, so assessments of niche usage within populations that contain individuals from both morphological groups would be useful in determining the role of microhabitat in shaping the morphology within this species. Indeed, it was shown that, within *Meroles knoxii*, individuals introduced into a novel habitat adapted to the new microhabitat in terms of their morphologies and performance capacities over a relatively short period of time (Chapter 5). This indicates that, despite very little genetic divergence between populations, phenotypic expression can change, and the lizards can adapt to a differing habitat, relatively quickly.

As selective pressures may act first upon the performance of the lizards, and then the morphologies are selected for that are best for optimal performance (Arnold, 1983), the investigations into the links between both morphological and performance capacities in relation to genetic relationships, and any possible links with environment, proved to be informative about the adaptive nature of the phenotypes to specific environmental factors. Understanding the link between the environment and the performance of the lizards may provide a good grounding for understanding the processes involved in shaping the

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morphologies and understanding why particular habitats drive selection for particular morphologies. As has been found previously (e.g. Revell *et al.*, 2007; Edwards *et al.*, 2012), convergent morphologies found between genetically disparate species in similar habitats may be driven by the habitat placing selective pressures on the lizards to perform in a particular way within that habitat, leading to directional selection toward similar morphologies. Thus, investigations into convergence of phenotypic traits benefit from the addition of performance analyses to morphological and genetic assessments. All in all, the performance capacities and associated morphological traits within lacertid lizards may be influenced more by the environment, than by genetic relationships, and this could lead to confusion when classifying species, which has conservation implications for this diverse group of lizards.

Taxonomic considerations

A number of taxonomic issues within the southern African lacertids need to be addressed due to the results of this dissertation. Two taxonomic revisions have been made on genetic bases, namely that *Ichnotropis squamulosa* was moved to *Meroles*, and a new genus was erected (*Vhembelacerta*) to accommodate the genetically divergent *Australolacerta rupicola* (Chapter 1; Edwards *et al.*, 2013). The analyses presented within this document constitute the first molecular phylogeny of *Nucras*, and it was interesting that the genetic relationships found between the *Nucras* individuals sampled correspond with the described species. The only classification issue within *Nucras* is the taxonomic level of *N. boulengeri*, as the sequence divergences between *N. boulengeri* and the other *Nucras* approximated the level of sequence divergences between other lacertid genera in this dissertation, and other studies (Mayer & Pavlicev, 2007; Podnar, Pinsker & Mayer, 2009). Thus, the taxonomic position of *Nucras boulengeri* needs to be investigated, but the current analyses can only be considered to be preliminary due to the small sample size. The three non-diving species within *Meroles* (*M. knoxii*, *M. squamulosus* and *M. suborbitalis*) each consist of multiple lineages that are geographically separate from one another. Whilst sequence divergence estimates indicated that the various lineages within the species were not at a species level, but rather closer to the population level, further investigations into the phylogeographical, ecological and morphological differences between the lineages would elucidate the level of divergence between the lineages, and also identify whether multiple lineages within a taxon would be better treated as one ESU. Similarly, within the *P. lineoocellata* species complex, the currently described *P. lineoocellata*, *P. pulchella* and the *P. l. inocellata* should be synonymised as one species (*P. lineoocellata*) that consists of four clades, although denser sampling could improve our understanding of the species boundaries and contact zones between lineages, and also the taxonomic status of Clade C in the Waterberg.

Historically, species have been described according to their external characteristics. Because morphology is labile and some of these characters are extremely variable within species, many species

that did not share a recent common ancestor were classified together (as in *Meroles squamulosa*, formerly *Ichnotropis* and *Vhembelacerta rupicola*, formerly *Australolacerta*; Edwards *et al.*, 2013). Species descriptions can be better informed using a multidisciplinary approach that includes genetic and morphological, and possibly also ecological, analyses, to avoid classifications that are not based on a shared evolutionary history. Species designations are tricky, at best, as evidenced by the numerous species concepts that are employed in the literature (de Queiroz, 2007). So, ultimately the use of a unified species concept would be the ideal starting point to standardise the classification of taxonomic groups (De Queiroz, 2011; Hausdorf, 2011; Vences *et al.*, 2013), and to avoid confusion leading to incorrect classifications (Kaiser *et al.*, 2013). Correct classifications have implications for conservation decisions (Frankham *et al.*, 2012) and thus the understanding of the influence that the environment has on the bauplans of organisms and their link with genetic relationships is important to accurately identify where species boundaries are.

Last thoughts...

To end off this dissertation, I would like to explore a few possibilities for future research that the current analyses have made conceivable. Firstly, the links found between performance capacities, morphology and environment indicate that the determination of species based solely upon external morphologies may lead to misclassifications, as phenotypic expression patterns between species may not mirror a shared evolutionary history. Thus, revisions of species historically described using only external traits and ecological associations are needed to elucidate the actual species boundaries and identify possible cryptic species. As such, species descriptions should be conducted using a multidisciplinary approach to accurately identify species boundaries (Padiál *et al.*, 2010).

In future dietary analyses, the inclusion of a measure of the niche breadth, in terms of the variety of prey items consumed, would add to the understanding of the links between morphology and dietary aspects within a species. Whether a species is a generalist or a specialist would determine whether the species has morphological traits that are adaptive to that particular diet. Also, the inclusion of analyses of performance (such as bite force and running capacities) would also aid in determining whether the species is specially adapted to capture and/or consume their particular type of dietary prey items.

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Life history traits also should be considered when investigating interspecific morphological trait differences, as particular behaviours necessary to optimally survive in a habitat may be driving the morphological aspects of a species (as in *Meroles*). Therefore, detailed information about a species life-history would greatly benefit functional morphological analyses and provide additional information about the underlying processes shaping the species bauplans. There remains a paucity of information on the life-history traits of many lizard species, particularly in Africa, due to lack of sampling in remote areas. Assessments of species' reproduction and behavioural traits would greatly aid in building a solid knowledge base about the patterns and processes in historical and current speciation events within the region. Investigations into the processes underlying speciation events in the past may be crucial to understanding a species' capacity to adapt to a changing climate, and in conclusion I suggest that using a multidisciplinary approach to flesh out the processes underlying speciation events will inform predictions of evolutionary changes in the herpetofauna of Africa and globally.

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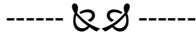
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APPENDIX A: SUPPLEMENTARY INFORMATION

Table A1: Primer sequences and gene region information, including the source from which the primer sequences were obtained.

Gene region name	Primer name	Forward (Fwd)/ Reverse (Rev)		T (°C)	Primer source
			Primer sequence		
16S	L2510	Fwd	5'-CGCCTGTTTATCAAAAACAT-3'	54	Palumbi, 1996
	H3080	Rev	5'-CCGGTCTGAACTCAGATCACGT-3'	54	Palumbi, 1996
ND4	ND4	Fwd	5'-TGACTACCAAAAAGCTCATGTAGAAGC-3'	57	Forstner <i>et al.</i> , 1995
	Leu1	Rev	5'-CATTACTTTACTTGGATTGCACCA-3'	57	Arévalo <i>et al.</i> , 1994
RAG1	RAG1-f0	Fwd	5'-AAAAGGGCTACATCCTGG-3'	52	Mayer & Pavlicev, 2007
	RAG1-R1	Rev	5'-AAAATCTGCCTTCCTGTTATTG-3'	52	Mayer & Pavlicev, 2007
KIAA-2018	KIAA 2018-F1	Fwd	5'-RCCCATCCYTACCTATGCAGCCATTA-3'	57	Portik <i>et al.</i> , 2011
	KIAA 2018-R1	Rev	5'-YTGCCCAGCCATTTGTGATATGCTYTGA-3'	57	Portik <i>et al.</i> , 2011

Table A2: List of specimens used in the phylogenetic analyses in Chapters 1, 2, 3 and 4 with genus and species names, ID numbers, Museum accession ID numbers and EMBL accession numbers for each gene. Sequence still to be added to EMBL are denoted by 'TBA'. Key to abbreviations within accession IDs underneath table.

Genus	Species	SANBI Herpbank Accession ID	Museum Accession ID*	EMBL accession number for		EMBL accession number for		EMBL accession number for		Chapter
				16S	ND4	RAG1	KIAA			
<i>Australolacerta</i>	<i>australis</i>	GW08	—	HF547772	HF547725	HF547691	HF547651	1,2		
		MH0531	—	DQ871152 ^S	HF547726	DQ871208 ^S	HF547652	1,2,3		
<i>Heliobolus</i>	<i>lugubris</i>	MCZ37870	MCZ37870	DQ871141 ^S	HF547729	DQ871199 ^S	—	1		
		MCZ37894	MCZ37894	DQ871142 ^S	HF547730	DQ871200 ^S	HF547655	1,2		
<i>Ichnotropis</i>	<i>bivittata</i>	KTH09-075	MBUR2074	HF547775	HF547731	HF547694	HF547656	1		
<i>Ichnotropis</i>	<i>capensis</i>	AMB6001	—	DQ871148 ^S	HF547732	DQ871206 ^S	HF547657	1,2,3		
		AMB6067	CAS209602	DQ871149 ^S	HF547733	DQ871207 ^S	HF547658	1,2,3		
		WP031	—	—	HF547734	HF547695	HF547659	1		
<i>Latastia</i>	<i>longicaudata</i>		—	AF080358	—	EF632229	—	2		
<i>Meroles</i>	<i>anchietae</i>	PEMR17286	PEMR17286	HF547779	—	—	—	1,3		
		WC09-011	PEMR17931	HF547780	HF547739	HF547702	HF547664	1,3		
		WP928	—	HF547781	HF547740	HF547703	—	1,3		
<i>Meroles</i>	<i>ctenodactylus</i>	AMB4632	—	HF547782	—	—	—	1		
		JM03609	—	HF547783	HF547741	HF547704	—	1,3		
		JM03611	—	—	HF547742	HF547705	HF547665	1,2,3		
		JM03613	—	HF547784	HF547743	HF547706	HF547666	1,3		
		MB20496	—	TBA	TBA	TBA	TBA	3		

Table A2: continued...

<i>Meroles</i>	<i>cuneirostris</i>	AMB4318	—	TBA	TBA	TBA	TBA	3		
		AMB5866	—	TBA	TBA	TBA	TBA	3		
		AMB6345	—	TBA	TBA	TBA	TBA	3		
		MB20484	MB20484	HF547785	HF547744	—	HF547667	1,3		
		MCZA38244	MCZA38244	HF547786	HF547745	HF547708	HF547668	1,3		
		PEMR17290	—	TBA	TBA	TBA	TBA	3		
		PEMR17291	—	TBA	TBA	TBA	TBA	3		
		WP914	—	HF547787	HF547746	HF547709	HF547669	1,3		
		WP920	—	TBA	TBA	TBA	TBA	3		
		WP921	—	HF547788	HF547747	HF547710	HF547670	1,3		
		<i>Meroles</i>	<i>knoxii</i>	AMB4705	—	TBA	TBA	TBA	TBA	3
				AMB5589	—	TBA	TBA	TBA	TBA	3
AMB5629	—			DQ871146\$	HF547748	DQ871204\$	HF547671	1,3		
ATKFMK	—			TBA	TBA	TBA	TBA	3		
ATTKMK1	—			TBA	TBA	TBA	TBA	3		
ATTKMK2	—			HF547789	HF547749	HF547711	HF547672	1,3		
H6160	—			TBA	TBA	TBA	TBA	3		
H6177	—			TBA	TBA	TBA	TBA	3		
H6179	H6179			HF547790	HF547750	HF547712	—	1,3		
SEL060	—			TBA	TBA	TBA	TBA	3		
SEL061	—			TBA	TBA	TBA	TBA	3		
SER008	—			TBA	TBA	TBA	TBA	3		
SER009	—			TBA	TBA	TBA	TBA	3		
SER017	—			HF547791	HF547751	—	—	1,3		
SVN070	—			TBA	TBA	TBA	TBA	3		
SVN071	—			TBA	TBA	TBA	TBA	3		
SVN084	PEMR18357			HF547792	HF547752	HF547713	—	1,3		
<i>Meroles</i>	<i>reticulatus</i>			AMB5921	—	TBA	TBA	TBA	TBA	3
		AMB7032	—	HF547793	—	—	—	1		
		WC09-005	PEMR17938	HF547794	HF547753	HF547714	HF547673	1,3		
		WP010	—	HF547795	HF547754	HF547715	HF547674	1,3		
		WP010	—	TBA	TBA	TBA	TBA	3		
		WP011	—	HF547796	HF547755	—	HF547675	1,3		
<i>Meroles</i>	<i>squamulosus</i>	FP264B	—	—	HF547735	HF547696	—	1,3		
		FP264C	—	TBA	TBA	TBA	TBA	3		
		MB21340	—	—	—	HF547698	HF547661	1,3		
		MBUR00872	—	TBA	TBA	TBA	TBA	3		
		MBUR00889	—	TBA	TBA	TBA	TBA	3		

Table A2: continued...

<i>Meroles</i>	<i>squamulosa</i>	RSP373	—	HF547777	HF547737	HF547699	HF547662	1,3		
		RSP374	—	TBA	TBA	TBA	TBA	3		
		RSP375	—	TBA	TBA	TBA	TBA	3		
		SVN362	—	HF547776	HF547736	HF547697	HF547660	1,3		
		WP122	—	—	—	HF547700	HF547663	1,3		
		WP125	—	HF547778	HF547738	HF547701	—	1,3		
<i>Meroles</i>	<i>suborbitalis</i>	AJC638	—	HF547797	HF547756	HF547716	HF547676	1,3		
		AMB4501	—	TBA	TBA	TBA	TBA	3		
		MB20609	PEMR16974	HF547798	HF547757	—	—	1,3		
		MB20698	—	TBA	TBA	TBA	TBA	3		
		MB21488	—	TBA	TBA	TBA	TBA	3		
		MB21589	—	HF547799	HF547758	HF547717	HF547677	1,3		
		MCZA38343	—	TBA	TBA	TBA	TBA	3		
		SVN049	PEMR18376	HF547800	HF547759	HF547718	HF547678	1,3		
		WP966	—	TBA	TBA	TBA	TBA	3		
		WP967	—	HF547801	HF547760	—	—	1,3		
		<i>Nucras</i>	<i>boulengeri</i>	JM02169	—	HG005184	HG005212	HG005233	HG005258	2
		<i>Nucras</i>	<i>holubi</i>	DM12	—	HG005185	HG005213	HG005234	HG005259	2
		MBUR00260	—	HG005188	HG005216	HG005237	HG005262	2		
		MBUR01002	—	HG005189	HG005217	HG005238	HG005263	2		
		MCZ38793	—	HG005186	HG005214	HG005235	HG005260	2		
		RSP420	—	HG005187	HG005215	HG005236	HG005261	2		
<i>Nucras</i>	<i>intertexta</i>	MB20952	—	HG005193	HG005221	HG005242	HG005267	2		
		MB21183	—	HG005194	HG005222	—	HG005268	2		
		MCZ38872	—	HG005192	HG005220	HG005241	HG005266	2		
		RSP030	PEMR18257	HG005191	HG005219	HG005240	HG005265	2		
		RSP277	—	HG005190	HG005218	HG005239	HG005264	2		
<i>Nucras</i>	<i>lalandii</i>	HB037	—	HF951554	HF951533	HF951538	HF951548	2		
		HB124	—	HF951553	HF951532	HF951537	—	2		
		HZ246	—	HF951555	HF951534	HF951539	HF951549	2		
		MB20982	—	HG005197	HG005225	HG005245	HG005271	2		
		MBUR00414	—	HG005195	HG005223	HG005243	HG005269	2		
		MBUR00483	—	HG005196	HG005224	HG005244	HG005270	2		
<i>Nucras</i>	<i>livida</i>	KTH08-071	—	HG005200	HG005227	HG005247	HG005273	2		
		MB21176	—	HG005201	HG005228	HG005248	HG005274	2		
		MB21225	—	HG005202	HG005229	HG005249	—	2		
		MBUR00670	—	HG005198	—	HG005246	HG005272	2		
		MBUR00687	—	HG005199	HG005226	—	—	2		

Table A2: continued...

<i>Nucras</i>	<i>ornata</i>	AMB8635	—	HG005206	—	HG005252	HG005277	2
		MB21672	—	HG005205	—	—	HG005276	2
		MBUR01226	—	HG005203	—	HG005250	—	2
		MBUR01230	—	HG005204	—	HG005251	HG005275	2
<i>Nucras</i>	<i>taeniolata</i>	HZ250	—	HG005207	—	HG005253	HG005278	2
		HZ251	—	HG005208	HG005230	HG005254	HG005279	2
		HZ252	—	HG005209	—	HG005255	HG005280	2
		PEMR18080	—	HG005210	HG005231	HG005256	HG005281	2
<i>Nucras</i>	<i>tessellata</i>	AMB5582	CAS 206723	DQ871143	—	DQ871201	—	2
		AMB5584	—	HG005211	HG005232	HG005257	HG005282	2
		KTH08-069	—	HF951559	—	HF951543	—	2
		MB20650	—	HF951556	HF951535	HF951540	HF951550	2
		MB20687	—	HF951557	HF951536	HF951541	HF951551	2
		MCZFS37819	—	TBA	TBA	—	—	4
<i>Pedioplanis</i>	<i>breviceps</i>	WP972	—	TBA	TBA	—	—	4
		KTH137	—	DQ871122 ^s	—	DQ871180 ^s	—	1
<i>Pedioplanis</i>	<i>burchelli</i>	MH0334	—	DQ871120 ^s	HF547761	DQ871178 ^s	HF547679	1
		<i>Pedioplanis</i>	<i>inornata</i>	ABE-393-mu	NHMW 35340:9	DQ871137 ^s	HF547762	DQ871195 ^s
<i>Pedioplanis</i>	<i>lineocellata</i>			KTH595	—	DQ871140 ^s	—	DQ871198 ^s
		ABA18	—	TBA	TBA	—	—	4
		ABA21	—	TBA	TBA	—	—	4
		ABA26	—	TBA	TBA	—	—	4
		AJC595	—	TBA	TBA	—	—	4
		AJC720	—	TBA	TBA	—	—	4
		AMB6214	—	TBA	TBA	—	—	4
		AMB6862	—	TBA	TBA	—	—	4
		AMB7656	—	TBA	TBA	—	—	4
		AMB7657	—	TBA	TBA	—	—	4
		AMB7658	—	TBA	TBA	—	—	4
		AMB8393	—	TBA	TBA	—	—	4
		ATKMPL01	—	TBA	TBA	—	—	4
		EL030	—	TBA	TBA	—	—	4
		H1685	—	TBA	TBA	—	—	4
		H1688	—	TBA	TBA	—	—	4
		H6158	—	TBA	TBA	—	—	4
H6176	—	TBA	TBA	—	—	4		
JM03543	—	TBA	TBA	—	—	4		

Table A2: continued...

<i>Pedioplanis</i>	<i>lineoocellata</i>	JM03547	—	TBA	TBA	—	—	4
		JM03557	—	TBA	TBA	—	—	4
		JM03559	—	TBA	TBA	—	—	4
		JM03560	—	TBA	TBA	—	—	4
		JM03565	—	TBA	TBA	—	—	4
		JM03566	—	TBA	TBA	—	—	4
		KTH08	—	TBA	TBA	—	—	4
		KTH08-076	—	TBA	TBA	—	—	4
		KTH151	—	TBA	TBA	—	—	4
		KTH502	—	TBA	TBA	—	—	4
		KTH512	—	TBA	TBA	—	—	4
		KTH535	—	TBA	TBA	—	—	4
		KTH590	—	TBA	TBA	—	—	4
		MB20891	—	TBA	TBA	—	—	4
		MB20903	—	TBA	TBA	—	—	4
		MB20905	—	TBA	TBA	—	—	4
		MB20906	—	TBA	TBA	—	—	4
		MB21239	—	TBA	TBA	—	—	4
		MB21247	—	TBA	TBA	—	—	4
		MB21259	—	TBA	TBA	—	—	4
		MB21318	—	TBA	TBA	—	—	4
		MB21330	—	TBA	TBA	—	—	4
		MB21331	—	TBA	TBA	—	—	4
		MB21381	—	TBA	TBA	—	—	4
		MBUR00629	—	TBA	TBA	—	—	4
		MBUR00641	—	TBA	TBA	—	—	4
		MBUR00702	—	TBA	TBA	—	—	4
		MBUR00728	—	TBA	TBA	—	—	4
		MBUR01004	—	TBA	TBA	—	—	4
		MBUR01570	—	TBA	TBA	—	—	4
		MCZA38271	—	TBA	TBA	—	—	4
		MCZA38342	—	TBA	TBA	—	—	4
		MCZA38364	—	TBA	TBA	—	—	4
		MCZA38797	—	TBA	TBA	—	—	4
		MCZA38798	—	TBA	TBA	—	—	4
		MH0141	—	TBA	TBA	—	—	4
		MH0632	—	TBA	TBA	—	—	4

Table A2: continued...

<i>Pedioplanis</i>	<i>lineoocellata</i>	MH0637	—	TBA	TBA	—	—	4
		PL13	—	TBA	TBA	—	—	4
		QQ0337	—	TBA	TBA	—	—	4
		RSP046	—	TBA	TBA	—	—	4
		RSP108	—	TBA	TBA	—	—	4
		RSP109	—	TBA	TBA	—	—	4
		RSP110	—	TBA	TBA	—	—	4
		RSP111	—	TBA	TBA	—	—	4
		RSP236	—	TBA	TBA	—	—	4
		RSP291	—	TBA	TBA	—	—	4
		RSP344	—	TBA	TBA	—	—	4
		RSP345	—	TBA	TBA	—	—	4
		RSP473	—	TBA	TBA	—	—	4
		RSP478	—	TBA	TBA	—	—	4
		SVN059	—	TBA	TBA	—	—	4
		SVN060	—	TBA	TBA	—	—	4
		SVN125	—	TBA	TBA	—	—	4
		SVN126	—	TBA	TBA	—	—	4
		SVN147	—	TBA	TBA	—	—	4
		SVN148	—	TBA	TBA	—	—	4
SVN334	—	TBA	TBA	—	—	4		
SVN337	—	TBA	TBA	—	—	4		
SVN366	—	TBA	TBA	—	—	4		
WRB106	—	TBA	TBA	—	—	4		
<i>Pedioplanis</i>	<i>lineoocellata lineoocellata</i>	ABA-20-mu	NHMW 35360:1	DQ871106 ^S	HF547763	DQ871164 ^S	HF547681	1,3,4
<i>Pedioplanis</i>	<i>lineoocellata pulchella</i>	MH0336	—	DQ871107 ^S	HF547764	DQ871165 ^S	—	1,3,4
		SVN189	—	HF547802	HF547765	HF547719	HF547682	1,3,4
<i>Pedioplanis</i>	<i>namaquensis</i>	AMB4541	—	DQ871099 ^S	HF547766	DQ871157 ^S	HF547684	1,4
		AMB4558	CAS 200033	DQ871101 ^S	HF547767	DQ871159 ^S	HF547685	1,4
<i>Philocortus</i>	<i>spinalis</i>	—	—	—	—	EF632238	—	2
<i>Pseuderemias</i>	<i>smithii</i>	—	—	—	—	EF632243	—	2

Table A2: continued...

<i>Tropidosaura</i>	<i>gularis</i>	EL036	—	HF547803	—	HF547720	HF547686	1
		RSP200	—	HF547804	HF547768	HF547721	HF547687	1
<i>Tropidosaura</i>	<i>montana montana</i>	HB082	—	HF547805	HF547769	HF547722	HF547688	1
<i>Tropidosaura</i>	<i>montana rangeri</i>	MBUR00544	—	HF547806	HF547770	HF547723	HF547689	1
		MBUR00552	—	HF547807	HF547771	HF547724	HF547690	1
<i>Vhembelacerta</i>	<i>rupicola</i>	MCZ38869	MCZ38869	HF547773	HF547727	HF547692	HF547653	1,3
		MCZ38874	MCZ38874	HF547774	HF547728	HF547693	HF547654	1

* N/A = Individuals were measured alive in the field and released, no voucher specimen deposited in a museum; TM = Ditsong museum; PEM = Port Elizabeth Museum; MCZ = Museum of Comparative Zoology, Harvard University; CAS = California Academy of Science; H = Ellerman Collection of Stellenbosch University
 \$ Makokha *et al.*, 2007.

Table A3: List of specimens used in morphometric analyses in Chapter 1, divided by genus. Accession numbers from either the Ditsong museum (TM), Port Elizabeth Museum (PEM), or field trips are shown.

Genus	Species	Number of individuals	Museum accession number
<i>Australolacerta</i>	<i>australis</i>	11	TM56019, AA02, AA03, AA04, AA06, AA07, AA09, AA10, AA11, AA12, GW08
<i>Australolacerta</i>	<i>rupicola</i>	18	FP310, HZ273, HZ278, HZ283, HZ284, T521, T522, T523, T524, T526, TM53553, TM53554, TM62696, TM62700, TM62701, TM62703, TM62704, TM62705
<i>Ichnotropis</i>	<i>capensis</i>	18	TM2201, TM2206, TM2209, TM2211, TM2213, TM4749, TM4783, TM4784, TM26871, TM26874, TM26875, TM31076, TM31077, TM31078, TM31304, TM38337, TM62754, TM79735
<i>Ichnotropis</i>	<i>squamulosa</i>	36	TM2293, TM4355, TM4552, TM4638, TM4639, TM14524, TM16822, TM16823, TM16824, TM16825, TM21542, TM21543, TM25606, TM30668, TM30823, TM30824, TM30825, TM34405, TM36061, TM61477, TM61478, TM61481, TM61485, TM61486, TM61500, TM61503, TM61516, TM61527, TM61529, TM61548, TM63137, TM80834, TM80835, TM80836, TM80837, TM80841
<i>Meroles</i>	<i>anchietae</i>	26	PEMR15913, PEMR15914, PEMR15915, PEMR17286, WP003, WP004, WP005, WP006, WP323, WP326, WP327, WP913, WP915, WP916, WP917, WP918, WP919, WP925, WP926, WP928, WP929, WP930, WP931, WP933, WP934, WP935
<i>Meroles</i>	<i>ctenodactylus</i>	17	PEMR526, PEMR2155, PEMR2156, PEMR2157, PEMR7403, PEMR11893, PEMR15810, PEMR15813, PEMR15924, PEMR16799, PEMR16800, PEMR17723, TM15772, TM15776, TM15779, TM15780, TM20986
<i>Meroles</i>	<i>cuneirostris</i>	39	PEMR2083, PEMR2084, PEMR2085, PEMR2086, PEMR2087, PEMR4848, PEMR6119, PEMR7422, PEMR7425, PEMR7426, PEMR7427, PEMR7460, PEMR7461, PEMR7463, PEMR7500, PEMR7531, PEMR7544, PEMR7557, PEMR7558, PEMR11948, PEMR15921, PEMR15922, PEMR15923, PEMR17288, PEMR17290, PEMR17291, WP001, WP320, WP322, WP324, WP325, WP912, WP914, WP920, WP921, WP923, WP924, WP936, WP937
<i>Meroles</i>	<i>knoxii</i>	80	2669, FB288, FB366, FB379, FB380, FB440, FB441, FB526, FB619, H3883, H3888, H3889, H6160, H6163, H6177, H6178, H6179, JM03397, JM03398, JM03401, JM03402, JM03403, JM03404, JM03405, PEMR525, PEMR529, PEMR2259, PEMR2260, PEMR2265, PEMR3496, PEMR5784, PEMR6711, PEMR6712, PEMR6713, PEMR6736, PEMR6737, PEMR7001, PEMR7077, PEMR7188, PEMR7545, PEMR7549, PEMR15873, PEMR15916, PEMR15917, PEMR15918, PEMR15919, PEMR15920, PEMR16797, PEMR16798, PEMR17233, PEMR17730, PEMR18351, PEMR18357, PEMR18379, SER097, SER098, SER100, SER101, SER105, SER106, SER107, SER108, SER109, SER111, SER112, SER113, SER114, SER115, SER116, SER117, SER119, SER121, SER122, SER123, SER124, SER132, SER135, SER136, SER137, SER149
<i>Meroles</i>	<i>reticulatus</i>	25	PEMR2015, PEMR2016, PEMR2017, PEMR2018, PEMR15949, PEMR15954, PEMR15956, PEMR15958, PEMR15959, PEMR15960, PEMR15963, PEMR15964, PEMR15965, PEMR15966, PEMR15967, PEMR17938, TM23959, TM23960, TM23962, TM23963, TM23990, TM23991, WP010, WP978, WP980
<i>Meroles</i>	<i>suborbitalis</i>	61	PEMR2100, PEMR2101, PEMR2103, PEMR2106, PEMR2107, PEMR2108, PEMR2109, PEMR2110, PEMR2112, PEMR3694, PEMR3696, PEMR4319, PEMR4320, PEMR4344, PEMR4345, PEMR4702, PEMR4734, PEMR4736, PEMR4737, PEMR4738, PEMR4747, PEMR4754, PEMR5065, PEMR6123, PEMR6737, PEMR7483, PEMR7484, PEMR7486, PEMR7488, PEMR11894, PEMR11919, PEMR11944, PEMR11946, PEMR15927, PEMR15928, PEMR15929, PEMR15930, PEMR15931, PEMR15932, PEMR15934, PEMR15938, PEMR15939, PEMR15940, PEMR15941, PEMR15942, PEMR18307, PEMR18376, WP012, WP013, WP014, WP015, WP016, WP017, WP963, WP966, WP967, WP968, WP969, WP970, WP971, WP976
<i>Pedioplanis</i>	<i>burchelli</i>	14	TM39736, TM39737, TM39739, TM61407, TM61409, TM61410, TM61411, TM80074, TM80083, TM39738, TM39740, TM61408, TM80071, TM80075

Table A3: continued...

<i>Pedioplanis</i>	<i>inornata</i>	18	WP939, WP940, WP942, WP943, WP944, WP945, WP946, WP947, WP948, WP949, WP950, WP951, WP952, WP953, WP954, WP955, WP956, WP962
<i>Pedioplanis</i>	<i>lineoocellata</i> <i>lineoocellata</i>	61	PEMR2128, PEMR2129, PEMR2138, PEMR2139, PEMR4415, PEMR4416, PEMR4742, PEMR4846, PEMR4847, PEMR10504, PEMR10506, PEMR10507, PEMR10508, PEMR10509, PEMR10511, PEMR10513, PEMR10514, PEMR10515, PEMR10516, PEMR10517, PEMR10527, PEMR10528, PEMR10529, PEMR10530, PEMR10531, PEMR10532, PEMR10533, PEMR10534, PEMR10632, PEMR10669, PEMR10670, PEMR10673, PEMR10674, PEMR10675, PEMR16865, PEMR16868, PEMR16869, PEMR18236, PEMR18252, PEMR18265, PEMR18286, PEMR18287, PEMR18288, PEMR18289, PEMR18297, PEMR18298, PEMR18304, TM4347, TM4348, TM4349, TM4350, TM4351
<i>Pedioplanis</i>	<i>lineoocellata pulchella</i>	30	PEMR4415, PEMR4416, PEMR6651, PEMR6652, PEMR6660, PEMR6662, PEMR6663, PEMR6664, PEMR6668, PEMR6669, PEMR6757, PEMR7063, PEMR7097, PEMR7104, PEMR8562, PEMR11220, PEMR11228, PEMR11258, PEMR11259, PEMR17231, PEMR17238, PEMR17265, PEMR17555, PEMR17856, PEMR17857, PEMR17880, PEMR17882, PEMR18230, PEMR18232, PEMR18369
<i>Pedioplanis</i>	<i>namaquensis</i>	18	TM14496, TM14497, TM25701, TM25717, TM26986, TM26987, TM27013, TM37682, TM37764, TM38942, TM49193, TM53589, TM54300, TM54317, TM54636, TM56418, TM63154, TM71483
<i>Tropidosaura</i>	<i>gularis</i>	10	TM19959, TM20174, TM20176, TM20215, TM20293, TM20294, TM20295, TM39734, TM39735, TM52522
<i>Tropidosaura</i>	<i>montana montana</i>	5	TM55618, TM55619, TM55620, TM56034, TM56035

* TM = Ditsong Museum, PEMR = Port Elizabeth Museum

^s H and FB = Ellerman collection (University of Stellenbosch), WP, FP, HZ, SER, T, AA, GW, JM = Field numbers of individuals caught during field work by SANBI staff, collaborators and myself

Table A4: List of specimens used in Chapter 2 for the morphometric analyses. Genus, species, museum and field accession numbers given, and an indication of whether the specimen was used in the linear morphometric, and geometric morphometric analyses.

Species	Linear morphometrics	Geometric morphometrics – dorsal view	Geometric morphometrics – lateral view
<i>Nucras boulengeri</i>	N=7 PEMR7147, PEMR10017, PEMR14030, PEMR16773, PEMR16780, PEMR16790, TM11913		
<i>N. caesicaudata</i>	N=8 TM28819, TM28894, TM28895, TM28954, TM28955, TM29279, TM29317, TM29467		
<i>N. holubi</i>	N=28 PEMR5079, PEMR10426, PEMR10427, PEMR10428, PEMR10430, PEMR10440, PEMR10441, PEMR10444, PEMR10445, PEMR10446, PEMR10447, PEMR10448, PEMR10449, PEMR10450, PEMR10451, PEMR10452, PEMR17430, PEMR18239 (RSP007), PEMR18240 (RSP008), PEMR18285, PEMR18290 (RSP122), PEMR18293 (RSP123), PEMR18296 (RSP121), PEMR18299 (RSP133), RSP420, WP128, WP134, WP137	N=19 PEMR5079, PEMR10427, PEMR10428, PEMR10430, PEMR10440, PEMR10441, PEMR10444, PEMR10446, PEMR10447, PEMR10448, PEMR10449, PEMR10450, PEMR10451, PEMR18239 (RSP007), PEMR18240 (RSP008), PEMR18290 (RSP122), PEMR18293 (RSP123), PEMR18296 (RSP121), PEMR18299 (RSP133)	N=20 PEMR5079, PEMR10427, PEMR10428, PEMR10430, PEMR10440, PEMR10444, PEMR10445, PEMR10446, PEMR10447, PEMR10448, PEMR10449, PEMR10450, PEMR17430, PEMR18239 (RSP007), PEMR18240 (RSP008), PEMR18285, PEMR18290 (RSP122), PEMR18293 (RSP123), PEMR18296 (RSP121), PEMR18299 (RSP133)
<i>N. intertexta</i>	N=29 PEMR8427, PEMR15970, PEMR18257 (RSP030), PEMR18258 (RSP031), TM14538, TM14958, TM28229, TM28820, TM44762, TM49438, TM57832, TM63058, TM67345, TM68838, TM68839, TM68840, TM78705, TM78706, TM78708, TM83339, TM83564, TM83566, RSP277, WP123, WP133, WP139, WP140, WP141, WP143	N=21 PEMR8427, PEMR15970, PEMR18257 (RSP030), PEMR18258 (RSP031), TM14538, TM14958, TM28229, TM28820, TM44762, TM49438, TM57832, TM63058, TM67345, TM68838, TM68839, TM68840, TM78706, TM78708, TM83339, TM83564, TM83566	N=19 PEMR8427, PEMR15970, PEMR18257 (RSP030), PEMR18258 (RSP031), TM14538, TM14958, TM28229, TM28820, TM57832, TM67345, TM68838, TM68839, TM68840, TM78705, TM78706, TM78708, TM83339, TM83564, TM83566
<i>N. lalandii</i>	N=34 HZ246, PEMR1939, PEMR2693, PEMR3043, PEMR3053, PEMR4576, PEMR7247, PEMR8055, PEMR8164, PEMR8168, PEMR13357, PEMR13358, PEMR16002, PEMR16003, PEMR16005, PEMR16007, PEMR16008, PEMR16012, PEMR16015, PEMR16016, PEMR16022, PEMR16023, PEMR16026, PEMR16027, PEMR16029, PEMR16032, PEMR16035, PEMR16036, PEMR16038, PEMR16039, PEMR16042, PEMR16492, PEMR16493, PEMR17435	N=27 HZ246, PEMR2693, PEMR3043, PEMR3053, PEMR4576, PEMR7247, PEMR8055, PEMR8164, PEMR8168, PEMR13357, PEMR13358, PEMR16002, PEMR16007, PEMR16012, PEMR16015, PEMR16016, PEMR16022, PEMR16023, PEMR16025, PEMR16029, PEMR16032, PEMR16035, PEMR16036, PEMR16038, PEMR16039, PEMR16042, PEMR16493	N=26 PEMR3043, PEMR3053, PEMR4576, PEMR7247, PEMR8055, PEMR8164, PEMR8168, PEMR13357, PEMR13358, PEMR16002, PEMR16003, PEMR16007, PEMR16012, PEMR16015, PEMR16016, PEMR16022, PEMR16025, PEMR16026, PEMR16029, PEMR16032, PEMR16035, PEMR16036, PEMR16038, PEMR16039, PEMR16492, PEMR16493
<i>N. livida</i>	N=16 PEMR542, PEMR4300, PEMR4382, PEMR4401, PEMR6547, PEMR6714, PEMR8186, PEMR8726, PEMR15531, PEMR15968, PEMR15969, TM20129, TM29997, TM36133, TM63817, TM70631		

Table A4: *continued...*

Species	Linear morphometrics	Geometric morphometrics – dorsal view	Geometric morphometrics – lateral view
<i>N. ornata</i>	N=25 PEMR5906, PEMR8421, PEMR8438, PEMR8439, PEMR8450, PEMR8478, PEMR8483, PEMR10425, PEMR10442, PEMR10453, PEMR10454, PEMR10458, PEMR10459, PEMR10463, PEMR10464, PEMR10466, PEMR10469, PEMR10470, PEMR10480, PEMR12000, PEMR12161, PEMR12162, PEMR17591, PEMR17595, PEMR17596	N=21 NANR25, PEMR5906, PEMR8421, PEMR8438, PEMR8439, PEMR8478, PEMR8483, PEMR10442, PEMR10453, PEMR10454, PEMR10458, PEMR10459, PEMR10463, PEMR10464, PEMR10466, PEMR10469, PEMR10470, PEMR10480, PEMR12000, PEMR17596	N=21 NANR25, PEMR5906, PEMR8421, PEMR8438, PEMR8439, PEMR8478, PEMR8483, PEMR10425, PEMR10442, PEMR10453, PEMR10454, PEMR10458, PEMR10459, PEMR10463, PEMR10464, PEMR10466, PEMR10470, PEMR10480, PEMR17591, PEMR17596
<i>N. taeniolata</i>	N=18 FP257, HZ250, HZ251, HZ252, HZ254, HZ256, HZ257, HZ259, PEMR4875, PEMR5075, PEMR10135, PEMR15974, PEMR15980, PEMR15983, PEMR15986, PEMR15988, PEMR17628, TM877		
<i>N. tessellata</i>	N=22 H5659, H6040, PEMR4763, PEMR4857, PEMR7070, PEMR7155, PEMR7590, PEMR7629, PEMR7681, PEMR8147, PEMR8719, PEMR11111, PEMR12410, PEMR13355, PEMR15990, PEMR15992, PEMR15993, PEMR15994, PEMR15997, PEMR16000, PEMR16872, PEMR16873,	N=17 H5659, H6040, PEMR4857, PEMR7070, PEMR7629, PEMR8147, PEMR11111, PEMR12410, PEMR13355, PEMR15990, PEMR15991, PEMR15993, PEMR15994, PEMR15997, PEMR16000, PEMR16872, PEMR16873,	N=19 H5659, H6040, PEMR4763, PEMR7070, PEMR7155, PEMR7590, PEMR7629, PEMR7681, PEMR8147, PEMR12410, PEMR13355, PEMR15990, PEMR15991, PEMR15993, PEMR15994, PEMR15997, PEMR16000, PEMR16872, PEMR16873,

* Key to accession numbers: PEMR = Port Elizabeth Museum; TM = Ditsong Museum (formerly the Transvaal Museum); RSP, HZ, FP, WP = field numbers for individuals collected by authors; H = field numbers for individuals collected by Prof. P. L. Mouton.

Table A5: List of specimens used for the performance analyses in Chapter 2. Species, sample size for performance analyses and field accession numbers given.

Species	Bite	Sprint	Individual accession ID numbers
<i>Nucras holubi</i>	5	5	RSP420, GF107, GF108, GF113, HZ603
<i>N. intertexta</i>	19	19	RSP277, 998, 999, GF154, GF176, GF202, GF218, GF221, GF253, GF279, GF286, GF287, HZ604, HZ613, HZ615, HZ619, HZ623, HZ635, 996, 997
<i>N. lalandii</i>	-	1	HZ246
<i>N. tessellata</i>	2	2	NI, 437

Table A6: Specimens used in Chapter 3 for the linear and geometric morphometric analyses.

Genus and species	Linear morphometrics	Dorsal cranial geometric morphometrics	Lateral cranial morphometrics
<i>Meroles anchietae</i>	N = 26 PEMR15913, PEMR15914, PEMR15915, PEMR17286, WP003, WP004, WP005, WP006, WP323, WP326, WP327, WP913, WP915, WP916, WP917, WP918, WP919, WP925, WP926, WP928, WP929, WP930, WP931, WP933, WP934, WP935	N = 27 PEMR15913, PEMR15914, PEMR15915, PEMR17286, PEMR17931, WP003, WP004, WP005, WP323, WP326, WP327, WP913, WP915, WP916, WP917, WP918, WP919, WP923, WP925, WP926, WP928, WP929, WP930, WP931, WP933, WP934, WP935	N = 19 PEMR15913, WP003, WP004, WP005, WP006, WP913, WP915, WP916, WP917, WP918, WP919, WP926, WP323, WP326, WP327, WP931, WP933, WP934, WP935
<i>Meroles ctenodactylus</i>	N = 21 PEMR526, PEMR2155, PEMR2156, PEMR2157, PEMR7403, PEMR11893, PEMR15810, PEMR15813, PEMR15924, PEMR16799, PEMR16800, PEMR17723, TM15772, TM15776, TM15779, TM15780, TM20986	N = 18 JM03605, JM03609, JM03611, JM03615, JM03616, JM03617, JM03622, JM03623, PEMR526, PEMR11893, PEMR15813, PEMR15924, PEMR16800, PEMR16799, PEMR17723, TM15772, TM15779, TM20986	N = 23 JM03605, JM03609, JM03611, JM03615, JM03616, JM03617, JM03621, JM03622, JM03623, PEMR526, PEMR2156, PEMR7403, PEMR11893, PEMR11933, PEMR15810, PEMR16799, PEMR16800, PEMR17723, TM15772, TM15776, TM15779, TM15780, TM20986
<i>Meroles cuneirostris</i>	N = 30 PEMR2083, PEMR2084, PEMR2085, PEMR2086, PEMR2087, PEMR4848, PEMR6119, PEMR7425, PEMR7426, PEMR7500, PEMR7531, PEMR7544, PEMR7558, PEMR11948, PEMR15921, PEMR15922, PEMR15923, PEMR17288, PEMR17290, PEMR17291, WP923, WP937, WP001, WP320, WP324, WP325, WP914, WP920, WP924, WP936	N = 31 PEMR2084, PEMR2086, PEMR4848, PEMR6119, PEMR7426, PEMR7422, PEMR7425, PEMR7427, PEMR7463, PEMR7500, PEMR7531, PEMR7544, PEMR7557, PEMR7558, PEMR11948, PEMR15921, PEMR17290, PEMR17291, WP001, WP320, WP322, WP324, WP325, WP912, WP914, WP920, WP921, WP923, WP924, WP936, WP937	N = 22 PEMR2084, PEMR6119, PEMR7422, PEMR7425, PEMR7426, PEMR7427, PEMR7500, PEMR7531, PEMR7544, PEMR7558, PEMR11948, PEMR17288, WP322, WP924, WP923, WP320, WP920, WP325, WP914, WP324, WP937, WP001

Table A6: continued...

<i>Merolles knoxii</i>	N = 33 2669, FB619, H6160, H6163, H6177, H6178, H6179, JM03397, JM03398, JM03401, JM03402, JM03403, JM03404, JM03405, PEMR525, PEMR529, PEMR2259, PEMR3496, PEMR6711, PEMR6712, PEMR6713, PEMR6737, PEMR7188, PEMR15873, PEMR15918, PEMR15919, PEMR15920, PEMR16797, PEMR16798, PEMR17730, PEMR18351, PEMR18357, PEMR18379	N = 81 2669, FB379, FB380, FB440, FB288, FB261, FB619, FB27, FB441, H3883, H3888, H3889, H3270, H3271, H3272, H3269, H6160, H6163, H6165, H6177, H6178, H6179, H6196, JM03397, JM03398, JM03401, JM03402, JM03403, JM03404, JM03405, JM03606, JM03608, JM03610, JM03618, JM03619, JM03620, PEMR529, PEMR2259, PEMR2260, PEMR2263, PEMR2265, PEMR3497, PEMR5784, PEMR6711, PEMR6712, PEMR6736, PEMR6737, PEMR7001, PEMR7077, PEMR7188, PEMR15917, PEMR15918, PEMR15919, PEMR15920, PEMR16796, PEMR16797, PEMR16798, PEMR17233, PEMR17730, PEMR18351, PEMR18357, SER107, SER108, SER109, SER110, SER111, SER113, SER114, SER115, SER116, SER117, SER121, SER122, SER123, SER124, SER132, SER132, SER135, SER136, SER137, SER149	N = 65 FB27, FB288, FB366, FB380, FB440, FB441, FB526, FB619, H3883, H3888, H6160, H6163, JM03397, JM03398, JM03401, JM03402, JM03404, JM03606, JM03608, JM03610, JM03612, JM03619, JM03620, PEMR525, PEMR529, PEMR2259, PEMR2265, PEMR5784, PEMR7001, PEMR7188, PEMR15873, PEMR15918, PEMR15919, PEMR15920, PEMR16797, PEMR16798, PEMR17233, PEMR18351, SER108, SER109, SER110, SER111, SER113, SER114, SER115, SER116, SER117, SER121, SER122, SER123, SER124, SER132, SER136, SER137, SER149
<i>Merolles micropholidotus</i>	N = 6 TM33034, TM44298, TM44773, TM53018, TM53061, TM53062	N = 8 TM33034, TM44298, TM44299, TM44773, TM51343, TM53018, TM53061, TM53062,	N = 8 TM33034, TM44298, TM44299, TM44773, TM51343, TM53018, TM53061, TM53062,
<i>Merolles reticulatus</i>	N = 25 PEMR2015, PEMR2016, PEMR2017, PEMR2018, PEMR15949, PEMR15954, PEMR15956, PEMR15958, PEMR15959, PEMR15960, PEMR15963, PEMR15964, PEMR15965, PEMR15966, PEMR15967, PEMR17938, TM23959, TM23960, TM23962, TM23963, TM23990, TM23991, WP010, WP978, WP980,	N = 26 PEMR2015, PEMR2016, PEMR2017, PEMR2018, PEMR15950, PEMR15956, PEMR15958, PEMR15959, PEMR15960, PEMR15963, PEMR15964, PEMR15965, PEMR15966, PEMR15967, PEMR17938, TM23959, TM23960, TM23962, TM23963, TM23964, TM23990, TM23991, WP010, WP011, WP978, WP980,	N = 24 PEMR2015, PEMR2016, PEMR2017, PEMR2018, PEMR15948, PEMR15950, PEMR15954, PEMR15956, PEMR15959, PEMR15960, PEMR15963, PEMR15964, PEMR15965, PEMR15966, PEMR15967, PEMR17938, TM23960, TM23962, TM23963, TM23964, TM23990, TM23991, WP010, WP011,

Table A6: continued...

<i>Meroles squamulosus</i>	N = 29 TM2293, TM4552, TM4638, TM4639, TM14524, TM21542, TM25606, TM30668, TM30823, TM30824, TM30825, TM34405, TM36061, TM61477, TM61478, TM61485, TM61486, TM61500, TM61503, TM61527, TM61529, TM61548, TM61516, TM63137, TM80834, TM80835, TM80836, TM80837, TM80841	N = 33 TM2293, TM4552, TM4638, TM4639, TM14524, TM16824, TM16825, TM21542, TM21543, TM25606, TM30668, TM34405, TM30823, TM30824, TM30825, TM36061, TM61477, TM61485, TM61486, TM61500, TM61503, TM61516, TM61527, TM61529, TM61548, TM61478, TM61548, TM63137, TM80834, TM80835, TM80836, TM80837, TM80841	N = 33 TM2293, TM4355, TM4552, TM4638, TM4639, TM14524, TM16824, TM16825, TM21543, TM25606, TM30668, TM30823, TM30824, TM30825, TM34405, TM36061, TM61477, TM61478, TM61485, TM61486, TM61500, TM61503, TM61516, TM61527, TM61529, TM61548, TM61548, TM63137, TM80835, TM80834, TM80836, TM80837, TM80841
<i>Meroles suborbitalis</i>	N = 32 PEMR2100, PEMR3694, PEMR3696, PEMR4320, PEMR4345, PEMR4736, PEMR4747, PEMR6123, PEMR7483, PEMR7484, PEMR7486, PEMR7488, PEMR11944, PEMR15927, PEMR15928, PEMR15931, PEMR15938, PEMR15939, PEMR15940, WP012, WP014, WP015, WP016, WP017, WP963, WP966, WP969, WP970, WP971, WP976, WP967, WP968	N = 58 PEMR2100, PEMR2101, PEMR2103, PEMR2106, PEMR2107, PEMR2108, PEMR2109, PEMR2110, PEMR3694, PEMR3696, PEMR4319, PEMR4320, PEMR4344, PEMR4345, PEMR4734, PEMR4736, PEMR4737, PEMR4738, PEMR4754, PEMR5065, PEMR6123, PEMR6737, PEMR7483, PEMR7484, PEMR7486, PEMR7488, PEMR11894, PEMR11919, PEMR11944, PEMR11946, PEMR15932, PEMR15927, PEMR15928, PEMR15929, PEMR15930, PEMR15931, PEMR15934, PEMR15938, PEMR15939, PEMR15940, PEMR15941, PEMR15942, PEMR18307, PEMR18376, WP012, WP013, WP014, WP015, WP016, WP017, WP968, WP963, WP967, WP969, WP971, WP970, WP977, WP976	N = 39 PEMR2101, PEMR2106, PEMR2107, PEMR2108, PEMR2109, PEMR3696, PEMR4320, PEMR4344, PEMR4345, PEMR4734, PEMR4737, PEMR4738, PEMR4747, PEMR6123, PEMR6737, PEMR7486, PEMR7488, PEMR11894, PEMR11919, PEMR11944, PEMR11946, PEMR15928, PEMR15929, PEMR15930, PEMR15931, PEMR15932, PEMR15938, PEMR15939, PEMR15940, PEMR15941, PEMR15942, PEMR18307, PEMR18376, WP012, WP013, WP966, WP967, WP969, WP971

* Key to accession numbers: PEMR = Port Elizabeth Museum; TM = Ditsong Museum (formerly the Transvaal Museum); RSP, HZ, JM, FP, WP = field numbers for individuals collected by author and collaborators; H, FB = field numbers for individuals collected by Prof. P. L. Mouton.

Table A7: Specimens used in Chapter 3 for the performance analyses. All specimens caught in the field by author and collaborators.

Genus and species	Bite force analyses	Sprinting analyses	Stamina analyses
<i>Ichnotropis squamulosa</i>	N = 20 RSP283, RSP360, RSP372, RSP374, RSP376, RSP409, RSP410, RSP414, RSP415, RSP464, RSP465, RSP466, RSP467, RSP468, RSP471, RSP472, RSP474, RSP476, RSP477, RSP480	N = 20 RSP283, RSP360, RSP372, RSP374, RSP376, RSP409, RSP410, RSP414, RSP415, RSP464, RSP465, RSP466, RSP467, RSP468, RSP471, RSP472, RSP474, RSP476, RSP477, RSP480	
<i>Meroles anchietae</i>	N = 18 WP003, WP006, WP327, WP916, WP929, WP004, WP326, WP913, WP914, WP915, WP919, WP925, WP926, WP928, WP931, WP933, WP934, WP935	N = 18 WP003, WP006, WP327, WP916, WP929, WP004, WP326, WP913, WP914, WP915, WP919, WP925, WP926, WP928, WP931, WP933, WP934, WP935	N = 21 WP003, WP004, WP005, WP006, WP323, WP326, WP327, WP913, WP914, WP915, WP916, WP917, WP918, WP925, WP926, WP928, WP929, WP931, WP933, WP934, WP935
<i>Meroles ctenodactylus</i>	N = 12 JM03603, JM03605, JM03609, JM03611, JM03613/JM03616, JM03614, JM03615, JM03617, JM03621, JM03622, JM03623, KOIN2	N = 12 JM03603, JM03605, JM03609, JM03611, JM03613/JM03616, JM03614, JM03615, JM03617, JM03617, JM03621, JM03622, JM03623, KOIN2	
<i>Meroles cuneirostris</i>	N = 11 WP001, WP320, WP322, WP324, WP912, WP920, WP921, WP923, WP924, WP936, WP937	N = 11 WP001, WP320, WP322, WP324, WP912, WP920, WP921, WP923, WP924, WP936, WP937	N = 10 WP001, WP320, WP324, WP325, WP914, WP920, WP923, WP924, WP936, WP937
<i>Meroles knoxii</i>	N = 19 422, 424, 425, 426, 427, 428, 429, 430, 431, 432, MK01, MK04, MK08, MK09, MK10, MK11, MK15, MK18, MK19	N = 19 422, 424, 425, 426, 427, 428, 429, 430, 431, 432, MK01, MK04, MK08, MK09, MK10, MK11, MK15, MK18, MK19	N = 22 SER121, SER122, SER123, SER124, SER132, SER135, SER136, SER137, SER172, SER173, SER174, SER175, SER176, SER177, SER179, SER180, SER181, SER182, SER184, SER185, SER186, SER187
<i>Meroles reticulatus</i>	N = 4 WP010, WP011, WP978, WP980	N = 4 WP010, WP011, WP978, WP980	
<i>Meroles suborbitalis</i>	N = 10 WP012, WP013, WP014, WP016, WP017, WP963, WP969, WP970, WP976, WP977	N = 10 WP012, WP013, WP014, WP016, WP017, WP963, WP969, WP970, WP976, WP977	N = 7 WP963, WP966, WP967, WP968, WP969, WP970, WP971

Table A8: Specimens used in Chapter 4 for the linear morphometric analyses.

Genus, species and subspecies	Museum accession IDs
<i>Pedioplanis lineoocellata lineoocellata</i>	N = 63 PEMR2128, PEMR2129, PEMR2131, PEMR2138, PEMR2139, PEMR5787, PEMR5794, PEMR10504, PEMR10506, PEMR10507, PEMR10508, PEMR10509, PEMR10510, PEMR10511, PEMR10513, PEMR10514, PEMR10515, PEMR10516, PEMR10517, PEMR10523, PEMR10526, PEMR10527, PEMR10528, PEMR10529, PEMR10530, PEMR10531, PEMR10532, PEMR10533, PEMR10534, PEMR10538, PEMR10632, PEMR10669, PEMR10670, PEMR10673, PEMR10674, PEMR10675, PEMR16864, PEMR16865, PEMR16868, PEMR16869, PEMR16968, PEMR16969, PEMR16970, PEMR16979, PEMR17357, PEMR17358, PEMR17555, PEMR17856, PEMR17857, PEMR17880, PEMR17882, PEMR18230, PEMR18232, PEMR18236, PEMR18252, PEMR18265, PEMR18286, PEMR18287, PEMR18288, PEMR18289, PEMR18297, PEMR18298, PEMR18304
<i>Pedioplanis lineoocellata pulchella</i>	N = 97 JM03406, JM03409, PEMR44, PEMR528, PEMR544, PEMR545, PEMR1561, PEMR1563, PEMR1564, PEMR1807, PEMR1942, PEMR1943, PEMR2130, PEMR2133, PEMR2135, PEMR2387, PEMR2440, PEMR3146, PEMR3191, PEMR3294, PEMR3697, PEMR4328, PEMR4415, PEMR4416, PEMR4524, PEMR4535, PEMR6562, PEMR6650, PEMR6651, PEMR6652, PEMR6655, PEMR6657, PEMR6658, PEMR6660, PEMR6662, PEMR6663, PEMR6664, PEMR6666, PEMR6668, PEMR6669, PEMR6757, PEMR7085, PEMR7097, PEMR7099, PEMR7104, PEMR8562, PEMR8715, PEMR8722, PEMR9364, PEMR10132, PEMR10133, PEMR10535, PEMR10539, PEMR10540, PEMR10541, PEMR10542, PEMR10543, PEMR10544, PEMR10598, PEMR10602, PEMR10603, PEMR10604, PEMR10607, PEMR10608, PEMR10612, PEMR10614, PEMR10615, PEMR10617, PEMR10633, PEMR10636, PEMR10638, PEMR10639, PEMR10640, PEMR10653, PEMR10658, PEMR10659, PEMR10664, PEMR11021, PEMR11022, PEMR11035, PEMR11220, PEMR11228, PEMR11233, PEMR11258, PEMR11259, PEMR11262, PEMR11262, PEMR11266, PEMR16511, PEMR17231, PEMR17238, PEMR17265, PEMR18369, PEMR18378, SVN337, SVN341, SVN366
<i>Pedioplanis lineoocellata inoocellata</i>	N = 3 PEMR4742, PEMR4846, PEMR4847

Table A9: List of individuals used in the analyses of Chapter 5, indicating which analyses they were used for (genetic, morphometric, performance and/or stomach contents analyses). Locality, Field collection ID numbers, sex, date caught, and EmblBank accession numbers for the ND4 gene shown.

Locality	Field ID	Sex	Date caught	Genetic	EmblBank accession number for ND4 gene fragment	Linear morphometric	Sprint speed performance	Stamina performance
Phillipi	SEL002	F	19-03-2012	Y	To be added	Y		
Phillipi	SEL004	F	19-03-2012	Y	To be added	Y		
Phillipi	SEL006	F	19-03-2012			Y		
Phillipi	SEL007	F	19-03-2012			Y		
Phillipi	SEL009	F	19-03-2012			Y		
Phillipi	SER078	F	18-05-2010	Y	To be added	Y		
Phillipi	SER084	F	21-05-2010	Y	To be added			
Phillipi	SER086	F	21-05-2010	Y	To be added	Y		
Phillipi	SER087	F	21-05-2010	Y	To be added			
Phillipi	SER088	F	21-05-2010	Y	To be added			
Phillipi	SER092	F	01-06-2010	Y	To be added	Y		
Phillipi	SER093	F	03-09-2010			Y		
Phillipi	SER099	F	14-09-2010	Y	To be added	Y		
Phillipi	SER120	F	26-01-2011	Y	To be added	Y		Y
Phillipi	SER151	F	01-08-2011	Y	To be added			
Phillipi	SER190	F	06-12-2011	Y	To be added	Y	Y	Y
Phillipi	SER191	F	06-12-2011	Y	To be added	Y	Y	Y
Phillipi	SER197	F	06-12-2011	Y	To be added	Y	Y	Y
Phillipi	SER201	F	06-12-2011	Y	To be added	Y	Y	Y
Phillipi	SER204	F	08-12-2011	Y	To be added	Y	Y	Y
Phillipi	MK17	M	05-11-2010			Y		
Phillipi	MK18	M	05-11-2010	Y	To be added	Y		Y
Phillipi	MK19	M	05-11-2010			Y		Y
Phillipi	SEL003	M	19-03-2012			Y		
Phillipi	SER079	M	18-05-2010	Y	To be added	Y		
Phillipi	SER080	M	18-05-2010	Y	To be added	Y		
Phillipi	SER081	M	19-05-2010	Y	To be added	Y		
Phillipi	SER082	M	21-05-2010	Y	To be added	Y		
Phillipi	SER083	M	21-05-2010	Y	To be added	Y		
Phillipi	SER085	M	21-05-2010	Y	To be added			
Phillipi	SER089	M	21-05-2010	Y	To be added	Y		
Phillipi	SER090	M	01-06-2010	Y	To be added	Y		
Phillipi	SER094	M	03-09-2010	Y	To be added	Y		
Phillipi	SER095	M	08-09-2010	Y	To be added	Y		
Phillipi	SER096	M	08-09-2010			Y		
Phillipi	SER102	M	16-09-2010	Y	To be added	Y		
Phillipi	SER103	M	21-09-2010	Y	To be added	Y		
Phillipi	SER133	M	01-02-2011	Y	To be added			
Phillipi	SER134	M	01-02-2011	Y	To be added			
Phillipi	SER150	M	01-08-2011	Y	To be added			
Phillipi	SER158	M	01-08-2011					
Phillipi	SER161	M	01-08-2011					
Phillipi	SER163	M	01-08-2011					
Phillipi	SER164	M	16-08-2011	Y	To be added			
Phillipi	SER192	M	06-12-2011	Y	To be added	Y	Y	Y
Phillipi	SER193	M	06-12-2011			Y	Y	Y
Phillipi	SER194	M	06-12-2011	Y	To be added	Y	Y	Y
Phillipi	SER195	M	06-12-2011	Y	To be added	Y	Y	Y
Phillipi	SER196	M	06-12-2011	Y	To be added	Y	Y	Y
Phillipi	SER199	M	06-12-2011	Y	To be added	Y	Y	Y

Table A9: *continued...*

Phillipi	SER203	M	08-12-2011			Y	Y	Y
Rietvlei	SEL012	F	22-03-2012			Y		
Rietvlei	SEL015	F	22-03-2012			Y		
Rietvlei	SER101	F	14-09-2010	Y	To be added	Y		
Rietvlei	SER109	F	13-12-2010	Y	To be added	Y		
Rietvlei	SER110	F	20-12-2010	Y	To be added	Y		
Rietvlei	SER112	F	20-12-2010	Y	To be added	Y		
Rietvlei	SER113	F	20-12-2010	Y	To be added	Y		
Rietvlei	SER114	F	20-12-2010	Y	To be added	Y		
Rietvlei	SER119	F	25-01-2011	Y	To be added	Y		
Rietvlei	SER122	F	28-01-2011	Y	To be added	Y		Y
Rietvlei	SER132	F	28-01-2011	Y	To be added	Y		Y
Rietvlei	SER135	F	03-02-2011	Y	To be added	Y		Y
Rietvlei	SER170	F	15-10-2011			Y		
Rietvlei	SER174	F	28-11-2011			Y	Y	Y
Rietvlei	SER177	F	28-11-2011			Y	Y	Y
Rietvlei	SER183	F	30-11-2011			Y	Y	
Rietvlei	SER185	F	30-11-2011			Y	Y	Y
Rietvlei	SER186	F	30-11-2011			Y	Y	Y
Rietvlei	SEL014	M	22-03-2012			Y		
Rietvlei	SER097	M	13-09-2010	Y	To be added	Y		
Rietvlei	SER098	M	13-09-2010			Y		
Rietvlei	SER100	M	14-09-2010	Y	To be added	Y		
Rietvlei	SER105	M	13-12-2010	Y	To be added	Y		
Rietvlei	SER106	M	13-12-2010	Y	To be added	Y		
Rietvlei	SER107	M	13-12-2010	Y	To be added	Y		
Rietvlei	SER108	M	13-12-2010	Y	To be added	Y		
Rietvlei	SER111	M	20-12-2010	Y	To be added	Y		
Rietvlei	SER115	M	24-12-2010	Y	To be added	Y		
Rietvlei	SER116	M	24-12-2010	Y	To be added	Y		
Rietvlei	SER117	M	24-12-2010	Y	To be added	Y		
Rietvlei	SER121	M	28-01-2011	Y	To be added	Y		Y
Rietvlei	SER123	M	28-01-2011	Y	To be added	Y		Y
Rietvlei	SER124	M	28-01-2011	Y	To be added	Y		Y
Rietvlei	SER136	M	03-02-2011	Y	To be added	Y		Y
Rietvlei	SER137	M	03-02-2011	Y	To be added	Y		Y
Rietvlei	SER149	M	05-02-2011	Y	To be added	Y		
Rietvlei	SER171	M	15-10-2011			Y		
Rietvlei	SER172	M	28-11-2011			Y	Y	Y
Rietvlei	SER173	M	28-11-2011			Y	Y	Y
Rietvlei	SER175	M	28-11-2011			Y	Y	Y
Rietvlei	SER176	M	28-11-2011			Y	Y	Y
Rietvlei	SER179	M	28-11-2011			Y	Y	Y
Rietvlei	SER180	M	28-11-2011			Y	Y	Y
Rietvlei	SER181	M	30-11-2011			Y	Y	Y
Rietvlei	SER182	M	30-11-2011			Y	Y	Y
Rietvlei	SER184	M	30-11-2011			Y	Y	Y
Rietvlei	SER187	M	30-11-2011			Y	Y	Y
Zandvlei	MK01	F	03-11-2010			Y		Y
Zandvlei	MK03	F	03-11-2010			Y		Y
Zandvlei	MK04	F	03-11-2010			Y		Y
Zandvlei	MK08	F	03-11-2010			Y		Y
Zandvlei	MK09	F	03-11-2010			Y		Y
Zandvlei	MK11	F	03-11-2010			Y		Y
Zandvlei	MK15	F	03-11-2010			Y		Y
Zandvlei	SEL019	F	23-03-2012			Y		
Zandvlei	SEL022	F	23-03-2012			Y		
Zandvlei	SEL024	F	23-03-2012			Y		

Table A9: *continued...*

Zandvlei	SER004	F	09-03-2010			Y		
Zandvlei	SER005	F	09-03-2010	Y	To be added	Y		
Zandvlei	SER006	F	09-03-2010	Y	To be added	Y		
Zandvlei	SER007	F	09-03-2010	Y	To be added	Y		
Zandvlei	SER008	F	09-03-2010	Y	To be added	Y		
Zandvlei	SER018	F	11-03-2010	Y	To be added	Y		
Zandvlei	SER021	F	11-03-2010	Y	To be added	Y		
Zandvlei	SER024	F	11-03-2010	Y	To be added	Y		
Zandvlei	SER025	F	11-03-2010	Y	To be added			
Zandvlei	SER026	F	11-03-2010	Y	To be added	Y		
Zandvlei	SER028	F	11-03-2010	Y	To be added			
Zandvlei	SER030	F	11-03-2010	Y	To be added	Y		
Zandvlei	SER032	F	15-03-2010	Y	To be added	Y		
Zandvlei	SER033	F	15-03-2010	Y	To be added	Y		
Zandvlei	SER035	F	15-03-2010	Y	To be added	Y		
Zandvlei	SER037	F	15-03-2010	Y	To be added	Y		
Zandvlei	SER040	F	16-03-2010	Y	To be added	Y		
Zandvlei	SER044	F	17-03-2010	Y	To be added	Y		
Zandvlei	SER052	F	06-04-2010	Y	To be added	Y		
Zandvlei	SER055	F	08-04-2010	Y	To be added	Y		
Zandvlei	SER058	F	08-04-2010	Y	To be added	Y		
Zandvlei	SER063	F	08-04-2010			Y		
Zandvlei	SER065	F	13-04-2010	Y	To be added	Y		
Zandvlei	SER071	F	13-04-2010	Y	To be added	Y		
Zandvlei	SER074	F	13-04-2010			Y		
Zandvlei	SER165	F	12-10-2011			Y		
Zandvlei	SER166	F	12-10-2011			Y		
Zandvlei	SER168	F	12-10-2011			Y		
Zandvlei	SER169	F	12-10-2011			Y		
Zandvlei	SER210	F	12-12-2011			Y	Y	Y
Zandvlei	SER211	F	12-12-2011			Y	Y	Y
Zandvlei	SER212	F	12-12-2011			Y	Y	Y
Zandvlei	SER214	F	12-12-2011			Y	Y	Y
Zandvlei	SER215	F	12-12-2011			Y	Y	Y
Zandvlei	SER218	F	12-12-2011			Y	Y	Y
Zandvlei	SER219	F	12-12-2011			Y	Y	Y
Zandvlei	SER221	F	12-12-2011			Y	Y	Y
Zandvlei	SER225	F	12-12-2011			Y	Y	Y
Zandvlei	SER009	J	09-03-2010	Y	To be added			
Zandvlei	SER010	J	09-03-2010	Y	To be added			
Zandvlei	SER012	J	09-03-2010	Y	To be added			
Zandvlei	SER013	J	09-03-2010	Y	To be added			
Zandvlei	SER017	J	10-03-2010	Y	To be added			
Zandvlei	SER022	J	11-03-2010	Y	To be added			
Zandvlei	SER023	J	11-03-2010	Y	To be added			
Zandvlei	SER029	J	11-03-2010	Y	To be added			
Zandvlei	MK10	M	03-11-2010			Y		Y
Zandvlei	SEB13	M	03-11-2010			Y		
Zandvlei	SEL018	M	23-03-2012			Y		
Zandvlei	SEL020	M	23-03-2012			Y		
Zandvlei	SEL021	M	23-03-2012			Y		
Zandvlei	SER001	M	09-03-2010	Y	To be added	Y		
Zandvlei	SER002	M	09-03-2010			Y		
Zandvlei	SER003	M	09-03-2010			Y		
Zandvlei	SER011	M	09-03-2010	Y	To be added	Y		
Zandvlei	SER014	M	09-03-2010	Y	To be added	Y		
Zandvlei	SER016	M	09-03-2010	Y	To be added			
Zandvlei	SER019	M	11-03-2010	Y	To be added	Y		

Zandvlei	SER020	M	11-03-2010	Y	To be added
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Table A9: *continued...*

Zandvlei	SER027	M	11-03-2010			Y		
Zandvlei	SER034	M	15-03-2010	Y	To be added	Y		
Zandvlei	SER041	M	17-03-2010	Y	To be added	Y		
Zandvlei	SER042	M	17-03-2010	Y	To be added	Y		
Zandvlei	SER043	M	17-03-2010	Y	To be added	Y		
Zandvlei	SER046	M	06-04-2010	Y	To be added	Y		
Zandvlei	SER047	M	06-04-2010			Y		
Zandvlei	SER048	M	06-04-2010	Y	To be added	Y		
Zandvlei	SER050	M	06-04-2010	Y	To be added	Y		
Zandvlei	SER053	M	08-04-2010	Y	To be added	Y		
Zandvlei	SER054	M	08-04-2010	Y	To be added	Y		
Zandvlei	SER056	M	08-04-2010	Y	To be added	Y		
Zandvlei	SER057	M	08-04-2010	Y	To be added	Y		
Zandvlei	SER059	M	08-04-2010	Y	To be added	Y		
Zandvlei	SER064	M	13-04-2010	Y	To be added	Y		
Zandvlei	SER066	M	13-04-2010	Y	To be added	Y		
Zandvlei	SER067	M	13-04-2010	Y	To be added	Y		
Zandvlei	SER068	M	13-04-2010	Y	To be added	Y		
Zandvlei	SER069	M	13-04-2010			Y		
Zandvlei	SER070	M	13-04-2010	Y	To be added	Y		
Zandvlei	SER167	M	12-10-2011			Y		
Zandvlei	SER213	M	12-12-2011			Y	Y	Y
Zandvlei	SER216	M	12-12-2011			Y	Y	Y
Zandvlei	SER220	M	12-12-2011			Y	Y	Y
Zandvlei	SER222	M	12-12-2011			Y	Y	Y
Zandvlei	SER223	M	12-12-2011			Y	Y	Y
Zandvlei	SER224	M	12-12-2011			Y	Y	Y
TOTALS				117		170	42	60

APPENDIX B: PAPERS PUBLISHED, IN PRESS OR IN PREPARATION

List of published papers attached in Appendix B:

Edwards S, Vanhooydonck B, Herrel A, Measey GJ, Tolley KA. 2012. Convergent evolution associated with habitat decouples phenotype from phylogeny in a clade of lizards. PLoS-One. 7 (12): e52636. DOI: 10.1371/journal.pone.0051636.

Edwards S, Branch WR, Vanhooydonck B, Herrel A, Measey GJ, Tolley KA. 2013. Taxonomic adjustments in the systematics of the southern African lacertid lizards (Sauria: Lacertidae). Zootaxa. 3669 (2): 101–114.

Edwards S, Tolley KA, Vanhooydonck B, Measey GJ, Herrel A. 2013. Is dietary niche breadth linked to morphology and performance in Sandveld lizards *Nucras* (Sauria: Lacertidae)? Biological Journal of the Linnean Society. 110(3):674-688

List of manuscripts in preparation:

Edwards S, Tolley KA, Vanhooydonck B, Herrel A. Prey avoidance behaviour determines morphology in the genus *Meroles* (Sauria: Lacertidae).

Edwards S, Tolley KA. Rapid evolution of morphology and performance after an introduction into a novel habitat in the lizard *Meroles knoxii* (Sauria: Lacertidae).

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Convergent Evolution Associated with Habitat Decouples Phenotype from Phylogeny in a Clade of Lizards

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Abstract

Convergent evolution can explain similarity in morphology between species, due to selection on a fitness-enhancing phenotype in response to local environmental conditions. As selective pressures on body morphology may be strong, these have confounded our understanding of the evolutionary relationships between species. Within the speciose African radiation of lacertid lizards (Eremiadini), some species occupy a narrow habitat range (e.g. open habitat, cluttered habitat, strictly rupicolous, or strictly psammophilic), which may exert strong selective pressures on lizard body morphology. Here we show that the overall body plan is unrelated to shared ancestry in the African radiation of Eremiadini, but is instead coupled to habitat use. Comprehensive Bayesian and likelihood phylogenies using multiple representatives from all genera (2 nuclear, 2 mitochondrial markers) show that morphologically convergent species thought to represent sister taxa within the same genus are distantly related evolutionary lineages (*Ichnotropis squamulosa* and *Ichnotropis* spp.; *Australolacerta rupicola* and *A. australis*). Hierarchical clustering and multivariate analysis of morphological characters suggest that body, and head, width and height (stockiness), all of which are ecologically relevant with respect to movement through habitat, are similar between the genetically distant species. Our data show that convergence in morphology, due to adaptation to similar environments, has confounded the assignment of species leading to misidentification of the taxonomic position of *I. squamulosa* and the *Australolacerta* species.

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Introduction

Convergent evolution is attributed to strong selection on a fitness-enhancing phenotype in response to local environmental conditions [1]. In reptiles, convergent evolution is found among species of *Anolis* lizards [2–4], amongst others, showing that similarities in environmental conditions and habitat use may elicit similar adaptive evolutionary responses by directional selection regardless of ancestry. Similar morphologies are also observed among distantly related rock-dwelling [5–8], burrowing [9–12], as well as arboreal lizards [13–15]. In each of these cases, adaptation is ascribed to selection on an animal's body plan in order to optimize performance in a given habitat. For example, rock-dwelling species typically have flat heads and bodies that allow them to fit into narrow cracks, yet long forelimbs adapted for climbing [8,16]. In contrast, some arboreal species that specialize on narrow substrates have short limbs and narrow, tall bodies [17–20].

Southern Africa has a diverse assemblage of macro-habitats, from tropical forest to desert, and ranges from sea level to more

than 3000 m. This complexity at the macro scale is interwoven with a diversity of micro-habitat structure that includes different substrates and vegetation organization, and the heterogeneity at both scales may be a strong factor in producing high diversity and endemism of reptiles in the region [21,22]. Indeed, many species are restricted and habitat specific at the micro scale (e.g. chameleons, cordylids), whilst others are apparent generalists (e.g. skinks). Morphological adaptation to this diversity in habitat structure should be reflected in phylogenies as lineages showing morphological convergence in species living in similar habitat structure, or divergence in species occupying different habitat structure.

Although phenotypic convergence is a common explanation for morphological similarity, such occurrences can be the result of phylogenetic history, chance and/or pre-existing constraints ('exaptation') rather than adaptation to similar environments [1]. Natural selection favors traits that increase fitness, even if the trait did not necessarily evolve in response to those selective pressures. While experimental conditions simulating environments can convincingly demonstrate whether natural selection drives con-

vergence in morphological traits [23], it is more difficult to test convergence through adaptation to shared environments within a natural setting [24]. Yet, repeated evolution of convergent phenotypes in divergent lineages inhabiting similar environments is often considered strong evidence of natural selection operating on morphological traits.

Here, we examine convergence of ecologically relevant phenotypic traits to habitat structure (cluttered and open vegetation) in a diverse group of lizards (Eremiadini, Lacertidae) from southern Africa. We predicted that ecologically relevant traits would converge in association with habitat similarity, regardless of evolutionary history. We postulated that species utilizing cluttered habitats would have relatively slender bodies and short limbs compared to species utilizing open habitats, to allow for efficient movement through the cluttered matrix [17,25,26]. To test this hypothesis, we investigated the evolutionary relationships in the Eremiadini using a multi-locus phylogenetic approach, in combination with principal components analysis and hierarchical clustering for morphological data on traits that are considered ecologically relevant to lizards [8,27]. The clusters were then compared *a priori* to habitat structure to examine occurrences of convergence in morphology between species.

Methods

Sampling

Taxa chosen for the study were genera from the southern African clade of the lacertid lizards from the tribe Eremiadini (five genera out of 20 total genera in Eremiadini). Samples for the genetic analyses were obtained either from field trips conducted by myself or from samples, collected by various researchers, housed in the collection at the South African National Biodiversity Institute. Some of the individuals sampled have been sequenced previously for the 16S and RAG1 genes, and accession numbers and references are provided in Table S1. Samples for the morphometric analyses included measurement of live lizards during field work, as well as voucher specimens housed at the Port Elizabeth Museum (PEM), the Ditsong Museum (TM) and the Ellerman Collection at Stellenbosch University.

Ethics Statement

Ethics clearance was obtained from University of Stellenbosch (permit no. 11NP-EDW01) and South African National Biodiversity Institute (permit no. 002/10), permitting the collection and handling of the lizards, as well as the sampling of tail tissue.

Laboratory Protocols

Genomic DNA was isolated from the tail or liver tissue preserved in 95–100% ethanol according to standard procedures involving a proteinase K digestion followed by salt-extraction [28]. Standard PCR procedures were utilized to amplify two mitochondrial (16S and ND4) and two nuclear genes (RAG1 and KIAA-2018). The nuclear genes were chosen because these genes have been shown to evolve at a rate that may allow high confidence in both the terminal and the deeper nodes [29,30]. For the mitochondrial genes, the primer pairs ND4 and tRNA^{Leu} [31], and L2510 and H3080 16S rRNA [32] were used to amplify the ND4 and 16S genes, respectively. The primers RAG1-F0 and RAG1-R1 [33], and KIAA2018-F1 and KIAA2018-R2 [30] were used to amplify the nuclear RAG1 and KIAA-2018 genes, respectively.

Amplification of the four genes was carried out with ~25–50 ng/ μ l genomic DNA and a 25 μ l reaction containing a thermophilic buffer (50 mM KCl, 10 mM Tris-HCl, pH 9.0),

1.5 mM MgCl₂, 0.2 μ M of each primer, 0.2 mM dNTPs, and 0.025 U/l Taq polymerase. Cycling profile for 16S, ND4 and KIAA-2018 genes included an initial denaturing step at 94°C for 4 minutes, followed by 35 cycles of 94°C for 30 s, 50–55°C for 30 s, and 72°C for 45 s, with a final extension at 72°C for 8 min. The amplification of the RAG1 gene region involved a step-down procedure [34]. The PCR products were sent to Macrogen Corp. (Seoul, Korea) for sequencing using the forward primers in all cases. Sequences were aligned in BioEdit Sequence Alignment Editor v. 7.0.5.2 [35]. All sequences have been deposited in EMBL-Bank (see Table S2 for all voucher information, with corresponding EMBL-Bank accession numbers).

Genetic Analyses

We first analyzed the mitochondrial (16S vs. ND4) and nuclear (RAG1 vs. KIAA2018) datasets separately to ensure that there was no conflict in the markers within each genome, using a partition homogeneity test [36,37] in PAUP* v4.0b10 [38]. The two mitochondrial and the two nuclear genes were not incongruent, so the partition homogeneity test was run again (nuclear vs. mitochondrial) to ensure that there was no conflict between the two genomes. Phylogenetic trees were constructed of the 1) mitochondrial gene dataset (Fig. S1), 2) the nuclear gene dataset (Fig. S1) and 3) the combined total evidence dataset (Fig. S2). The saturation of the codon positions was assessed using the program Dambe v.5.2.65 [39]. Even though the third codon position of the ND4 gene was found to be saturated, it was not excluded from the analyses, but rather it was coded as a separate partition. Two individuals of *Heliobolus lugubris* were used as the outgroup, as it is within the sister clade to the southern African radiation within Eremiadini [33,40]. Sequence divergences were determined by estimating the uncorrected p-distances between and within species using the program MEGA v.4 [41].

Two different algorithms were utilized to obtain phylogenetic trees (Figs. 1 and S1). Bayesian inference (BI) was performed using the program MrBayes v.3.1.0 [42,43]. Priors in MrBayes were set according to the evolutionary model which best fits the dataset using the program MrModeltest v.3.6 [44], and uniform priors were kept for all other parameters. The MCMC were run with 2 parallel runs for 10 million generations each, with trees sampled every 1000 generations. The number of generations to discard as burn-in was determined by examining the number of generations 1) at which the standard deviation of split frequencies stabilized (at less than 0.001), 2) at which the log-likelihood tree scores reached stationarity, and 3) the effective sample sizes (ESS) of all parameters which were ≥ 600 (using the program Tracer v.1.5 [45]). A 50% majority rule tree was constructed with the burn-in excluded using the “sumt” command in MrBayes, and nodes with ≥ 0.95 posterior probability were considered supported. A Shimodaira–Hasegawa (SH) test [46,47] was performed to compare the consensus tree with a tree where *I. squamulosa* was constrained to be closely related to *Ichnotropis*.

A partitioned maximum likelihood (ML) analysis was also run in RAxML v.7.2.8 [48], at the CIPRES Science Gateway (www.phylo.org/sub_sections/portal/) using the same partitions as the Bayesian analysis, a GTR+I+G model of evolution, and automatic halting of bootstrapping [48,49].

Characterization of Habitat

Two broad habitat types (open and cluttered) were defined for our analysis based on the general characteristics of vegetation structure associated with each species sampled. Open habitat lacks vegetation completely (i.e. dunes) or is sparsely vegetated, and mainly characterized by open sand, gravel or rock patches briefly

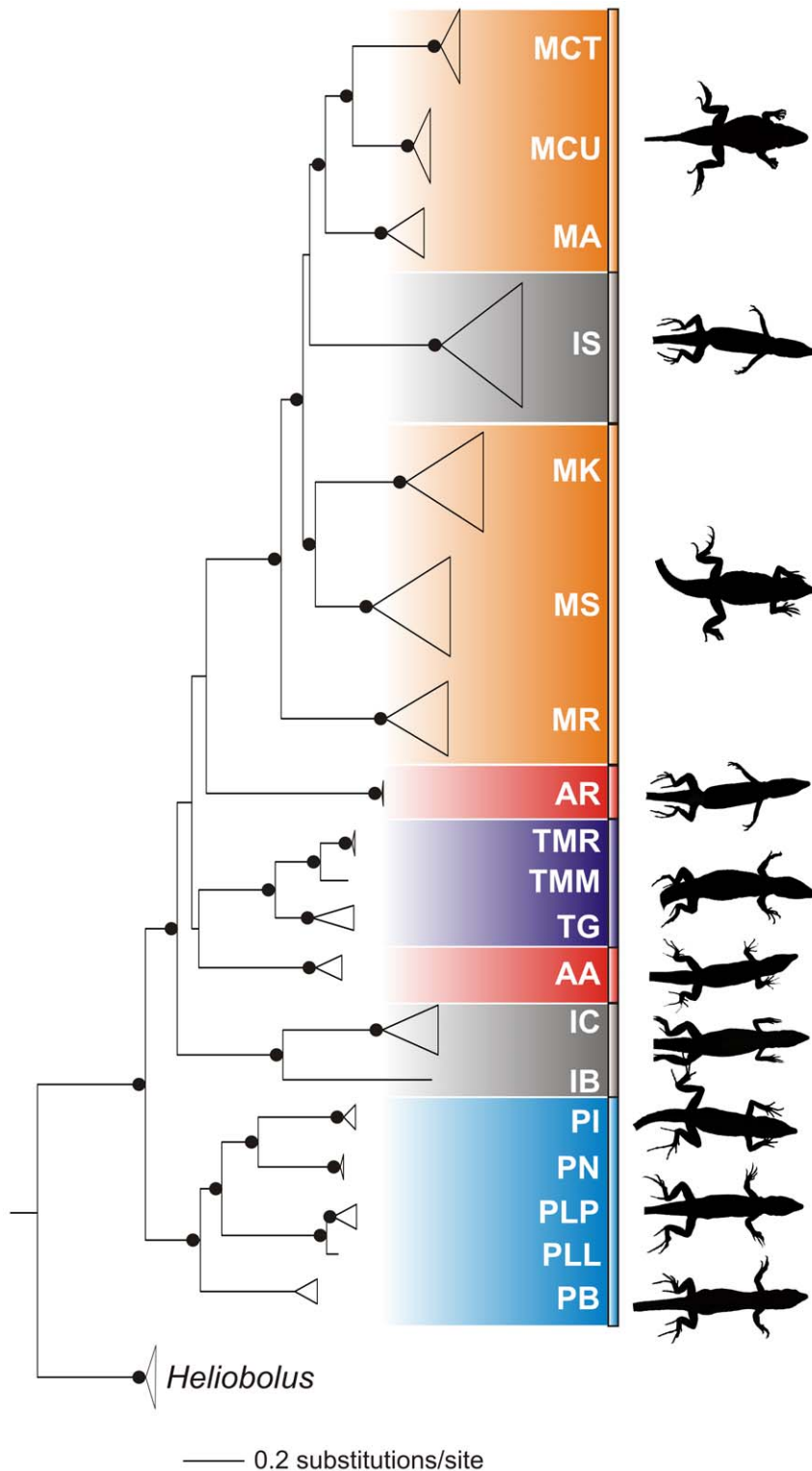


Figure 1. Phylogeny of the southern African lacertids. Phylogenetic reconstruction (left) using Bayesian inference (BI) of the southern African radiation of the lacertid subfamily Eremiadini based on the combined partial *16S*, *ND4*, *RAG1* and *KIAA* gene regions and inferred by BI and maximum likelihood (ML). Nodes with filled circles indicate BI posterior probabilities ≥ 0.95 and ML bootstrap values $\geq 75\%$. Representatives of the body shapes for each general clade are included (right) to show differences in bauplan of the main genetic clades. Key to the color coding for genera and species abbreviations: *Australolacerta* (red): AA = *Australolacerta australis*, AR = *A. rupicola*; *Ichnotropis* (gray): IB = *Ichnotropis bivittata*, IC = *I. capensis*, IS = *I. squamulosa*; *Merole* (orange): MA = *Merole anchietae*, MCT = *M. ctenodactylus*, MCU = *M. cuneirostris*, MK = *M. knoxii*, MS = *M. suborbitalis*; *Pedioplanis* (light blue): PB = *Pedioplanis burchelli*, PI = *P. inornata*, PLL = *P. lineocellata lineocellata*, PLP = *P. I. pulchella*, PN = *P. namaquensis*; *Tropidosaura* (blue): TG = *Tropidosaura gularis*, TMM = *T. montana montana*, TMR = *T. m. rangeri*.
doi:10.1371/journal.pone.0051636.g001

interspersed with bushes or grass tufts. A cluttered habitat is densely vegetated (i.e. with low vegetation such as grasses, sedges and restios, with an abundance of bushes in various sizes), with intermittent open patches (Fig. S3).

Morphometric Analyses

Body length (snout-vent length; SVL) and biometric characters on the head, hind limbs and fore limbs were measured externally using digital calipers for each individual. Measurements on the crania that related to the length of the head have seldom been investigated in lizards in terms of habitat openness, however the height and width of the crania have been linked to the use of specific refuges in cluttered environments (e.g. crevices in rocky habitats [8]). Measurements taken on the head were: head length (HL) from snout-tip to the back of the parietal bone, head width (HW) measured as the widest part of the head, head height (HH) measured as the height from the top of the interparietal scale to the bottom of the lower jaw (including muscles), lower-jaw length (LJL), coronoid to snout-tip length (CT), and quadrate to snout-tip length (QT). Limb measurements were taken of both the hind- and fore-limbs, as cluttered habitats have been cited as a factor in limb reduction [26,50], and longer limbs may be necessitated by an open habitat for higher sprint speeds, in order to effectively escape predators [17,25,26]. Measurements taken on the limbs were as follows: the femur length (FM), tibia length (TB), humerus length (HM) and radius length (RD). Other body dimensions measured were body height (BH) and body width (BW). Accession numbers for each individual and number of individuals measured for each species is detailed in Table S2.

Hierarchical clustering of the species was performed in the program R Studio v.0.94.84 [51], to identify morphological clusters. The mean value per species (17 species) of each size-regressed measurement (12 measurements) was calculated (package: 'base', function: 'mean' [50]) and the mean values per species for each measurement were regressed onto the mean snout-vent length (SVL) using a linear model to eliminate the effect of size (package: 'stats', functions: 'lm' and 'resid' [52]). Hierarchical clustering of the residual distances was performed (package: 'pvclust', function: 'pvclust' [53]) in which the distance matrix was calculated using the "correlation" option, the clustering dendrogram was constructed using the "complete" option, and support values for the nodes were estimated using 1000 bootstrap replicates.

To examine trait differences among the morphological groups obtained in the hierarchical clustering, a principal components analyses (PCA) on the residuals was performed in the program SPSS v.15 (SPSS, Inc.). Varimax rotation was used and three principal components (PC) with eigenvectors greater than 1 were extracted, which accounted for *ca.* 74.45% of the total variance (Table 1). The KMO test indicated sampling was adequate (i.e. in excess of 0.5), all communalities were high (i.e. in excess of 0.5) suggesting that all variables were reliable contributors to the analysis, there were sizeable correlations between all original variables, and low correlations in the residual correlation matrix [54]. The three PC's extracted (Table 1) loaded highest with body and head width (PC1), head lengths (PC2), and limbs (PC3). Boxplots (Figs. 2 & S4) were constructed using the PC scores for these same groups (package: 'stats', function: 'boxplot' [51]). Analysis of variance (ANOVA) was carried out on the three principal components extracted with the morphological cluster as the fixed factor (package: 'stats', function: 'anova' [51]).

Results

The combined mitochondrial and the nuclear topologies (BI and ML) were congruent (Figs. 1 and S1) and largely consistent with previous work [33,40]. Our data however shows two notable exceptions due to the inclusion of additional taxa (*Ichnotropis spp.* and *Australolacerta spp.*), both of which suggest that factors independent of ancestry are driving morphological evolution in the Eremiadini. Firstly, the two species of *Australolacerta* are separate evolutionary lineages, and form part of a deep basal polytomy at the generic level (Figs. 1 & S1), despite the ecological and morphological similarities that were used to place them in the same genus (Fig. 2 [55]). Secondly, the phylogeny shows that *Ichnotropis squamulosa* shares its most recent ancestry with members of the genus *Meroles* (Figs. 1 & S1), rather than with species in the morphologically similar genus *Ichnotropis* (Fig. 2), leading to a misclassification at the generic level. There was a significant difference between the Bayesian consensus tree and the tree where *I. squamulosa* was constrained as part of *Ichnotropis* (SH test: $P < 0.01$). Sequence divergences also show that *Ichnotropis squamulosa* is highly divergent from other *Ichnotropis* species examined (16S: $10.96 \pm 2.27\%$, ND4: $21.80 \pm 2.62\%$, RAG1: $5.09 \pm 0.88\%$, KIAA: $3.38 \pm 0.1\%$). In both cases, convergence in bauplan is coupled to traits associated with body/head width and limb dimensions (Fig. 2).

The phylogenetic analyses show that the two species, *A. australis* and *A. rupicola*, are separate evolutionary lineages, and form a basal polytomy with all other Eremiadini genera except *Meroles*. The SH test was not performed with *Australolacerta* constrained as monophyletic group, due to the unresolved relationship between the two species. The sequence divergence between these lineages were high (16S: $9.55 \pm 2.08\%$; ND4: $22.69 \pm 1.60\%$; RAG1: $3.74 \pm 0.76\%$; KIAA: $1.90 \pm 0.47\%$), consistent with generic divisions in southern African Lacertidae (16S: $7.57 \pm 1.38\%$; ND4: $21.21 \pm 1.33\%$; RAG1: $4.07 \pm 0.54\%$; KIAA: $2.84 \pm 0.60\%$, this study) as well as others (combined RAG1 & C-MOS: 1.40% between *Archaeolacerta* and *Zootoca*, [33]). Due to the high sequence divergences, we suggest that they have been incorrectly placed together in a single genus due to their similar body plans.

The adaptive nature of convergence in Eremiadini is demonstrated by the significant association of ecologically relevant traits and habitat structure. Hierarchical clustering of morphological features resulted in two major clusters that correspond to A) cluttered and B) open habitats (Fig. 2). These morphological clusters do not correspond to the evolutionary history of these taxa, but instead are significantly different with respect to sets of ecologically relevant characteristics related to habitat structure. Each cluster was further subdivided into either three (Cluster A: A1, A2 and A3) or two (Cluster B: B1 and B2) subclusters. Some of the subclusters can be linked to particular microhabitats within a cluttered or open habitat. For example, Cluster B2 species are dune-dwelling, whilst species of Cluster A2 and A3 are rupicolous.

Multivariate analyses (principal components analysis and analysis of variance) indicate that the two morphological clusters differ significantly in terms of body/head slenderness (PC1: $F = 430.19$, $p < 0.001$, 50.74% of the variation; Table 1), with species inhabiting cluttered habitats being slender and more elongate compared to those in more open habitats (Figs. 1 & S5). The two morphological clusters did not differ significantly for the second principal component (PC2: $F = 2.60$, $p = 0.11$, 14.24% of the variation) that loaded positively with most head measurements, particularly lengths (Table 1). An exception is that dune-dwelling species (B2) have significantly longer heads compared to clusters B1 ($F = 98.86$, $p < 0.0001$) and A2 ($F = 24.73$, $p < 0.0001$) (Table 2).

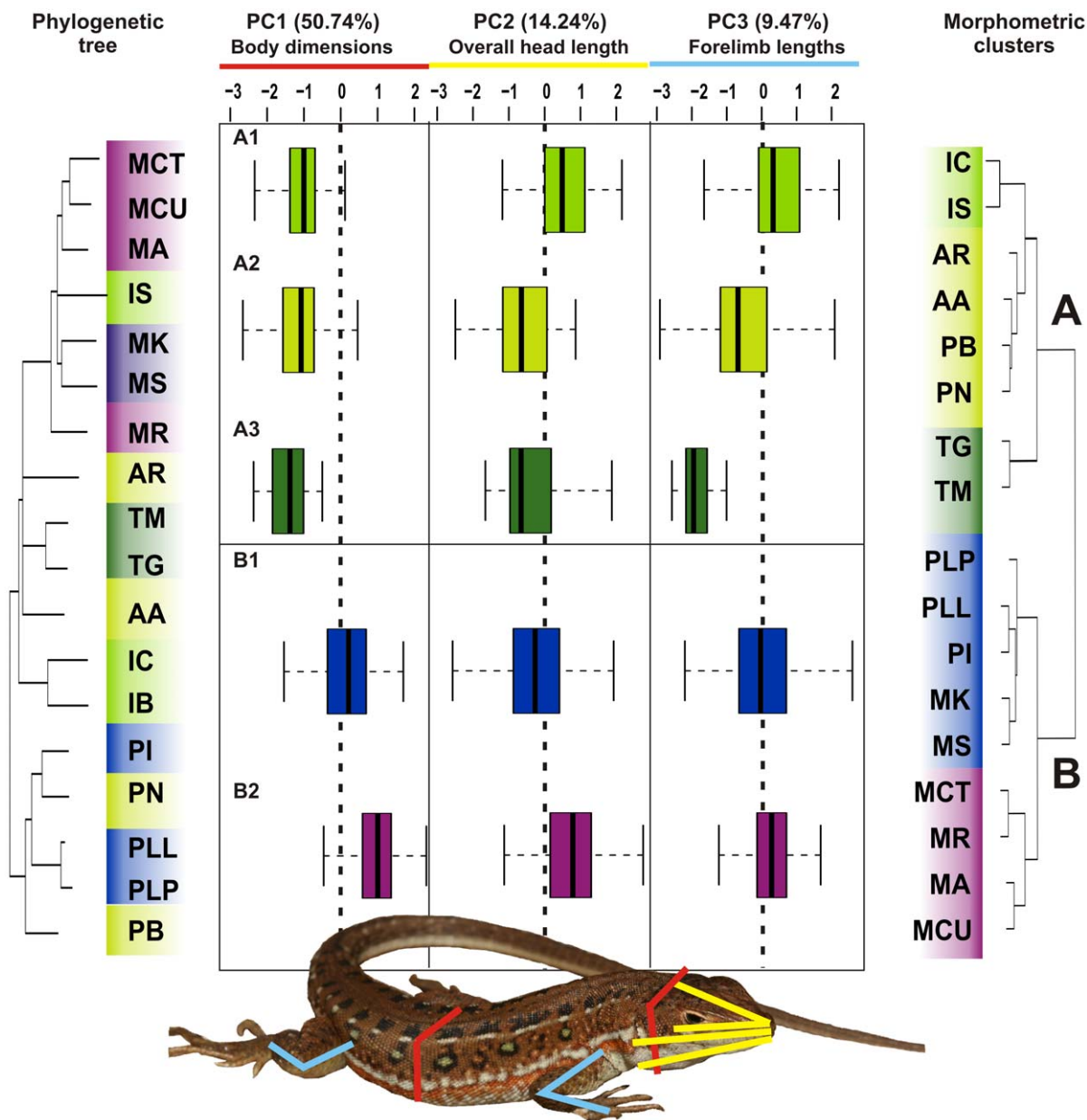


Figure 2. Clustering and principal components analysis of morphological markers. Boxplots of the first three principal component axes (center) for each morphological group (A, B) retrieved by hierarchical clustering (shown right). Positive values of the PC axes indicate larger body dimensions, whilst negative values indicate smaller body dimensions. Morphological groupings are shaded as follows: A1 = bright green, A2 = lime green, A3 = green, B1 = blue, B2 = purple. The phylogenetic tree (left) is color coded by species according to its morphological group membership. Morphological measurements are shown on lizard schematic, and line colors correspond to sets of original variables that loaded onto each PC (PC1 = red, PC2 = yellow, PC3 = light blue). Percentage of variation contributed to each PC axis is given. Key to the species abbreviations as in Fig. 1. doi:10.1371/journal.pone.0051636.g002

The two clusters differed significantly for PC3 ($F = 15.77$, $p < 0.001$; 9.47% of the variation), however this may be due to the relatively shorter forelimbs of *Tropidosaura* (A3).

Discussion

Whilst morphological characters are traditionally used to define species, descriptions that incorporate multidisciplinary approaches, including morphological, genetic, behavioral and ecological aspects, are typically better informed (e.g. [56]). Our data shows that among the morphological similarities upon which taxonomic classifications for Eremiadini are based [57], some are

the result of convergence due to habitat structure and not shared ancestry. We show that convergent evolution of morphological characters has led to genetically distant, but partially sympatric (*Ichnotropis* spp.) and parapatric (*Australolacerta* spp.) species being considered as sister taxa. Such examples of misclassification due to phenotypic similarities between species are increasingly familiar, suggesting that morphological adaptation in response to similar environments is pervasive, rather than exceptional. Even what might appear to be obvious cases of shared evolutionary history based on morphology, have turned up surprising developments revealing incorrect classifications at the generic level (e.g. geckos of

Table 1. Principal components analysis loadings of size-regressed measurements.

Residuals	PC1	PC2	PC3
Body width (BW)	0.89	0.07	0.03
Head width (HW)	0.79	0.32	0.27
Body height (BH)	0.77	0.14	-0.02
Head height (HH)	0.60	0.53	0.21
Lower jaw length (LJL)	0.27	0.81	0.10
Quadrate-Tip length (QT)	0.29	0.78	0.29
Head length (HL)	0.40	0.76	0.23
Coronoid-Tip length (CT)	-0.07	0.70	0.27
Radius length (RD)	0.02	0.23	0.88
Humerus length (HM)	0.02	0.21	0.87
Tibia length (TB)	0.52	0.27	0.66
Femur length (FM)	0.58	0.30	0.59
% variance	50.74	14.27	9.47
F-value	430.19 (***)	2.60 (ns)	15.77 (***)

Principal components analysis of size-regressed measurements, with loadings of each measurement for the three axes that had eigenvalues >1.0. Characters that loaded most strongly with each principal component are in bold. F-values from the analysis of variance between two main morphological clusters are shown. ***P<0.001; ns-not significant. doi:10.1371/journal.pone.0051636.t001

the genera *Pachydactylus/Elasmodactylus* [58]; chameleons of the genera *Archaius/Rieppoleon* [59].

Convergence in phenotype can be the result of random evolutionary change [1], however the observed morphological convergence in the southern African lacertids suggests adaptation to particular environments. The high genetic divergence between morphologically and ecologically similar species suggests that vegetation density (i.e. habitat clutter) is a major driving force in the evolution of phenotypic diversity in these lizards, irrespective of ancestry. Within the lacertid lizards, the phylogenetic position of species inhabiting particular environments (i.e. xeric or mesic environments) was investigated previously [33], and a unique monophyletic trend from mesic to xeric species within the

Lacertidae could not be demonstrated, despite previous morphological phylogenies which showed this trend [57,60]. With the comparison of the molecular tree to the broad environmental categories, it was suggested that there are multiple origins of xeric-adapted species within Eremiadini. However, here we show that morphology of a lizard is likely to be driven by its microhabitat, with less association to broad scale biome features. In fact, for many reptiles, geographic proximity influences phylogenetic position (e.g. [13,61]) making it unsurprising that a link exists between broad scale environmental classifications and phylogenetic position. For example, within *Meroles*, *M. anchietae* and *M. cuneirostris* are in the same clade, have a similar body plan and both inhabit a xeric environment. However, the lack of phylogenetic independence means that similarities due to a common ancestor which inhabited the xeric region prior to diversification cannot be ruled out. Conversely, *M. reticulatus* is not within the same clade as *M. anchietae* and *M. cuneirostris*, but the bauplans of all three species are similar suggesting a separate origin of this morphology due to similarity in microhabitat (open habitat) within the xeric macrohabitat.

Whilst the morphological clusters were significantly different with respect to overall body slenderness (PC1) and linked to habitat openness, the lack of a significant difference for PC2 (Table 1) indicates that head shape is driven by factors other than habitat structure such as diet or sexual selection (e.g. [62,63]). Convergence in head shape within the dune-dwelling species (B2) may be as a result of their preference to sand-dive or to utilize burrows, both of which are behavioral adaptations for predator avoidance and thermoregulation [27,64]. The *Ichnotropis* (A1) head dimensions are not significantly different from the dunes cluster (B2) (F = 2.78, p = 0.10), and this could be due to a propensity for digging burrows for shelter and reproduction [65], thereby evolving the same relative head morphology [65]. Another possibility is that *Ichnotropis* may have a similar diet to the sand-dwelling species, which may be driving the similarity in head shape [65].

In terms of limb lengths, the two morphological clusters were significantly different (PC3), in particular because of the short limbs in *Tropidosaura*. The shorter forelimbs in conjunction with their slender bodies may allow *Tropidosaura* to optimize maneuvering performance while negotiating cluttered vegetation (e.g. [17,27]), whereas the long limbs of the *Ichnotropis* spp. (A1) and

Table 2. Analysis of variance (ANOVA) results for morphological clusters.

PC1	Df	Sum-Sq	Mean-Sq	F-value	P	PC2	Df	Sum-Sq	Mean-Sq	F-value	P	PC3	Df	Sum-Sq	Mean-Sq	F-value	P			
A1	A2	1	0.67	0.67	1.54	0.22	A1	A2	1	37.50	37.50	56.04	<0.001	A1	A2	1	28.32	28.32	34.28	<0.001
	A3	1	1.84	1.84	6.64	0.01		A3	1	10.92	10.92	17.71	<0.001		A3	1	61.63	61.63	108.44	<0.001
	B1	1	63.95	63.95	147.48	<0.001		B1	1	27.60	27.60	36.36	<0.001		B1	1	6.91	6.91	7.09	0.01
	B2	1	139.27	139.26	409.44	<0.001		B2	1	1.90	1.90	2.78	0.10		B2	1	1.16	1.16	2.41	0.12
A2	A3	1	0.71	0.71	1.30	0.26	A2	A3	1	0.39	0.39	0.49	0.48	A2	A3	1	20.25	20.25	24.28	<0.001
	B1	1	89.80	89.80	181.98	<0.001		B1	1	6.21	6.21	7.79	0.01		B1	1	17.64	17.64	17.15	<0.001
	B2	1	175.14	175.14	384.49	<0.001		B2	1	73.35	73.35	97.15	<0.001		B2	1	25.77	25.77	42.71	<0.001
A3	B1	1	36.02	36.02	77.89	<0.001	A3	B1	1	0.44	0.44	0.54	0.46	A3	B1	1	50.91	50.91	50.98	<0.001
	B2	1	73.60	73.60	197.33	<0.001		B2	1	18.76	18.76	24.73	<0.001		B2	1	58.64	58.64	156.77	<0.001
B1	B2	1	44.42	44.42	100.31	<0.001	B1	B2	1	77.69	77.69	98.86	<0.001	B1	B2	1	3.45	3.45	4.07	<0.001

Analysis of variance (ANOVA) results for morphological clusters (A1, A2, A3, B1 and B2; as in Fig. 2). Significant differences (P<0.05) are indicated in bold, italic font. PC=principal component, Df=degrees of freedom, Sum-Sq=sum of squares value, Mean-Sq=mean sum of squares value, P=significance value. doi:10.1371/journal.pone.0051636.t002

those inhabiting more open habitats (B1 and B2) should increase sprint performance (e.g. [26,27,66–68]). Relative forelimb and hindlimb dimensions, however, need to be investigated in conjunction with substrate type and structure, as opposed to habitat structure, in order to better understand the evolution of limb dimensions in Eremiadini.

Although sub-sets of taxa from *Meroles* and *Ichnotropis* were investigated as part of higher level lacertid phylogenies, the placement of *I. squamulosa* within *Meroles* was not identified previously due to the inclusion of only a single *Ichnotropis* (*I. squamulosa*) and various *Meroles* (*M. knoxii*, *M. suborbitalis* or *M. ctenodactylus*) in those analyses [33,40,69]. Despite their placement in the phylogeny, *I. capensis* and *I. squamulosa* do not differ significantly morphologically, and cluster together when body dimensions, head measurements and limbs measurements are investigated. Both of these species possess more slender bodies relative to *Meroles*. In addition, they share characters not possessed by *Meroles* (rough scales and the absence of a nuchal collar). Because these two species have partially sympatric distributions, their overlapping niche might explain the observed morphological similarities. For example, limb dimensions could reflect adaptation to substrate type, while head shape similarities could reflect adaptation to similar diets. Although neither have a nuchal collar, this is also absent in other *Meroles* (i.e. *M. anchietae*), as well as other lacertids (e.g. *Tropidosaura*). Thus, the presence/absence of the collar is unlikely to be a synapomorphy (Fig. S1). Similarly, the presence of a gular fold (similar to a nuchal collar, but does not extend all the way around the head) does not appear to be a character than can be used to indicate shared ancestry (Fig. S1). The other characteristic feature that has linked these species in the past is the presence of rough (strongly keeled) scales. However, this is also not a synapomorphy as other lizards and even lacertids (e.g. *Tropidosaura*) are known to have rough scales suggesting shared scale micro-ornamentation is not an indication of a shared ancestry in lacertid lizards but rather related to microhabitat use [70].

There are several interesting implications of the placement of *I. squamulosa* within *Meroles*, rather than *Ichnotropis*. Sympatry often leads to competition for resources particularly between closely related species. *Ichnotropis squamulosa* is sympatric with *I. capensis* in the northern regions of its distribution, but is allopatric with all *Meroles*. Whilst *Meroles* are primarily sand-dwellers, *Ichnotropis* are classified as terrestrial [71], with a propensity for sandy habitats in mesic and arid savannah [65]. The reproductive cycles of *I. squamulosa* and *I. capensis* are not concordant [65,72,73], which is thought to prevent interspecific competition [72,73]. Both species are considered to be annual breeders, although the breeding times are staggered [73], and life-spans are unusually short for lacertid lizards. *Ichnotropis squamulosa* lives approximately eight to nine months, mating in late summer and hatchlings appear in spring [65,73]. *Ichnotropis capensis* may live only marginally longer (13–14 months), mating in spring with hatchlings appearing in late summer [65,73]. It has been suggested that this staggered reproductive pattern arose to prevent interspecific competition between closely related species [74]. However, because these species are not closely related, this shared life-history trait cannot be associated with a reduction of competition between sister taxa, but rather suggests an independent evolution of a similar but temporally disjunct reproductive strategy. The reasons for this are not clear, particularly because *I. squamulosa* still exhibits the same reproductive strategy in regions where the two species are not sympatric (e.g. in Upington, South Africa [73]) suggesting that the staggered reproduction of the two species is not driven by interspecific competition.

Morphological adaptation to a particular microhabitat may confer a greater fitness to individuals through their performance (for a review see [75]). We show that habitat openness determines the morphological shape of southern African lacertid species and we expect that these differences in morphology will, in turn, be associated to performance differences between the species. Those species adapted to open dunes may be better sprinters than those inhabiting cluttered rocky environments, whilst the rock-dwellers may be better climbers than sand dwellers. A closer investigation into associations between body and limb shape and performance in southern African lizards is needed to understand the functional implications of the morphological shape differences in southern African lacertid lizards.

Supporting Information

Figure S1 Phylogenetic trees of the southern African radiation of the lacertid subfamily Eremiainae based on the partial (A) *16S*, (B) *ND4*, (C) *RAG1* and (D) *KLAA* gene regions and inferred by BI. Sample numbers are indicated at terminal tips, and species names are given (right). Posterior probabilities ≥ 0.95 are above the nodes. Key to the species abbreviations: *Australolacerta* (red): AA = *Australolacerta australis*, AR = *A. rupicola*; *Ichnotropis* (gray): IB = *Ichnotropis bivittata*, IC = *I. capensis*, IS = *I. squamulosa*; *Meroles* (orange): MA = *Meroles anchietae*, MCT = *M. ctenodactylus*, MCU = *M. cuneirostris*, MK = *M. knoxii*, MS = *M. suborbitalis*; *Pedioplanis* (light blue): PB = *Pedioplanis burchelli*, PI = *P. inornata*, PLL = *P. lineocellata lineocellata*, PLP = *P. l. pulchella*, PN = *P. namaquensis*; *Tropidosaura* (blue): TG = *Tropidosaura gularis*, TMM = *T. montana montana*, TMR = *T. m. rangeri*. (TIF)

Figure S2 Phylogenetic tree of the southern African radiation of the lacertid subfamily Eremiainae based on the combined partial *16S*, *ND4*, *RAG1* and *KLAA* gene regions and inferred by BI and ML (Bayesian topology shown). Sample numbers are indicated at terminal tips, and species names are given. Posterior probabilities ≥ 0.95 are above the nodes and bootstrap values $\geq 75\%$ are below nodes. Filled stars next to species names indicate presence of both a gular fold and a nuchal collar in the species, open stars indicate presence of nuchal collar only, filled circles indicate presence of gular fold only. (TIF)

Figure S3 Photographs of cluttered (A) and open habitat (B), as examples of the two habitat categories defined for this study (Photos by SE). (TIF)

Figure S4 Hierarchical clustering of size-regressed morphological measurements, with “approximately unbiased” support values above the nodes. Support values ≥ 0.95 are considered supported. For key to cluster abbreviations see Figure 2 and key to the species abbreviations see Figure S2. (TIF)

Figure S5 Scatterplots of the principal components analysis (PCA) scores for the first and second (bottom), and first and third (top) principal component axes. Colors of the symbols correspond to the hierarchical clustering: green = A1, light green = A2, dark green = A3, light blue = B1, dark blue = B2. Boxplots next to axes show the mean and 95% confidence intervals of each morphological cluster for each PC axis, and label abbreviations as in Figure 2. Boxplots of PC1 below the scatterplots, PC2 are bottom-left and PC3 are top-left. Divisions for the boxplots are indicated by the color and at the axis. (TIF)

Table S1 List of specimens used in the phylogenetic analyses with genus and species names, ID numbers, Museum accession ID numbers and EMBL accession numbers for each gene. (DOCX)

Table S2 List of specimens used in morphometric analyses, genus and species names, ID numbers from either the Ditsong museum (TM), Port Elizabeth Museum (PEM), or field trips. (DOCX)

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Author Contributions

Conceived and designed the experiments: SE,KT,JM. Performed the experiments: SE. Analyzed the data: SE KT. Contributed reagents/materials/analysis tools: SE AH BV JM KT. Wrote the manuscript: SE AH BV JM KT. Other (please specify): Funding of field work: SE AH BV JM KT.

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Taxonomic adjustments in the systematics of the southern African lacertid lizards (Sauria: Lacertidae)

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Abstract

Molecular phylogenetic analyses of southern African lacertid lizards (Eremiadini) using mitochondrial and nuclear markers revealed two examples of generic assignments incompatible with monophyletic clades. *Australolacerta* Arnold 1989, a genus endemic to South Africa and to which two isolated species have been referred, is paraphyletic at the generic level. In addition, the species *Ichnotropis squamulosa* Peters 1854 was found to be embedded within the genus *Meroles*. To resolve the paraphyly in *Australolacerta* we erect a new genus, *Vhembelacerta* Edwards, Branch, Herrel, Vanhooydonck, Measey, & Tolley, **gen. nov.**, to accommodate *Lacerta rupicola* FitzSimons 1933. To maintain a monophyletic *Ichnotropis* Peters 1854, *Ichnotropis squamulosa* Peters 1854 is transferred to *Meroles* Gray 1838, now named *Meroles squamulosus* **comb. nov.** Where necessary the genera affected by these actions are re-characterized.

Key words: Lacertidae, Eremiadini, *Ichnotropis squamulosa*, *Australolacerta*, paraphyly, mitochondrial and nuclear DNA

Introduction

Lacertids are a diverse group of lizards, ubiquitous throughout much of the Old World and occur in a wide variety of habitats; e.g. high mountain tundra, heath lands, Mediterranean scrub, tropical forest, semi-desert and desert (FitzSimons 1943; Arnold 1989; Branch 1998). However, they have an unusual distribution, with only a limited penetration into south-east Asia, and are absent from Australia and Oceania. Lacertids are also absent from Madagascar but occur throughout mainland Africa, with high regional endemism at both genus and species level (Branch 1998; Spawls *et al.* 2002). Although diverse lacertid faunas occur in southern Africa (at least 8 genera and 37 species; Branch 1998; Conradie *et al.* 2012) and eastern Africa (10 genera, 19 species; Spawls *et al.* 2002; Greenbaum *et al.* 2011), only three species (*Nucras ornata* (Gray 1864), *Ichnotropis capensis* (Smith 1838) and *I. squamulosa* Peters 1854) occur in both regions and then only marginally, with the southern African species just entering the southern parts of East Africa.

Early classification of lacertids, as with that of most organisms, relied almost exclusively on morphological characteristics, occasionally supplemented with other types of biological data. Phylogenetic hypotheses of lacertid

relationships were originally based on morphology, and Arnold (1986; 1989) presented a generic level phylogeny which recognised two Afrotropical groups: a South African clade containing *Tropidosaura* Fitzinger 1826, *Pedioplanis* Fitzinger 1843, *Meroles* Gray 1838 and *Ichnotropis* Peters 1854; and another comprising *Nucras* Gray 1838 and a subclade consisting of *Latastia* Bedriaga 1884, *Heliobolus* Fitzinger 1843 and *Philochortus* Matschie 1893, referred to as the Northeast African group.

The use of molecular phylogenetics as a tool for systematics has revealed some surprising relationships for lizards, which are often incongruent with taxonomy based on morphological characters (e.g. lack of genetic distinction between morphologically different agamids: *Agama agama* and *A. finchi*; Leaché *et al.* 2009; *Agama boueti* and *A. castroviejoi*; Gonçalves *et al.* 2012). Morphological traits can be labile, and the phenotype may be influenced by factors such as microhabitat and environment (e.g. Vanhooydonck & Van Damme 1999; Herrel *et al.* 2002; Revell *et al.* 2007; Barros *et al.* 2011; Hopkins & Tolley 2011; Edwards *et al.* 2012; Herrel *et al.* 2013), dietary preferences (e.g. Measey *et al.* 2009), sexual selective pressures (Herrel *et al.* 2011), or a combination of these factors. If a particular environment places a selective pressure on a lizard to evolve a specific trait, then lizards living in similar environments may evolve convergent phenotypes (e.g. Revell *et al.* 2007; Edwards *et al.* 2012), confounding taxonomy. Arnold (1991) investigated why phylogenies based on morphology varied considerably in quality, based (in part) on what he considered to be a “robust and explicit morphological phylogeny” for *Meroles* and a poorly-supported morphological phylogeny for *Pedioplanis*. He found the former to most likely result from exposure to different ecological conditions, resulting in pectinate phylogenies or what is often termed an ‘adaptive radiation’. In *Meroles* this was postulated to reflect the increasing acquisition of morphological synapomorphies in species inhabiting increasingly more aeolian habitats.

To ensure that systematics and taxonomy reflect evolutionary history, molecular phylogenies are routinely used as a guide. Recent molecular studies have indicated primary divisions within the Lacertidae, although there has been debate as to the taxonomic hierarchy assigned to the divisions. Harris *et al.* (1998) divided the family into three subfamilies: Gallotiinae, Eremiainae and Lacertinae, but it now seems more appropriate to recognize Gallotiinae as a clade sister to Lacertinae. The latter contains the tribes Eremiadini Szczerbak 1975 and Lacertini Oppel 1811 (Arnold *et al.* 2007; Kapli *et al.* 2011; Salvi *et al.* 2011), of which only the Eremiadini occurs in sub-Saharan Africa.

Various phylogenies, based on molecular markers, have generally agreed on relationships between southern African lacertid genera within Eremiadini (Mayer & Pavlicev 2007; Hipsley *et al.* 2010; Kapli *et al.* 2011), and Salvi *et al.* (2011) showed a sister-group relationship within the Eremiadini of *Australolacerta* Arnold 1989 and *Tropidosaura*. However, these phylogenies used only a few representatives from each genus, and thus interspecific relationships within genera remained largely unknown. To date only phylogenies for the southern African genera *Meroles* (Harris *et al.* 1998; Lamb & Bauer 2003; Edwards *et al.* 2012) and *Pedioplanis* (Makokha *et al.* 2007; Conradie *et al.* 2012) have been investigated. The evolutionary history of both genera, as well as of other lizards in the subcontinent (Lamb *et al.* 2003; Bauer & Lamb 2005), was thought to be driven by habitat changes induced by climate aridification during the Mid-Miocene (Siesser 1978; 1980).

Southern African lacertid lizards inhabit a wide variety of microhabitats, differing in substrate, openness, elevation and inclination (or slope) (Branch, 1998). In instances where unrelated species are convergent in morphology due to occurrence in similar habitats, species may be incorrectly classified. Recent molecular phylogenies using mitochondrial and nuclear markers for southern African lacertid lizards (Eremiadini) revealed two examples of existing generic assignments incompatible with evolutionary history (Edwards *et al.* 2012; Engleder *et al.* 2013). The first was that *Ichnotropis squamulosa*, a species previously not included in higher level phylogenies, grouped within a clade containing nearly all of the described *Meroles*. This species did not group with other *Ichnotropis* previously incorporated in phylogenies, including the type species *I. capensis*. The second was that the two known *Australolacerta* species, *A. australis* Hewitt 1926 and *A. rupicola* FitzSimons 1933, showed high levels of genetic divergence and were paraphyletic with respect to *Tropidosaura*, *Ichnotropis*, and *Meroles*. These unexpected results were interpreted to be due to convergence in morphology between species from different lineages (Edwards *et al.* 2012). These phylogenetic results have taxonomic consequences. We therefore conducted a re-analysis of evolutionary relationships within southern African lacertids within a taxonomic framework.

Material and methods

Sampling and laboratory protocols. We obtained complete genus level taxon sampling of southern African Eremiadini (*Meroles*, *Australolacerta*, *Pedioplanis*, *Tropidosaura*, *Ichnotropis*, *Nucras* and *Heliobolus*), which included complete sampling for *Australolacerta*, and near complete species level taxon sampling for *Meroles*. In order to re-investigate the phylogenetic relationships of all southern African lacertid lizard taxonomic groups, individuals used in Edwards *et al.* (2012) were included and additional individuals from *Pedioplanis* and *Nucras* (highlighted in grey in Table 1) were sequenced using standard PCR techniques for four genes (mitochondrial: 16S, ND4 and nuclear: RAG1, KIAA2018) as in Edwards *et al.* (2012). Sequences were aligned using Clustal Omega v.1.1.0 (Sievers *et al.* 2011) and checked in BioEdit Sequence Alignment Editor v. 7.0.5.2 (Hall 1999). All sequences have been deposited in EMBL-Bank (see Table 1 for all voucher information, with corresponding EMBL-Bank accession numbers).

Genetic analyses. We first analysed the mitochondrial (16S vs. ND4) and nuclear (RAG1 vs. KIAA2018) datasets separately and then analyzed the combined dataset (nuclear vs. mitochondrial), using a partition homogeneity test (Farris *et al.* 1994; 1995) in PAUP* v4.0b10 (Swofford 2002), to ensure that there was no conflict in the markers within each genome. The saturation of the codon positions was assessed (Dambe v.5.2.65; Xia *et al.* 2003) and the third codon position of the ND4 gene was found to be saturated, so it was coded as a separate partition in the maximum likelihood and Bayesian analyses using nucleotide substitution models (thus five partitions in total: 16S, ND4-1, ND4-2, RAG1 and KIAA2018). Individuals from two genera (*Nucras* and *Heliobolus*) were used as outgroup, as they are nested within the sister clade to the southern African lacertids within the Eremiadini (Mayer & Palicev 2007; Kapli *et al.* 2011). Sequence divergences (uncorrected *p*-distances) were determined in MEGA v.4 (Tamura *et al.* 2007).

Phylogenetic trees were constructed based on the combined total evidence dataset using two different algorithms (Figure 1). Bayesian inference (BI; MrBayes v.3.1.0; Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) was performed using the best-fit models of nucleotide substitution for all five gene partitions (Modeltest v.3.6; Posada & Crandall 1998). The best-fit models of nucleotide substitution for all the mitochondrial gene partitions were GTR+I+G and all the nuclear gene partitions were GTR+G, and uniform priors were kept for all other parameters. A second Bayesian inference was performed, using a codon substitution model for all three partitions of coding genes (ND4, RAG1 and KIAA-2018) and the best-fit model of nucleotide substitution (GTR+I+G) for the 16S gene fragment partition. The nucleotide substitution parameters within the codon models were of the 6-rate variety (inferring different rates for all nucleotide pairs, GTR-like), with empirical codon frequencies. The MCMCs were run with 2 parallel runs for 20×10^6 generations each, sampling trees every 1000 generations. The number of generations to discard as burn-in was determined by examining the number of generations 1) at which the standard deviation of split frequencies stabilized (at less than 0.001), 2) at which the log-likelihood tree scores reached stationarity, and 3) the effective sample sizes (ESS) of all parameters which were ≥ 600 (Tracer v.1.5; Rambaut & Drummond 2007). A 50% majority rule tree was constructed with the burn-in excluded using the 'sumt' command in MrBayes, and nodes with ≥ 0.95 posterior probability values were considered supported. A partitioned maximum likelihood (ML) analysis was also run in RAxML v.7.2.8 (Stamatakis 2006), at the CIPRES Science Gateway (www.phylo.org/sub_sections/portal/) using the same partitions as the Bayesian analysis, a GTR+I+G model of evolution, and automatic halting of bootstrapping (Stamatakis 2006; Stamatakis *et al.* 2008).

Competing phylogenetic hypotheses of monophyly for *Ichnotropis* and *Australolacerta* were investigated using a Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa 1999; Goldman *et al.* 2000) and the approximately unbiased (AU) test (Shimodaira, 2002) generating maximum likelihood scores for the trees (1000 replicates) using PAUP* v.4.0b10 (Swofford 2002) and bootstrapping *p* values for the SH and AU tests in Consel (Shimodaira 2002). The Bayesian consensus topology obtained was compared to a topology which constrained 1) *I. squamulosa* to be within *Ichnotropis*, and 2) *Australolacerta australis* and *A. rupicola* as monophyletic.

Results

Phylogenetic trees were obtained using an aligned sequence dataset of a total of 2683 nucleotide base pairs (bp) from the four genes (16S: 515bp, ND4: 678bp, RAG1: 679bp, KIAA: 813bp) for the 64 taxa. The number of variable sites

for each gene were as follows: 16S = 134, ND4 = 434, RAG1 = 357, KIAA = 199 (1076 total variable sites). The topologies of the phylogenetic trees obtained with the additional taxa were similar to the one obtained by Edwards *et al.* (2012), with the trees obtained using the codon- substitution models being the best resolved with the highest node support values (Figure 1). The generic-level polytomy was again found when nucleotide-substitution models were employed. However the polytomy between *Tropidosaura*, *Ichnotropis*, *Australolacerta* and *Meroles* was resolved when a codon-substitution model was used. The two examples of conflict with existing classification observed in the earlier study were again recovered within this phylogenetic study: a) *Ichnotropis squamulosa* falls within *Meroles*, not *Ichnotropis*; and b) the two *Australolacerta* species are genetically distinct and do not form a monophyletic clade.

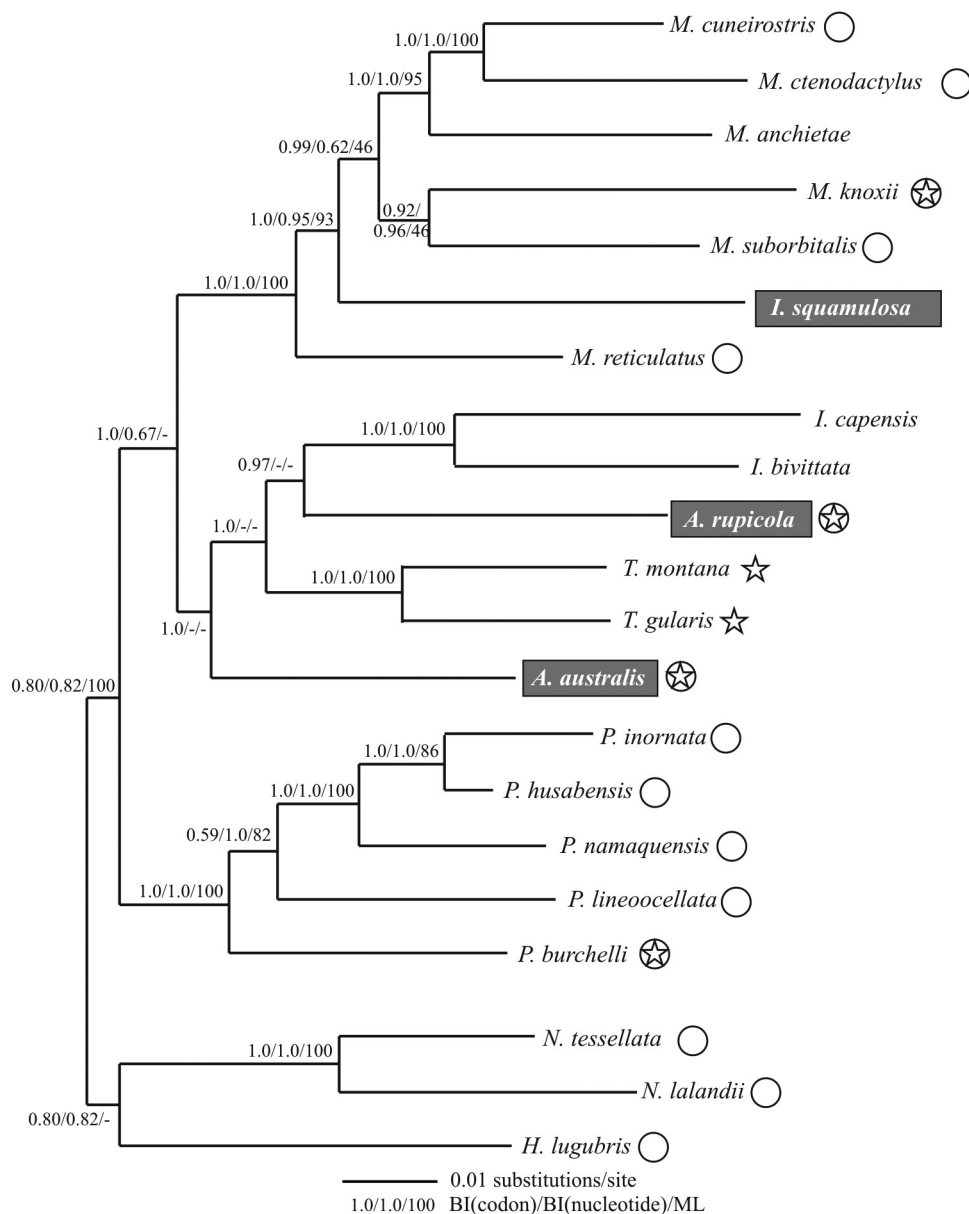


FIGURE 1. Phylogenetic relationships of the southern African clade of lacertid lizards (Lacertidae: Eremiadini) estimated from four mitochondrial and nuclear markers (Bayesian topology estimated using a nucleotide substitution model shown). Nodes that are supported using Bayesian inference (posterior probabilities > 0.95) using nucleotide substitution models and maximum likelihood (bootstrap values >75%) using GTR+I+G nucleotide substitution model are shown at nodes (post. prob. using nucleotide-substitution model/bootstrap value for ML). A dash indicates that the node was not supported for the particular analysis. Species highlighted in grey are those species which are reclassified in this study. Stars next to species names indicate presence of gular fold; circles indicate presence of collar and a star within a circle indicate the presence of both a gular fold and a collar.

TABLE 1. List of specimens used in the phylogenetic analyses with genus and species names, ID numbers, Museum accession ID numbers and EMBL accession numbers for each gene. All sequences listed here were used in Edwards *et al.* (2012), except for those highlighted in grey, which were additional individuals sequenced for this study.

Genus	Species	SANBI Herpbank Accession ID	Museum Accession ID*	EMBL accession (16S)	EMBL accession (ND4)	EMBL accession (RAG1)	EMBL accession (K1AA)
<i>Australolacerta</i>	<i>australis</i>	GW08	N/A	HF547772	HF547725	HF547691	HF547651
		MH0531	N/A	DQ871152 ^{\$}	HF547726	DQ871208 ^{\$}	HF547652
<i>Australolacerta</i>	<i>rupicola</i>	MCZ38869	MCZ38869	HF547773	HF547727	HF547692	HF547653
		MCZ38874	MCZ38874	HF547774	HF547728	HF547693	HF547654
<i>Heliobolus</i>	<i>lugubris</i>	MCZ37870	MCZ37870	DQ871141 ^{\$}	HF547729	DQ871199 ^{\$}	—
		MCZ37894	MCZ37894	DQ871142 ^{\$}	HF547730	DQ871200 ^{\$}	HF547655
<i>Ichnotropis</i>	<i>bivittata</i>	KTH09-075	MBUR2074	HF547775	HF547731	HF547694	HF547656
<i>Ichnotropis</i>	<i>capensis</i>	AMB6001	N/A	DQ871148 ^{\$}	HF547732	DQ871206 ^{\$}	HF547657
		AMB6067	CAS209602	DQ871149 ^{\$}	HF547733	DQ871207 ^{\$}	HF547658
<i>Ichnotropis</i>	<i>squamulosa</i>	WP031	N/A	—	HF547734	HF547695	HF547659
		MB21340	N/A	—	—	HF547698	HF547661
		RSP373	N/A	HF547777	HF547737	HF547699	HF547662
		SVN362	N/A	HF547776	HF547736	HF547697	HF547660
		WP122	N/A	—	—	HF547700	HF547663
		WP125	N/A	HF547778	HF547738	HF547701	—
<i>Merolus</i>	<i>anchietae</i>	PEMR17286	PEMR17286	HF547779	—	—	—
		WP928	N/A	HF547781	HF547740	HF547703	—
		WC09-011	PEMR17931	HF547780	HF547739	HF547702	HF547664
<i>Merolus</i>	<i>ctenodactylus</i>	JM03609	N/A	HF547783	HF547741	HF547704	—
		JM03611	N/A	—	HF547742	HF547705	HF547665
		JM03613	N/A	HF547784	HF547743	HF547706	HF547666
<i>Merolus</i>	<i>cuneirostris</i>	WP921	N/A	HF547788	HF547747	HF547710	HF547670
		WP914	N/A	HF547787	HF547746	HF547709	HF547667
		MB20484	MB20484	HF547785	HF547744	—	HF547667
		MCZA38244	MCZA38244	HF547786	HF547745	HF547708	HF547668
<i>Merolus</i>	<i>knoxii</i>	H6179	H6179	HF547790	HF547750	HF547712	—
		AMB5629	CAS:HERP:206782	DQ871146 ^{\$}	HF547748	DQ871204 ^{\$}	HF547671
		ATTKMK2	N/A	HF547789	HF547749	HF547711	HF547672
		SER017	N/A	HF547791	HF547751	—	—
		SVN084	PEMR18357	HF547792	HF547752	HF547713	—

.....continued on the next page

TABLE 1. (Continued)

Genus	Species	SANBI Herpbank Accession ID	Museum Accession ID*	EMBL accession (16S)	EMBL accession (ND4)	EMBL accession (RAG1)	EMBL accession (K1AA)
<i>Meroles</i>	<i>reticulatus</i>	WP010	N/A	HF547795	HF547754	HF547715	HF547674
		WC09-005	PEMR17938	HF547794	HF547753	HF547714	HF547673
		WP011	N/A	HF547796	HF547755	—	HF547675
<i>Meroles</i>	<i>suborbitalis</i>	AJC638	N/A	HF547797	HF547756	HF547716	HF547676
		MB20609	PEMR16974	HF547798	HF547757	—	—
		MB21589	N/A	HF547799	HF547758	HF547717	HF547677
		SVN049	PEMR18376	HF547800	HF547759	HF547718	HF547678
<i>Nucras</i>	<i>lalandii</i>	HB124	N/A	HF951553	HF951532	HF951537	—
		HB037	N/A	HF951554	HF951533	HF951538	HF951548
		HZ246	N/A	HF951555	HF951534	HF951539	HF951549
<i>Nucras</i>	<i>tessellata</i>	MB20650	PEMR16873	HF951556	HF951535	HF951540	HF951550
		MB20687	PEMR16872	HF951557	HF951536	HF951541	HF951551
		MB21061	N/A	HF951558	—	HF951542	HF951552
		KTH08-069	N/A	HF951559	—	HF951543	—
<i>Pedioplanis</i>	<i>burchelli</i>	KTH137	N/A	DQ871122 ^{\$}	—	DQ871180 ^{\$}	—
		MH0334	N/A	DQ871120 ^{\$}	HF547761	DQ871178 ^{\$}	HF547679
<i>Pedioplanis</i>	<i>husabensis</i>	MCZFS37127	MCZ R184164	DQ871139 ^{\$}	—	DQ871197 ^{\$}	—
		ABE473	N/A	DQ871138 ^{\$}	—	DQ871196 ^{\$}	—
<i>Pedioplanis</i>	<i>inornata</i>	ABE-393-mu	NHMW 35340:9	DQ871137 ^{\$}	HF547762	DQ871195 ^{\$}	HF547680
		KTH595	N/A	DQ871140 ^{\$}	—	DQ871198 ^{\$}	—
<i>Pedioplanis</i>	<i>lineoocellatilineoocellata</i>	ABA-20-mu	NHMW 35360:1	DQ871106 ^{\$}	HF547763	DQ871164 ^{\$}	HF547681
<i>Pedioplanis</i>	<i>lineoocellata pulchella</i>	MH0336	N/A	DQ871107 ^{\$}	HF547764	DQ871165 ^{\$}	—
		SVN189	N/A	HF547802	HF547765	HF547719	HF547682
<i>Pedioplanis</i>	<i>namaquensis</i>	AMB4541	N/A	DQ871099 ^{\$}	HF547766	DQ871157 ^{\$}	HF547684
		AMB4558	CAS:HERP-200033	DQ871101 ^{\$}	HF547767	DQ871159 ^{\$}	HF547685
<i>Tropidosaura</i>	<i>gularis</i>	EL036	N/A	HF547803	—	HF547720	HF547686
<i>Tropidosaura</i>	<i>montanamontana</i>	RSP200	N/A	HF547804	HF547768	HF547721	HF547687
<i>Tropidosaura</i>	<i>montanarangeri</i>	HB082	N/A	HF547805	HF547769	HF547722	HF547688
		MBUR00544	N/A	HF547806	HF547770	HF547723	HF547689
		MBUR00552	N/A	HF547807	HF547771	HF547724	HF547690

* N/A = Individuals were measured alive in the field and released, no voucher specimen deposited in a museum; TM = Ditsong museum; PEM = Port Elizabeth Museum; MCZ = Museum of Comparative Zoology, Harvard University; CAS = Californian Academy of Science; H = Ellerman Collection of Stellenbosch University
^{\$} Makokha JS, et al. (2007) Mol. Phylogenet. Evol. 44 (2), 622-633
[#] Harris DJ, Batista V, Carretero MA (2004) *Amphibia-Reptilia* 25: 227-232
^{##} Harris, DJ, Arnold, EN Thomas, RH (1998) Mol. Phylogenet. Evol. 10 (1), 37-48
^{**} Mayer W, Pavlicev M (2007) Mol. Phylogenet. Evol. 44 (3): 1155-1163

The members of *Pedioplanis* formed a well-supported monophyletic clade, as was expected (Edwards *et al.* 2012). The genera *Ichnotropis*, *Australolacerta* and *Tropidosaura* formed a well-supported clade (using the codon substitution model), within which *Australolacerta* was polyphyletic. Divergence between the two *Australolacerta* species was high compared to other inter-specific divergence levels within this study (16S: $9.55 \pm 2.08\%$; ND4: $22.69 \pm 1.60\%$; RAG1: $3.74 \pm 0.76\%$; KIAA: $1.90 \pm 0.47\%$), although a monophyletic *Australolacerta* could not be rejected using the SH and AU tests. *Meroles* formed a well-supported clade, within which the sand-diving, psammophilic species (*M. anchietae*, *M. cuneirostris*, *M. ctenodactylus*) formed a well-supported subclade. As was found previously (Edwards *et al.* 2012), *I. squamulosa* grouped with *Meroles* with strong support, and inclusion of this species within a monophyletic *Ichnotropis* can be rejected by the SH and AU tests ($P < 0.01$, $P < 0.001$, respectively).

Discussion

The phylogenetic analyses in this study shows that the two *Australolacerta* species are not monophyletic and that *Ichnotropis squamulosa* is placed within *Meroles*. The re-analysis using a codon-substitution model, instead of nucleotide-substitution model of evolution, also increased support for some nodes at the generic-level between the southern African lacertid lizards.

Codon-based models (such as GY94; Goldman & Yang 1994; Muse & Gaut 1994) may be the most biologically realistic models of coding sequence evolution as they explicitly incorporate information about the genetic code (Shapiro *et al.* 2006). However, the use of codon-substitution models in phylogenetic analyses is not as wide-spread as the use of nucleotide-substitution models, due to commonly used model selection programs, such as jModeltest (Posada & Crandall 1998), which do not include the codon-substitution models. Here, we found that the employment of a codon-substitution model produced the most resolved and best supported tree, clearly showing the paraphyly of *Australolacerta* and the placement of *I. squamulosa* within *Meroles*.

Monophyly of *Australolacerta*

The two species of *Australolacerta* are endemic to South Africa (Branch 1998), and both were originally placed within *Lacerta*, creating a zoogeographic paradox as most congeners were restricted to Eurasia (Arnold 1989). Arnold (1989), when describing *Australolacerta*, gave only a minimal diagnosis, noting that "... the South African species share a number of features with other Ethiopian lacertids which are not found in the apparent closest Palaearctic relatives, namely *Lacerta jayakari* etc.". The latter, now transferred to *Omanosaura*, was initially considered to form a basal lineage within the Eremiadini (Harris *et al.* 1998), although fuller taxon sampling of African lacertids (Arnold *et al.* 2007; Hipsley *et al.* 2009; Kapli *et al.* 2011) shows it to cluster with a suite of mainly north African genera (e.g. *Acanthodactylus*, *Mesalina*, *Ophisops*), with *Atlantolacerta* basal within the Eremiadini (Arnold *et al.* 2007). The sister relationship of *Australolacerta* and *Tropidosaura* proposed by Salvi *et al.* (2011) and Kapli *et al.* (2011) was based on the inclusion only of *A. australis*, and the inclusion of *A. rupicola* (Edwards *et al.* 2012; Engleder *et al.* 2013; this study) revealed the paraphyly of *Australolacerta* and the basal position of *A. australis* in a subclade including *Ichnotropis*, *Tropidosaura* and *A. rupicola*.

Both species are rupicolous and Kirchhof and Richter (2009) and Kirchhof *et al.* (2010a,b; 2012) give details of the species' biology. They are morphologically similar, albeit that many of these similarities are plesiomorphic within lacertids (Arnold 1989). Due to their high-altitude and small ranges (Branch 1998), the two species have been difficult to collect and therefore little morphological data exists for either species. Recent morphological analyses (Edwards *et al.* 2012) confirm the similarity between the two species. Yet, important features of hemipenial ornamentation and everted hemipenis structure remain unknown. Whether these similar morphologies reflect adaptive convergence to rupicolity or the retention of plesiomorphic features remains unknown.

The two species are allopatric and geographically separated from one another by a distance of approximately 1700km. Few other genera in southern Africa are known to show such large geographical disjunctions between congeners, and analysis of previous examples has often revealed deep genetic divergence best reflected in generic re-assignment. Examples include: the erection of the genera *Kinyongia* and *Nadzikambia* for non-South African

dwarf chameleons previously included in *Bradypodion* (Tilbury *et al.* 2006), and *Inyokia* for the problematic Swazi rock snake that was shown to be sister to the tropical forest snake *Homonotus modestus* (Kelly *et al.* 2011). In one of the few exceptions of congeneric range disjunctions within the subcontinent, cordylid flat lizards of the *Platysaurus capensis* complex are separated geographically from other *Platysaurus* by approximately 850km (Branch & Whiting 1997). Other described lacertid species are also separated from congeners by large distances, for example *Heliobolus lugubris* is separated from its congeners (*H. spekii*, and *H. nitidus*) by >2000km and *Ichnotropis chapini* is separated from other *Ichnotropis* by approximately 2000km (Branch, 1998; Spawls *et al.* 2002). However, the Central African region is undersampled and it is possible that with increased sample collection new species may be discovered or that ranges of described species may increase, lessening the geographic gap between congeners.

Although there were no significant differences between the obtained trees and the constrained trees in the SH or AU test when the topology was constrained to monophyly for *Australolacerta* sequence divergence estimates and the long branch lengths in the phylogenetic analyses (Figure 1) all other evidence strongly suggests that the two *Australolacerta* species do not share a recent evolutionary history (Edwards *et al.* 2012). To provide consistency between taxonomic divisions in the Eremiadini, we propose that the two species of *Australolacerta* should be placed in separate genera. The type species of *Australolacerta* is *Lacerta australis* (Arnold 1989), and we therefore erect a new genus for the remaining species *Lacerta rupicola*, based on morphology and genetic divergence.

***Vhembelacerta* Edwards, Herrel, Vanhooydonck, Measey, Tolley & Branch, gen. nov.**

Type species. *Lacerta rupicola* FitzSimons 1933

Content. *Vhembelacerta rupicola* (FitzSimons 1933)

Characterization and diagnosis. The monophyly of the monotypic genus *Vhembelacerta* is established on the basis of a suite of mitochondrial and nuclear markers (Edwards *et al.* 2012; this study). Morphologically similar to *Australolacerta* (differences noted in brackets below), it can be distinguished from all other lacertids by the following combination of characteristics (FitzSimons 1943; Jacobsen 1989; Branch 1998; Kirchoff & Richter 2009): size small, (SVL ~52mm), maximum snout-vent length (SVL) 70mm, tail somewhat depressed basally, cylindrical distally, nearly 1.5x SVL (up to 2x SVL); upper head shields smooth; nostril pierced between a supranasal, 2 postnasals and narrowly separated from first upper labial (in contact with first upper labial); supranasals in contact behind rostral; frontonasal much broader than long; prefrontals in contact; frontal hexagonal; supraoculars 4, 1st smallest and separated from frontal; parietals in contact with 4th supraocular; shallow parietal foramen present (absent); rostral not entering nostril; 5 (4) upper labials anterior to subocular, whose lower border is not distinctly narrowed (lower border much shorter than upper) and only feebly keeled; lower eyelid scaly but with a about 3 enlarged and elongate scales in the middle (no enlarged scales in lower eyelid); elongate tympanic shield on upper anterior border of large, exposed ear-opening; five pairs of chin shields, first smallest, first 3 in contact in midline; gular fold distinct (present, but not strongly marked); collar present, straight, free, composed of 7–8 scales; dorsal scales flat, hexagonal, faintly keeled posteriorly and in 34–43 rows across midbody (small, granular, non-keeled and about 68 across midbody); ventral plates quadrangular, feebly imbricate and in 6 longitudinal and about 26 (28) transverse rows; a very large preanal plate, bordered by smaller plates (enlarged preanal preceded by two smaller ones); femoral pores 15–19; subdigital lamellae smooth, about 26 below 4th toe (23–25); and the adpressed hindlimb reaches the armpit (to collar).

Coloration: top of head and dorsum dark brown, back with paired narrow reddish brown vertebral stripes, and a white dorsolateral stripe that extends from the eye to the tail base (head and dorsum olive-green, body with numerous spots that are yellow dorsally and white on flanks, demarcated by a dorsolateral series of orange spots).

Distribution. Endemic to the Soutpansberg mountain range in Limpopo Province, South Africa (Branch 1998).

Etymology. The species is endemic to the Vhembe region of Limpopo Province, South Africa, after which the genus name is partially constructed. The second part of the name “lacerta” (L. lizard) also retains the historical link to the genus *Lacerta* to which the single species was originally referred.

Australolacerta Arnold 1989

Type species. *Lacerta australis* Hewitt 1926

Content. *Australolacerta australis* (Hewitt 1926)

Characterization and diagnosis. With the transfer of *Lacerta rupicola* to *Vhembelacerta*, a re-diagnosis of *Australolacerta* is required. The monophyly of the monotypic genus *Australolacerta* is established on the basis of a suite of nuclear and mitochondrial markers (Edwards *et al.* 2012). Morphologically closest to *Vhembelacerta*, it can be distinguished from all other lacertids by the following combination of characteristics (FitzSimons 1943; Branch 1998): head moderately depressed, body feebly so; SVL 50–65mm, maximum 70mm; adpressed hindlimb reaches collar; tail cylindrical; head shields normal with upper head shields smooth, occipital region flat; snout pointed, shorter than postocular part of head; nostril pierced between the nasal and one or two postnasals, and 1st upper labial, with nasals in contact with one another behind rostral; frontoparietals paired in contact; parietals in contact with the 4th of four supraoculars and separated from the postoculars; parietal foramen absent; interparietal about twice as long as broad, in good contact with occipital; a series of 9 granules between supraoculars and supraciliaries; 4 upper labials anterior to subocular, which has a strongly-marked keel along upper border and a lower border that is much shorter than upper; elongate temporal shield posterior to the subocular, followed by 3 smaller rounded ones; temporal scales small and granular, similar to dorsal scales; ear-opening large, exposed, bordered anteriorly by an elongate tympanic shield and with no auricular denticulation; lower eyelid scaly, lacking vertically-enlarged scales in the middle; 6 lower labials and five pairs of enlarged chin-shields, 1st smallest, 4th largest, and 1st three pairs in median contact with one another; gular fold present, but not strongly marked; collar composed of 8 plates, straight, free, and even-edged; dorsal scales small, granular, smooth, similar to laterals and about 68 across midbody; ventral plates quadrangular, feebly imbricate, in 6 longitudinal and 28 transverse series; preanal plate enlarged, preceded by two smaller scales; Forelimb with small granular scales on upper surface of forearm and a series of strongly enlarged, smooth and imbricate plates along anterior surface of humerus; hindlimb with granular scales on upper surface of tibia; a series of much enlarged and vertically elongate plates run along anterior surface of thigh and on the lower surface of tibia; 16–19 femoral pores; sub-digital lamellae smooth; scales on tail enlarged, quadrangular, elongate; more or less smooth dorsally, becoming keeled distally, and below scales smooth basally, more strongly keeled and bluntly mucronate distally.

Coloration: head and dorsum dark brown to olive, with numerous pale spots arranged in more or less regular longitudinal series that are yellow on back, white on flanks, and separated by a dorsolateral series of orange spots; upper surface head with pale green to yellow vermiculations; distinct pale vertical stripes on temporal region; indistinct pale spots on tail; venter bluish green; labials, chin-shields and throat pale greenish, with small black spots and mottling.

Distribution. Found in the southwestern Cape Fold Mountains in Western Cape Province, South Africa (Branch 1998).

Taxonomic position of *Ichnotropis squamulosa* Peters, 1854

Both the present phylogenetic analysis (Figure 1) and that of Edwards *et al.* (2012) clearly place *I. squamulosa* within *Meroles* with strong support, and with genus-level sequence divergences between *I. squamulosa* and *Ichnotropis*. There was a significant difference between the Bayesian topology and that in which *I. squamulosa* was constrained within *Ichnotropis* (SH and AU test: $P < 0.01$) supporting the conclusion that *I. squamulosa* should be moved to *Meroles*. A relationship between *I. squamulosa* and *Meroles* was found previously using nuclear markers (Mayer & Pavlicev 2007) and combined mitochondrial and nuclear datasets (Harris *et al.* 1998; Kapli *et al.* 2011). However, the taxonomic significance was not previously appreciated due to the incomplete taxon sampling for *Ichnotropis* in those analyses.

Confusion of *I. squamulosa* with members of the genus *Ichnotropis* is understandable as they are very similar morphologically, and cluster together when body dimensions, head measurements and limbs measurements are investigated (Edwards *et al.* 2012). The species possess a more slender body than most *Meroles*, and in addition displays characters not possessed by other *Meroles* (e.g. strongly keeled, imbricate body scalation and rugose head shields). The geographic range and habitat of *I. squamulosa* overlaps with that of a number of *Ichnotropis*, but not

that of other *Meroles* (Branch 1998; Spawls *et al.* 2002). An overlapping niche may explain the morphological similarities between *I. squamulosa* and other *Ichnotropis*, where limb dimensions reflect adaptation to substrate, and similar head shape are adaptation to similar diets. The absence of a nuchal collar is also unusual within *Meroles*, but also occurs in *M. anchietae* as well as in other lacertids (e.g. *Tropidosaura*) and its loss may be secondary and not indicative of shared ancestry. Both *Meroles* and *Ichnotropis* have symmetrical armed hemipenes (Arnold 1986). However, due to intra-generic variation in hemipenial morphology in both *Ichnotropis* and *Meroles* (Arnold 1986), the hemipenis of *I. squamulosa* gives no insight to its generic placement. Indeed, the phylogenetic placement of *M. suborbitalis* (Figure 1) indicates that even the hemipenial armature in this species has been secondarily lost. Thus, we conclude that the similarity in body plan between *I. squamulosa* and *Ichnotropis* is a result of convergence and not shared ancestry (Edwards *et al.* 2012), and in light of genetic monophyly (Figure 1) we therefore transfer this species to the genus *Meroles*.

***Meroles* Gray 1838**

Type species. *Meroles knoxii* (Milne-Edwards 1829)

Content. *Meroles anchietae* (Bocage 1867), *Meroles ctenodactylus* (Smith 1838), *Meroles cuneirostris* (Strauch 1867), *Meroles knoxii* (Milne-Edwards 1829), *Meroles micropholidotus* Mertens 1938, *Meroles reticulatus* (Bocage 1867), *Meroles squamulosus* (Peters 1854), *Meroles suborbitalis* (Peters 1869)

Characterization and diagnosis. The inclusion of *M. squamulosus* requires the genus to be redefined. Head shields normal and usually smooth (rugose in *squamulosus*), but occipital often very small or absent; nostril pierced between three nasals and widely separated from 1st upper labial; subocular not bordering mouth; lower eyelid scaly, without window; collar distinct (absent in *squamulosus*); gular fold absent; dorsal scales granular, juxtaposed or subimbricate, (but rhombic, strongly keeled and imbricate in *squamulosus*); ventral plates smooth, not or feebly imbricate, posterior borders straight; digits subcylindrical, compressed or depressed (feebly compressed in *squamulosus*), laterally serrated, denticulated or fringed (except in *squamulosus*); subdigital lamellae smooth or keeled (pluricarinate and spinulose in *squamulosus*), femoral pores present; parietal foramen present (absent or feebly marked in *squamulosus*); and tail long and cylindrical (in *knoxii*, *suborbitalis* and *squamulosus*) or depressed basally and feebly compressed distally.

Remark. As the gender of *Meroles* is masculine the specific ending of *squamulosa* must be adjusted accordingly to *squamulosus*.

***Ichnotropis* Peters 1854.**

Type species. *Ichnotropis macrolepidota* (Peters 1854); = *I. capensis* (Smith 1838)

Content. Uetz (2012) recognizes six species (excluding *squamulosus*): *Ichnotropis bivittata* Bocage 1866, *Ichnotropis capensis* (A. Smith 1838), *Ichnotropis chapini* Schmidt 1919, *Ichnotropis grandiceps* Broadley 1967, *Ichnotropis microlepidota* Marx 1956, *Ichnotropis tanganicana* Boulenger 1917.

Characterization and diagnosis. The monophyly of the genus *Ichnotropis* remains to be established with complete taxon sampling of the referred species. The removal of *M. squamulosus* from *Ichnotropis* does not significantly alter the diagnosis for the genus given in FitzSimons (1943), as morphological variation within the remaining species still incorporates that of *M. squamulosus*.

Remark. No modern revision of the genus has been undertaken, and the status of a number of taxa remains equivocal, e.g. *Ichnotropis bivittata pallida* Laurent 1964; *Ichnotropis capensis nigrescens* Laurent 1952; *Ichnotropis microlepidota* Marx 1956, and the generic assignment of many requires molecular confirmation.

Revised key to genera of Southern African Lacertidae

- | | | |
|---|--|---|
| 1 | Tail cylindrical, without a lateral fringe; rock-living or terrestrial | 2 |
| - | Tail flattened, with a lateral fringe of large, flat scales; arboreal | <i>Holaspis</i> (Blue-tailed tree lizard) |
| 2 | Smooth or tubercular lamellae beneath toes | 3 |

	Keeled lamellae beneath the toes	6
3	A distinct collar present; dorsal scales small, granular or flattened and not overlapping.	4
-	No distinct collar; dorsal scales large, strongly keeled and overlapping	<i>Tropidosaura</i> (Mountain lizards)
4	Nostril pierced between 2–4 nasals; temporal scale elongate; rock-living	5
-	Nostril pierced between 2–3 nasals and well separated from first upper labial; temporal scale rounded; terrestrial	<i>Nucras</i> (Sandveld lizards)
5	Nostril in contact with first upper labial; four upper labials anterior to subocular, whose lower border is much shorter than upper; dorsal scales small, granular, non-keeled and about 68 across midbody.	<i>Australolacerta</i> (Southern rock lizard)
-	Nostril narrowly separated from first upper labial; five upper labials anterior to subocular, whose lower border is not distinctly narrowed; dorsal scales flat, hexagonal, faintly keeled posteriorly and in 34–43 rows across midbody	<i>Vhembelacerta</i> (Soutpansberg rock lizard)
6	Belly plates in 10 or more long rows	7
-	Belly plates in 6 long rows	<i>Heliobolus</i> (Bushveld lizards)
7	Dorsal scales large, keeled and overlapping; head shields striated and keeled.	8
-	Dorsal scales small or granular; head shields smooth or slightly rough	9
8	Subocular borders lip	<i>Ichnotropis</i> (Rough-scaled lizards)
-	Subocular does not border lip	<i>Meroles squamulosus</i> (Rough-scaled Desert Lizard)
9	Toes without a serrated or fringed edge: subocular bordering lip.	<i>Pedioplanis</i> (Sand lizards)
	Toes with a serrated or fringed edge; subocular not bordering lip	<i>Meroles</i> (Desert lizards) part.

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Is dietary niche breadth linked to morphology and performance in Sandveld lizards *Nucras* (Sauria: Lacertidae)?

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The functional characteristics of prey items (such as hardness and evasiveness) have been linked with cranial morphology and performance in vertebrates. In lizards particularly, species with more robust crania generally feed on harder prey items and possess a greater bite force, whereas those that prey on evasive prey typically have longer snouts. However, the link between dietary niche breadth, morphology, and performance has not been explicitly investigated in lizards. The southern African genus *Nucras* was used to investigate this link because the species exhibit differing niche breadth values and dietary compositions. A phylogeny for the genus was established using mitochondrial and nuclear markers, and morphological clusters were identified. Dietary data of five *Nucras* species, as reported previously, were used in correlation analyses between cranial shape (quantified using geometric morphometrics) and dietary niche breadth, and the proportion of hard prey taken and bite force capacity. Dietary niche breadth and the proportion of hard prey eaten were significantly related to cranial shape, although not once phylogeny was accounted for using a phylogenetic generalized least squares regression. The proportion of evasive prey eaten was a significant predictor of forelimb length when phylogeny was taken into account. We conclude that, in *Nucras*, the percentage of evasive prey taken co-evolves with forelimb morphology, and dietary niche breadth co-evolves with cranial shape. However, although head width is correlated with the proportion of hard prey eaten, this appears to be the result of shared ancestry rather than adaptive evolution. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, 110, 674–688.

ADDITIONAL KEYWORDS: bite force – co-evolution – geometric morphometrics – phylogenetic generalized least squares regression – phylogeny – southern Africa – sprintspeed.

INTRODUCTION

Adaptations to particular habitats can be physiological, morphological or behavioural, and are often driven by a multitude of factors, such as habitat

structure (Vitt, 1981; Vitt *et al.*, 1997; Revell *et al.*, 2007; Goodman & Isaac, 2008; Goodman, 2009; Measey, Hopkins & Tolley, 2009; Edwards *et al.*, 2012), prey composition (Herrel *et al.*, 2008), and seasonality (Huey, Pianka & Hoffman, 1977), amongst others. Variation in morphology may be driven by a number of factors, such as sexual selection (Braña, 1996), competition (Langkilde, 2009), foraging

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method (Huey & Pianka, 1981; Huey *et al.*, 1984; Verwajen & Van Damme, 2007a, b, 2008; McBrayer & Wylie, 2009), and prey availability (Herrel *et al.*, 2001; Verwajen, Van Damme & Herrel, 2002). The dietary composition, particularly the type of prey taken, may influence the head morphology of lizards (Herrel *et al.*, 2001; Verwajen *et al.*, 2002). Lizard species that consume harder prey have been shown to have relatively wider, more robust heads (in lacertid lizards: Herrel *et al.*, 2001), which are assumed to allow more space for jaw adductor muscles (Herrel *et al.*, 1999a) or a more vertical orientation of the jaw adductors (Herrel, Aerts & De Vree, 1998). Selective pressures on the functional aspects of the organism (i.e. organismal performance) may lead to the evolution of particular phenotypes, which may lead to greater fitness (Arnold, 1983). Functionally, relatively larger and more robust crania have been linked to greater bite forces in lizards (*Anolis*: Herrel *et al.*, 2007; *Podarcis*: Herrel *et al.*, 2001; Huyghe, Vanhooydonck & Van Damme, 2009), and a greater bite force may be advantageous for lizards in that they may be able to feed on harder and larger prey (Herrel *et al.*, 1999a). Other aspects of the crania, such as snout lengths, have been linked to the capture of evasive prey items. For example, in anoles, longer jaws are assumed to facilitate easier capture of flying insects (Herrel, McBrayer & Larson, 2007; Herrel *et al.*, 2011). Other functional aspects of lizards, such as the sprint speed and endurance, have been linked to the capture of evasive prey (Vanhooydonck, Herrel & Van Damme, 2007).

Although feeding on hard and/or evasive prey has been linked to head shape and functional aspects of head and limb morphology in lizards (Vanhooydonck *et al.*, 2007; Measey *et al.*, 2011), the relationship between dietary niche breadth (range of prey taken) and morphology has not been explicitly investigated. If a lizard species is specialized (low niche breadth value) to feed on a particular type of prey (e.g. hard or evasive prey), it may have particular phenotypic and behavioural traits that allow for the capture of that prey. On the other hand, if the species is a generalist, feeding on a large range of prey items, its morphology would be versatile, enabling the processing of a large range of prey types (e.g. hard or soft and/or evasive or sedentary prey). Investigations of the relationship between body size and niche breadth in lizards have been undertaken (Costa *et al.*, 2008), where a negative relationship was found between body size and niche breadth in 159 lizard species. This was contrary to positive body size-niche breadth relationships in birds (Brändle *et al.*, 2002b), butterflies and moths (Wasserman & Mitter, 1978; Brändle, Ohlschlager & Brandl, 2002a) and herbivorous insects (Novotny & Basset, 1999), although the negative relationship in

lizards was attributed to the overall frequency distribution of body sizes in lizards. Little information, however, is available on the link between dietary niche breadth and morphology in lizards, and the associated variation in performance.

The southern African lacertid genus *Nucras* (Eremiadini, Lacertidae) was used to investigate the link between dietary niche breadth and morphology because the species of this genus differ in dietary niche breadth (Van Der Meer, Whiting & Branch, 2010). *Nucras* are predominantly insectivorous, supplementing their diet with spiders, scorpions, and centipedes, and each species preys upon arthropods of varying degrees of hardness and evasiveness (Branch, 1998; Spawls, Howell & Drewes, 2006; Van Der Meer *et al.*, 2010). All *Nucras* are described as active foragers (Branch, 1998), and thus morphological differences between species are likely not driven by foraging methods but, instead, by other factors (such as diet). There are ten described species from East and southern Africa (Branch, 1998); however, dietary data for only five species are available to date (Van Der Meer *et al.*, 2010).

In the present study, we hypothesized that cranial shape in lizards of the genus *Nucras* is related to dietary niche breadth, and that functional capacities are linked to dietary composition. Although all *Nucras* are described as active foragers (as opposed to sit-and-wait foragers), the type of prey that they are able to prey upon may be determined by their morphology. We predicted that species specializing on hard prey items would have more robust crania and higher bite forces, and that those species feeding on evasive prey would have longer limbs and better sprinting capacities. We constructed a phylogeny for the genus, using both mitochondrial and nuclear markers, aiming to determine the evolutionary history of the genus and to investigate potential phylogenetic effects driving morphological similarity between species. We used linear morphometric techniques to identify morphologically similar groups of species. Using the five species for which dietary data are available, we first investigated the relationships between cranial morphology (using geometric morphometric techniques), dietary niche breadth, prey characteristics, and bite force. We then investigated the relationship between limb lengths and sprinting capacity, and the proportion of evasive prey taken.

MATERIAL AND METHODS

DNA EXTRACTION AND SEQUENCING

For the phylogenetic comparative methods, we estimated the phylogeny of *Nucras* using 48 individuals

from eight of the ten described species (*Nucras scalaris* and *Nucras caesicaudata* were not included due to lack of samples; see Supporting information, Table S1). Thirty individuals were collected in the field and tissue was stored in 95–100% ethanol. The dataset was supplemented with sequences from six individuals available on GenBank/EMBL. Individuals from seven related genera within the Eremiadini (*Australolacerta*, *Heliobolus*, *Ichnotropis*, *Latastia*, *Meroles*, *Philocortus*, and *Pseudereimias*) obtained from GenBank were used as outgroup taxa (Mayer & Pavlicev, 2007; Kapli *et al.*, 2011). For all newly sequenced individuals, genomic DNA was isolated from tail or liver tissue in accordance with a standard salt-extraction protocol (Bruford *et al.*, 1992). Standard polymerase chain reaction (PCR) procedures were utilized to amplify two mitochondrial (16S and ND4) and two nuclear genes (RAG1 and KIAA-2018). For the mitochondrial genes, the primer pairs L2510 and H3080 16S rRNA primers (Palumbi, 1996) and ND4 (Forstner, Davis & Arevalo, 1995) and Leu1 (Arévalo, Davis & Sites, 1994) primers were used to amplify the 16S and ND4 genetic markers, respectively. The primers RAG1-F0 and RAG1-R1 (Mayer & Pavlicev, 2007), and KIAA2018-F1 and KIAA2018-R2 (Portik *et al.*, 2011) were used to amplify the partial nuclear RAG1 and KIAA-2018 genes, respectively. For amplification of the four genetic markers, 25- μ L PCR mixes contained approximately 50 ng of genomic DNA, 1 \times SuperTherm reaction buffer, 1.5 mM MgCl₂, 0.2 μ M of each primer, 200 μ M dNTPs, and 0.025 U/ μ L Taq polymerase (SuperThermTaq; Southern Cross Biotechnologies). For the 16S, ND4, and KIAA-2018 gene fragments, a standard PCR protocol was followed, with a cycling profile including an initial denaturing step at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 50–55 °C for 30 s, and 72 °C for 45 s, and with a final extension at 72 °C for 8 min. Methods for the amplification of the RAG1 gene region involved the use of a step-down procedure (Groth & Barrowclough, 1999). The products were sent directly to Macrogen for clean up and sequencing, using the forward primers in all cases. Sequences were aligned using CLUSTALOMEGA, version 1.1.0 (Sievers *et al.*, 2011) and checked in BIOEDIT, version 7.0.5.2 (Hall, 1999). A 168-bp portion of the 16S marker that could not be unambiguously aligned was excluded from the analyses. Details of the samples and EMBL accession numbers are provided in the Supporting information (Table S1).

PHYLOGENETIC TREE ESTIMATIONS

A partition homogeneity test (Farris *et al.*, 1994, 1995) was implemented in PAUP*, version 4.0b10 (Swofford, 2002), and no conflict was found between

markers within each genome, nor between genomes. Sequence divergences were determined by estimating the uncorrected *p*-distances between and within species using MEGA, version 4 (Tamura *et al.*, 2007).

Phylogenetic trees were constructed from the combined total evidence dataset from all four markers. Bayesian inference (BI) was performed with uniform priors for all parameters (MRBAYES, version 3.1.0; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The third codon position of the ND4 gene was found to be saturated (DAMBE, version 5.2.65; Xia *et al.*, 2003), and so it was partitioned separately from the other two codon positions of the ND4 gene (1, the first and second codon positions; 2, the third codon position). The remaining markers were partitioned separately resulting in five partitions in total. Evolutionary models best fitting the individual marker datasets were chosen (MODELTEST, version 3.7; Posada & Crandall, 1998) and model priors were set accordingly (16S: GTR+G, ND4: GTR+I+G, RAG1: HKY+G, KIAA-2018: HKY+G). Two parallel runs for 20 \times 10⁶ generations each were run for Markov chain Monte Carlo analysis, with trees sampled every 1000 generations. The number of generations to discard as burn-in (1 \times 10⁶ generations) was determined by examining the number of generations (1) at which the standard deviation of split frequencies stabilized (at less than 0.001); (2) at which the log-likelihood tree scores reached stationarity; and (3) the effective sample sizes of all parameters were \geq 400 (TRACER, version 1.5; Rambaut & Drummond, 2007). A 50% majority rule tree was constructed with the burn-in excluded using the ‘sumt’ command in MRBAYES, and nodes with \geq 0.95 posterior probability were considered supported. A partitioned maximum likelihood (ML) analysis was also run (RAXML, version 7.2.7, via the Cipres Portal; Stamatakis, 2006; Stamatakis, Hoover & Rougemont, 2008) using the same partitions as the Bayesian analysis, a GTR+I+G model of evolution, and automatic halting of bootstrapping (Stamatakis, 2006; Stamatakis *et al.*, 2008).

LINEAR MORPHOMETRIC ANALYSIS

For the linear morphometric analyses, 187 individuals of nine *Nucras* species were measured using digital callipers (approximately 20 per species, *N. scalaris* was not included because of a lack of specimens; see Supporting information, Table S2). Measurements taken on the body and limbs were: body length from snout–vent length (SVL), femur length (FM), tibia length (TB), humerus length (HM), and radius length (RD). Head measurements taken were: head length (HL), head width at the widest part

of the temporal region (HW), head height of the posterior part of the cranium (HH), and lower jaw length (LJL). Unless otherwise specified, all analyses were performed using R STUDIO, version 0.97.248 (R Core Team, 2012; R Studio, 2012). To eliminate the effect of size in the traditional morphometric analyses, \log_{10} -transformed head and limb measurements were regressed onto the geometric means of the particular set of measurements using a linear model (package: 'stats', functions: 'resid' and 'lm'; R Core Team, 2012). The absolute values and the size-corrected residuals for each morphometric character were used in further analyses. To identify whether the morphology of the lizards was linked to their genetic relationships, hierarchical clustering of the means of the size-corrected residuals for each species (package: 'stats', function: 'mean'; R Core Team, 2012) was performed to identify the morphological clusters and support for the nodes was obtained using 1000 bootstrap replicates (package: 'pvclust', function: 'pvclust', method.hclust: 'complete', method.dist: 'euclidean', nboot: 1000; R Core Team, 2012). If the morphological clusters do not correspond to genetic clusters, then differences in morphology may be driven by environmental factors such as diet or substrate and not solely by phylogenetic relationships, and further investigations into these factors would be warranted.

DIETARY ANALYSIS

Five species (*Nucras holubi*, *Nucras intertexta*, *Nucras lalandii*, *Nucras ornata*, and *Nucras tessellata*; hereafter referred to as the 'dietary species') were used to investigate the relationship between diet and head shape because dietary information on these species was available (Table 1; adapted from Van Der Meer *et al.*, 2010). These species can be considered as being characteristic for major patterns in the genus because they are distributed across the southern African landscape (Branch, 1998), are representatives from each major genetic clade within the genus (see Results for phylogenetic analysis), and are also representatives of each major morphometric cluster (for hierarchical cluster analysis, see Results). The percentage volume in the diet for each insect order was used in the analyses (adapted from Van Der Meer *et al.*, 2010). In the dietary analyses, sexes were combined because there were no significant differences in the percentage volume of the different prey eaten by the two sexes (Van Der Meer *et al.*, 2010). Although the diet of both sexually mature and sexually immature individuals was examined in the analyses by Van Der Meer *et al.* (2010), mean prey volume was significantly correlated with SVL for *N. intertexta* and *N. ornata* but not for

N. holubi, *N. lalandii* and *N. tessellata* (Van Der Meer *et al.*, 2010), indicating that possibly ontogenetic effects are at play in terms of the percentage volume of prey consumed by each age class in *N. intertexta* and *N. ornata*. Because the differences in prey volume, number or type between age-classes were not explicitly examined by Van Der Meer *et al.* (2010), we cannot exclude ontogenetic effects on prey consumption.

Dietary niche breadth values (hereafter referred to as the niche breadth) for each species were estimated using the inverse of Simpson's diversity index (Simpson, 1949):

$$B = 1 / \sum_{i=1}^N p_i^2$$

where B is the niche breadth value, i is the resource category, N is the total number of categories, and p is the proportion of resource category i . These niche breadth values, ranging from one to n , indicate whether the species preys upon a large range of arthropod orders (high value, close to n) or specializes on a limited range of arthropod orders (low value, close to one). Each arthropod order was categorized as either hard or soft, sedentary or evasive (Herrel, Van Damme & De Vree, 1996; Andrews & Bertram, 1997; Herrel *et al.*, 1999a; Herrel, Verstappen & De Vree, 1999b; Herrel *et al.*, 2001; Verwajen *et al.*, 2002; Aguirre *et al.*, 2003; Herrel *et al.*, 2006; Vanhooydonck *et al.*, 2007) and the percentage volumes of two prey categories were calculated for each studied species of *Nucras* (Table 1).

GEOMETRIC MORPHOMETRIC ANALYSIS

Geometric morphometric analyses of the crania were performed to investigate the cranial shape of the five species used in the dietary analyses (14–22 individuals per species, totalling 100 individuals; see Supporting information, Table S2). The heads were photographed using digital cameras (Fuji Finepix S2000HD, resolution 10.0 MP; Canon 50D, resolution 10.0 MP and macro lens F18/100). The dorsal and lateral profiles were used because head width, head height, and snout length have been shown to be important in species feeding on hard and/or evasive prey; dimensions that would not have been apparent from other views of the crania (such as the ventral view). Homologous landmarks were chosen to appropriately describe the shape of the whole cranium, and landmarks on the cheek region were included and digitized (TPSUTIL, version 1.26, Rohlf, 2004; TPSDIG2, version 2.05, Rohlf, 2005; Fig. 1). A generalized Procrustes analysis (Rohlf & Slice, 1990; Rohlf, 1999) was performed in which the sizes were

Table 1. Percentage volume of prey consumed per species used in the dietary analyses (*sensu* Van Der Meer *et al.*, 2010), as well as prey hardness and evasiveness categories (Vanhooydonck *et al.*, 2007), and niche breadth values (calculated in the present study) for each species

Categories	Prey hardness	Prey evasiveness	<i>Nucras holubi</i>	<i>Nucras intertexta</i>	<i>Nucras lalandii</i>	<i>Nucras ornata</i>	<i>Nucras tessellata</i>
Prey order							
Araneae	Soft	Sedentary	1.90	6.70	1.40	15.30	1.30
Blattaria	Soft	Evasive	2.50	13.00	0.00	0.50	0.00
Chilopoda	Soft	Evasive	3.50	10.10	1.30	17.60	0.30
Coleoptera	Hard	Evasive	15.60	11.50	18.20	1.10	16.40
Diplopoda	Soft	Sedentary	0.00	0.00	0.50	0.20	0.00
Diptera	Soft	Evasive	3.30	0.90	0.00	2.70	0.00
Hemiptera	Hard	Evasive	0.80	4.40	0.00	0.40	0.60
Hymenoptera	Hard	Evasive	1.00	0.50	0.80	0.00	0.00
Ants	Hard	Sedentary	0.20	0.00	0.00	0.30	53.00
Insect eggs	Soft	Sedentary	0.00	0.00	1.40	0.00	0.00
Isoptera	Soft	Sedentary	39.30	7.60	3.30	8.50	11.30
Lepidoptera	Soft	Evasive	2.50	11.70	0.00	1.30	1.60
Mantodea	Soft	Sedentary	1.30	0.30	0.00	0.00	0.00
Neuroptera	Soft	Evasive	0.00	0.00	0.00	0.00	1.80
Orthoptera	Hard	Evasive	24.60	15.40	63.30	49.90	13.70
Scorpiones	Soft	Evasive	1.60	2.70	10.50	1.40	0.00
Solifugae	Hard	Sedentary	1.20	4.90	0.00	0.00	0.00
Niche breadth			4.10	10.75	2.24	3.21	2.94
Proportions							
Hard prey percentage			0.44	0.41	0.82	0.52	0.84
Evasive prey percentage			0.56	0.78	0.93	0.76	0.34
Performance							
Maximum bite forces (N)			8.20 ± 2.01	24.23 ± 7.19	-	-	13.85 ± 4.93
Residual bite force			0.25	0.21	-	-	0.02
Maximum sprint speeds (m s ⁻¹)			2.88 ± 0.43	3.03 ± 0.61	4.17	-	2.39 ± 0.16
Residual sprint speeds			0.03	-0.003	0.30	-	-0.19

Percentage volume of hard and evasive prey consumed per species used in the dietary analyses and performance values (maximal bite force and sprint speeds) for each species. Values for the performance means are given as the mean ± SD.

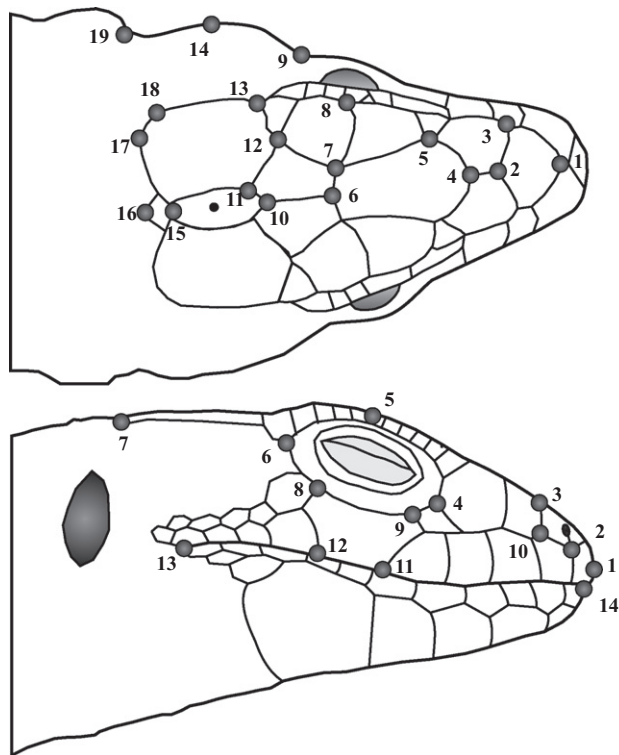


Figure 1. Diagram depicting the homologous landmarks that were digitized for the geometric morphometric analyses for the dorsal (top) and lateral (bottom) views of the *Nucas* crania.

standardized and the landmark configurations were translated and rotated. A relative warps analysis (similar to a principal components analysis) was performed on the residuals to identify which portions of the crania show the most variation between individuals and species (TPSRELW; Rohlf, 2003). Deformation grids (thin-plate splines) were used to visualize changes in cranial shape.

PERFORMANCE ANALYSIS

The performance capacities of four *Nucas* species (*N. holubi*, *N. intertexta*, *N. lalandii*, and *N. tessellata*; see Supporting information, Table S3), caught and measured in the field, were used to identify the functional relationship between morphology and diet (sample sizes: *N. holubi* = 5, *N. intertexta* = 19, *N. lalandii* = 1, and *N. tessellata* = 2). The maximal bite force out of five trials was determined by having the lizard bite two metal plates connected to an isometric force transducer and a charge amplifier (Herrel *et al.*, 1999a, 2001). For the bite force analyses, *N. lalandii* was not included as a result of the poor biting performance of the single individual obtained during field work. To eliminate the effect of size, the \log_{10} -transformed maximal bite force

values were regressed onto the \log_{10} -transformed geometric means of the head measurements (i.e. the mean of the sum of HL, HW, HH, and LJL) using a linear model (package: 'stats', functions: 'resid' and 'lm'; R Core Team, 2012) and the mean residuals for each species were used in subsequent analyses.

To determine the maximal sprint speed for each species, the lizards were allowed to rest in an incubator at 35 °C for 1 h before each trial to standardize body temperature. The temperature was chosen according to the preferred body temperatures for other lacertid lizards (Huey *et al.*, 1977; Bauwens *et al.*, 1995; Castilla, Van Damme & Bauwens, 1999; Vanhooydonck, Van Damme & Aerts, 2001) because optimal body temperature for performance trials have not been identified for all *Nucas* species (only *N. intertexta* and *N. tessellata*; Huey *et al.*, 1977). The sprint speeds were determined using a 2-m long cork-covered racetrack with sensors placed at 25-cm intervals along the track (Vanhooydonck *et al.*, 2001). Runs were repeated three times, and lizards were allowed to rest for at least 1 h between each run, and the maximum of the sprint speeds for each individual were taken (measured in metres per second). The \log_{10} -transformed maximal sprint speed values were regressed onto the \log_{10} -transformed geometric means of the limb measurements to eliminate the effect of size (package: 'stats', function: 'resid' and 'lm'; R Core Team, 2012) and the mean residuals for each species were used in further analyses.

STATISTICAL ANALYSIS

Correlation analyses were performed between the mean morphometric variables for each species (both size-corrected linear morphometric residuals and geometric relative warp scores), dietary niche breadth values, proportions of hard and evasive prey, and mean size-corrected performance residuals for each species (package: 'stats', functions: 'cor.test' and 'summary.lm'; R Core Team, 2012).

PHYLOGENETIC COMPARATIVE ANALYSIS

A phylogenetic generalized least squares analysis (PGLS; Grafen, 1989; Hansen & Martins, 1996; Hansen, 1997; Martins & Hansen, 1997; Martins & Housworth, 2002) was employed to identify the coevolution of morphological traits and dietary composition, and performance variables (package: 'nlme', function: 'gls', method: 'REML'; R Core Team, 2012). The mean species values of the both absolute and relative \log_{10} -transformed morphometric and performance traits were used in the analyses. The PGLS method statistically accounts for the expected covariance of the measured variables between species

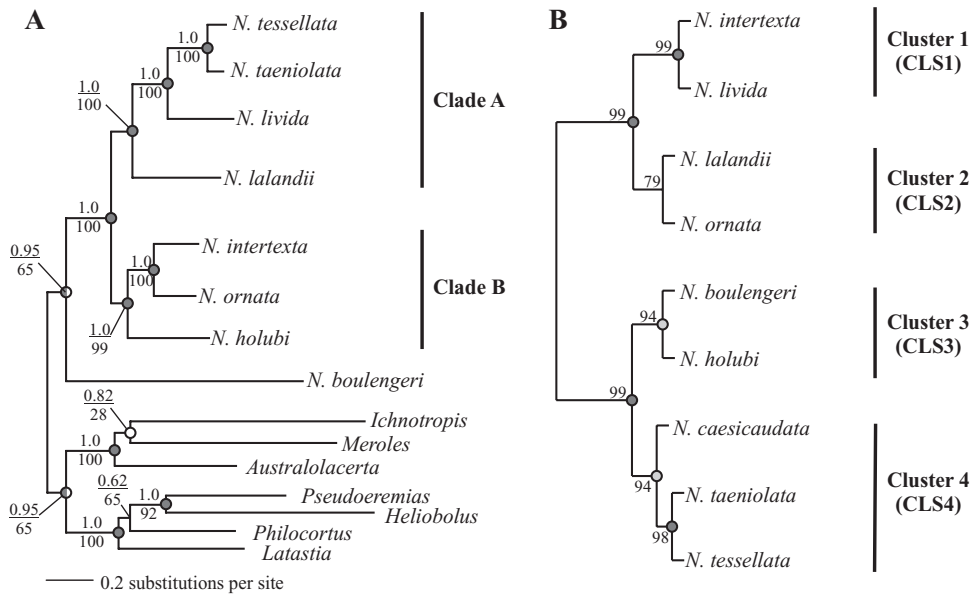


Figure 2. Phylogenetic tree shown (A) inferred from Bayesian analyses (BI) and likelihood methods (ML) using a combined dataset of mtDNA (16S, ND4) and nuclear DNA (RAG1, KIAA-2018) (topology from BI shown). Support values shown at the nodes and indicated by the circles at the nodes: Bayesian posterior probabilities > 0.90 (above node; left fill of circle) and ML bootstrap values > 50% (below node; right fill of circle). If a node is supported using both algorithms, the circle at the node is filled completely. Hierarchical clustering dendrogram (B) of the morphometric measurements, showing the four morphological clusters (CLS1–4) obtained. Supported values [AU (approximately unbiased) *P*-values] shown at the nodes, and dark-grey filled circles indicate nodes with strong support (AU > 95%), and light-grey filled circles indicate nodes with moderate support (95% > AU > 90%).

resulting from phylogenetic relationship for regression-based or analyses of variance, at the same time as incorporating an explicit model of evolution. A significant result indicates that the relationship holds once phylogeny has been accounted for. The phylogenetic covariance matrix was estimated using the branch lengths from the phylogenetic tree and the expected pattern of phylogenetic covariance specified by the Brownian Motion (BM) model of evolution (package: ‘ape’, function: ‘corBrownian’, Paradis, 2012). PGLS analyses were not performed for bite force values, as the low sample size (three mean values) would give spurious results.

RESULTS

PHYLOGENETIC RELATIONSHIPS AND MORPHOLOGICAL CLUSTERING OF ALL *NUCRAS*

Phylogenetic trees constructed using both methods (BI and ML) had the same topology with high support values for the clades recovered (Fig. 2A; Fig. S2). All described species were recovered as monophyletic, with high sequence divergences (uncorrected *p*-distances) between them (16S: $5.80 \pm 2.47\%$; ND4: $13.31 \pm 1.12\%$; RAG1: $1.07 \pm 0.51\%$; KIAA: $0.58 \pm 0.29\%$). The separate clades are geographically

proximate: the single sample of *Nucras boulengeri* (the only species from East Africa) is sister to the remaining *Nucras* species, which are themselves split into two well-supported main clades: Clade A (coastal and south-interior of southern Africa) and Clade B (savannah biome of southern Africa) (Fig. 2A; see also Supporting information, Fig. S1). The sequence divergences between *N. boulengeri* and the other *Nucras* (16S: $5.98 \pm 1.44\%$; ND4: $16.95 \pm 1.03\%$; RAG1: $5.41 \pm 0.84\%$; KIAA: $1.25 \pm 0.41\%$) approximated the level of sequence divergence between other genera in this study (16S: $10.10 \pm 1.79\%$; ND4: $16.58 \pm 1.01\%$; RAG1: $5.59 \pm 0.80\%$; KIAA: $2.61 \pm 0.53\%$). Four morphological clusters were obtained using hierarchical clustering analyses (Fig. 2B) but with little support for the four clusters, whereas relationships between species within the clusters was highly supported. Morphological clusters did not correspond to genetic clades, indicating that morphology may not only be driven by the shared ancestry, but also by other factors, such as diet.

DIETARY, MORPHOLOGICAL, AND PERFORMANCE ANALYSIS OF FIVE *NUCRAS* SPECIES

Two significant relationships were found between (1) niche breadth and the means of first dorsal cranial

Table 2. The mean \pm SD of the linear morphometric measurements (mm) for the species used in the dietary analyses

Categories	<i>Nucras holubi</i>	<i>Nucras intertexta</i>	<i>Nucras lalandii</i>	<i>Nucras ornata</i>	<i>Nucras tessellata</i>
Sample number (<i>N</i>)	28	29	36	25	23
Snout–vent length (SVL)	51.19 \pm 4.83	68.73 \pm 10.51	83.86 \pm 11.67	76.84 \pm 17.85	59.10 \pm 9.03
Head length (HL)	11.62 \pm 1.00	14.99 \pm 1.87	15.79 \pm 1.88	17.08 \pm 3.52	13.66 \pm 1.98
Head width (HW)	7.50 \pm 0.94	8.18 \pm 1.26	10.04 \pm 1.55	10.99 \pm 2.44	7.43 \pm 1.29
Head height (HH)	6.21 \pm 0.98	6.99 \pm 1.17	8.43 \pm 1.16	8.48 \pm 2.06	5.88 \pm 1.08
Lower jaw length (LJL)	12.67 \pm 0.98	15.76 \pm 1.89	17.05 \pm 1.99	19.85 \pm 4.40	14.25 \pm 1.98
Femur length (FM)	8.25 \pm 0.99	11.31 \pm 1.87	11.07 \pm 1.39	11.37 \pm 2.46	9.87 \pm 1.49
Tibia length (TB)	7.53 \pm 1.16	9.74 \pm 1.41	9.35 \pm 1.16	9.90 \pm 2.08	8.59 \pm 1.66
Humerus length (HM)	5.55 \pm 0.80	7.62 \pm 1.12	7.31 \pm 1.01	8.30 \pm 1.59	6.23 \pm 1.24
Radius length (RD)	5.16 \pm 0.57	6.67 \pm 0.98	6.46 \pm 1.00	7.20 \pm 1.57	5.42 \pm 0.94

view relative warp scores (positive relationship; Table 3) and (2) between the proportion of hard prey eaten and absolute head width (positive relationship; Fig. 4 and Tables 2 and 3). Bite force was significantly positively related to body size (SVL) and linear head measurements (HL, HW, HH, and LJL; Table 3). The proportion of evasive prey was not significantly related to either absolute or relative limb measurements, or sprint speeds (Table 4). Sprint speeds were positively related to absolute but not relative limb measurements, which was expected as larger individuals will have longer stride-lengths and therefore will be able to run faster than smaller individuals (Table 4).

The first three relative warps of the dorsal cranial view described the width and elongation of the cheek of the five *Nucras* species (Fig. 3). The first dorsal view relative warp (DC-RW1) was positively related to niche breadth in the nonphylogenetic correlations (Fig. 4, Table 3), indicating that species that are more specialized, in this case specialist feeders on hard prey (*N. tessellata* and *N. lalandii*; Table 1), have cheek regions that are not as wide, and are more posteriorly elongated (landmarks 8, 9, 13, 14, 18, and 19; Fig. 3), compared to more generalist species (*N. intertexta*) (Fig. 3). The proportion of hard prey consumed was not related to any of the relative warps components, although it was significantly positively related to the absolute head width. There was no relationship between bite force and linear head measurements in the phylogenetic correlations, although this is likely a result of the low sample size (three data points = species means) used in the analyses. The lateral-view relative warp scores, describing the elongation of the snout (LC-RW1: landmarks 1–4, 10, 11, 14) and posterior cranial height (LC-RW2 and -RW3: landmarks 6–8, 11, 12) (Fig. 3), were not related to either niche breadth or proportion of hard prey taken, which was similar to results for absolute

and relative linear measurements of head length and height (Table 3).

PHYLOGENETIC COMPARATIVE ANALYSIS

There were no significant relationships between the proportion of hard prey eaten and cranial morphology once phylogeny was taken into account (Table 3), indicating that the relationships between these variables in the nonphylogenetic correlations may be influenced by a shared ancestry. Interestingly, although there were no significant relationships between the proportion of evasive prey and limb morphology, once phylogeny was taken into account, there were significant relationships between forelimb dimensions and the proportion of evasive prey taken (Table 4).

DISCUSSION

In the genus *Nucras*, we show a link between head shape, diet, and underlying functional performance at the whole-organism level, before phylogeny is taken into account. Clustering based on morphology did not correspond to the clades identified in the molecular phylogeny, indicating that factors other than phylogeny influence the evolution of morphology in *Nucras* lizards. When the diet of selected species was compared with morphology and performance, dietary niche breadth and the proportion of hard prey eaten were found to be correlated with cranial shape, although not when phylogeny was accounted for, suggesting that cranial shape in the five species investigated is somewhat constrained by evolutionary history. Absolute values of performance (bite force and sprint speeds) were significantly positively related to absolute head and limb measurements, respectively. When phylogeny was accounted for, the relationship between forelimbs and proportion of evasive prey was

Table 3. Nonphylogenetic and phylogenetic correlations between niche breadth, proportion of hard prey eaten, bite force capacity (absolute and relative) and cranial morphometrics (geometric morphometric scores, and relative and absolute linear morphometric measurements)

Independent	Dependent*	Nonphylogenetic			Phylogenetic			
		Variances (R^2)	Slope	Correlation (r)	P -value	Slope	Correlation (r)	P -value
Niche breadth (prey range)	Snout-vent length (SVL)	0.001	-0.002	-0.04	0.95	0.03	-0.69	0.33
	LC-RW1	0.07	-0.002	-0.26	0.68	0.00	-0.69	0.09
	LC-RW2	0.003	0.0003	0.06	0.93	0.00	-0.69	0.85
	LC-RW3	0.03	-0.001	-0.18	0.77	0.00	-0.69	0.39
	DC-RW1	0.84	0.004	0.91	0.03	0.004	-0.74	0.03
	DC-RW2	0.08	0.001	0.28	0.65	0.002	-0.74	0.33
	DC-RW3	0.01	0.0003	0.09	0.88	0.001	-0.74	0.66
	Head length (HL)	0.0004	0.001	0.02	0.98	0.01	-0.69	0.73
	Head width (HW)	0.08	-0.01	-0.28	0.65	0.02	-0.69	0.43
	Head height (HH)	0.02	-0.007	-0.15	0.80	0.02	-0.69	0.34
	Lower jaw length (LJL)	0.005	-0.003	-0.07	0.91	0.01	-0.69	0.75
	Relative HL	0.21	0.004	0.45	0.44	0.00	-0.69	0.51
	Relative HW	0.35	-0.01	-0.59	0.29	0.01	-0.69	0.38
	Relative HH	0.01	-0.002	-0.10	0.88	0.01	-0.69	0.32
	Relative LJL	0.02	0.001	0.16	0.80	0.00	-0.69	0.23
Proportion hard prey	SVL	0.57	0.71	0.75	0.14	-0.03	-0.95	0.97
	LC-RW1	0.60	0.08	0.78	0.12	-0.02	-0.95	0.76
	LC-RW2	0.17	-0.03	-0.41	0.49	0.01	-0.95	0.86
	LC-RW3	0.03	-0.01	-0.16	0.80	-0.07	-0.95	0.10
	DC-RW1	0.43	-0.05	-0.65	0.24	0.02	-0.91	0.60
	DC-RW2	0.09	-0.02	-0.31	0.62	0.03	-0.91	0.40
	DC-RW3	0.01	0.005	0.10	0.88	0.02	-0.91	0.59
	HL	0.54	0.48	0.74	0.16	0.20	-0.95	0.67
	HW	0.82	0.73	0.91	0.03	-0.03	-0.95	0.96
	HH	0.67	0.62	0.82	0.09	-0.21	-0.95	0.73
	LJL	0.64	0.62	0.80	0.10	0.23	-0.95	0.69
	Relative HL	0.08	-0.05	-0.28	0.65	0.11	-0.95	0.41
	Relative HW	0.06	0.06	0.25	0.68	-0.15	-0.95	0.41
	Relative HH	0.02	-0.04	-0.13	0.84	-0.33	-0.95	0.06
	Relative LJL	0.06	0.03	0.24	0.70	0.13	-0.95	0.08
Bite force (N)	SVL	0.79	1.00	0.89	< 0.0001	-	-	-
	HL	0.89	0.21	0.94	< 0.0001	-	-	-
	HW	0.77	0.08	0.88	< 0.0001	-	-	-
	HH	0.74	0.11	0.86	< 0.0001	-	-	-
	LJL	0.88	0.20	0.94	< 0.0001	-	-	-
	Relative HL	0.03	0.03	0.19	0.38	-	-	-
	Relative HW	0.09	-0.09	-0.30	0.16	-	-	-
	Relative HH	0.001	0.01	0.02	0.91	-	-	-
	Relative LJL	0.001	-0.005	-0.03	0.91	-	-	-

*LC, lateral cranial view; DC, dorsal cranial view; RW, relative warp component. Phylogeny was taken into account using the Brownian Motion (BM) model in a phylogenetic generalized least squares analysis. Variances (R^2), slope of the correlation, and Pearson's correlation indices (r) are shown for correlations between variables (without taking phylogeny into account). Significant correlations ($P < 0.05$) are indicated in bold.

Table 4. Nonphylogenetic and phylogenetic correlations between proportion of evasive prey eaten, sprint speed capacity (absolute and relative) and limb measurements (relative and absolute)

Independent	Dependent	Nonphylogenetic				Phylogenetic			
		Variances (R^2)	Slope	Correlation (r)	P -value	Slope	Correlation (r)	P -value	
Proportion evasive prey	Snout–vent length (SVL)	0.66	0.69	0.81	0.09	0.64	−0.93	0.16	
	Femur length (FM)	0.40	0.36	0.64	0.25	0.37	−0.93	0.22	
	Tibia length (TB)	0.46	0.30	0.68	0.21	0.33	−0.93	0.14	
	Humerus length (HM)	0.51	0.48	0.71	0.18	0.58	−0.93	0.05	
	Radius length (RD)	0.60	0.46	0.77	0.13	0.54	−0.93	0.03	
	Relative FM	0.03	−0.02	−0.16	0.79	−0.06	−0.93	0.38	
	Relative TB	0.57	−0.07	−0.75	0.14	0.09	−0.93	0.24	
	Relative HM	0.32	0.08	0.56	0.32	−0.09	−0.93	0.03	
	Relative RD	0.18	0.06	0.42	0.48	0.13	−0.93	0.03	
Sprint speed ($m \cdot s^{-1}$)	SVL	0.97	23.15	0.48	< 0.0001	0.31	−0.97	0.07	
	FM	0.97	3.86	0.26	< 0.0001	0.23	−0.97	0.14	
	TB	0.98	3.52	0.21	< 0.0001	0.10	−0.97	0.47	
	HM	0.97	2.46	0.49	< 0.0001	0.07	−0.97	0.46	
	RD	0.97	2.25	0.41	< 0.0001	0.11	−0.97	0.37	
Relative sprint speed	Relative FM	0.05	0.00	−0.23	0.27	0.11	−0.97	0.31	
	Relative TB	0.07	−0.05	−0.26	0.23	−0.03	−0.15	0.78	
	Relative HM	0.04	0.08	0.19	0.38	−0.06	−0.15	0.31	
	Relative RD	0.04	0.06	0.19	0.37	0.06	−0.15	0.19	

Phylogeny was taken into account using the Brownian Motion (BM) model in a phylogenetic generalized least squares analysis. Variances (R^2), slope of the correlation, Pearson’s correlation indices (r) and P -value shown for correlations between variables (without taking phylogeny into account). Significant correlations ($P \leq 0.05$) are indicated in bold.

significant, indicating that forelimb lengths have co-evolved with the proportion of evasive prey taken.

The morphological cluster dendrogram was not congruent with the molecular phylogeny. Two species, *N. tessellata* and *Nucras livida*, once considered subspecies of *N. tessellata* (Fitzsimons, 1943), are morphologically and genetically distinct, which is consistent with the current species designations (Branch & Bauer, 1995). The phylogeny shows that *Nucras taeniolata*, *N. holubi*, and *N. ornata*, once considered subspecies of *N. taeniolata* (Broadley, 1972) are separate lineages, and are also in separate morphological clusters, which is also consistent with the current species designations (Jacobsen, 1989; Branch, 1998). Although related species are geographically proximate to each other, the morphological topology is incongruent with the phylogeny (see Supporting information, Fig. S1). The phylogeny indicates the evolutionary patterns of radiations within the genus, whereas the morphology may be driven by other factors, such as diet, causing the topologies to differ.

Niche breadth (i.e. range of arthropod orders taken) was significantly correlated with cranial shape, indicating that species preying on a large number of arthropod orders have wider cheek regions (as in

N. intertextata) and higher bite forces, whereas those species that specialize (low niche breadth values) on hard prey items have more robust crania (shorter snouts) but narrower cheek regions (as in *N. lalandii* and *N. tessellata*), and lower biting capacities. There was also a positive relationship between absolute head width and the proportion of hard prey consumed in *Nucras*. Previously, it was shown in other lacertid lizards that those species consuming harder prey have wider heads as a result of the larger jaw adductor muscles (e.g. Herrel *et al.*, 2001; Verwajen *et al.*, 2002; Huyghe *et al.*, 2009) facilitating a greater relative bite force. It was expected that those *Nucras* species specializing on hard prey would show harder bite forces; however, this was not the case. By contrast, the dietary niche breadth (the variety of prey taken) determined how hard a species bit. Although puzzling at first, variation in prey size may explain this result. Because hardness is known to increase with prey size (Herrel *et al.*, 2001; Aguirre *et al.*, 2003), species eating only hard, yet small prey may not need very high bite forces. On the other hand, generalist species may profit from high bite forces because this would allow them to consume a wide range of prey varying in size and hardness. With the

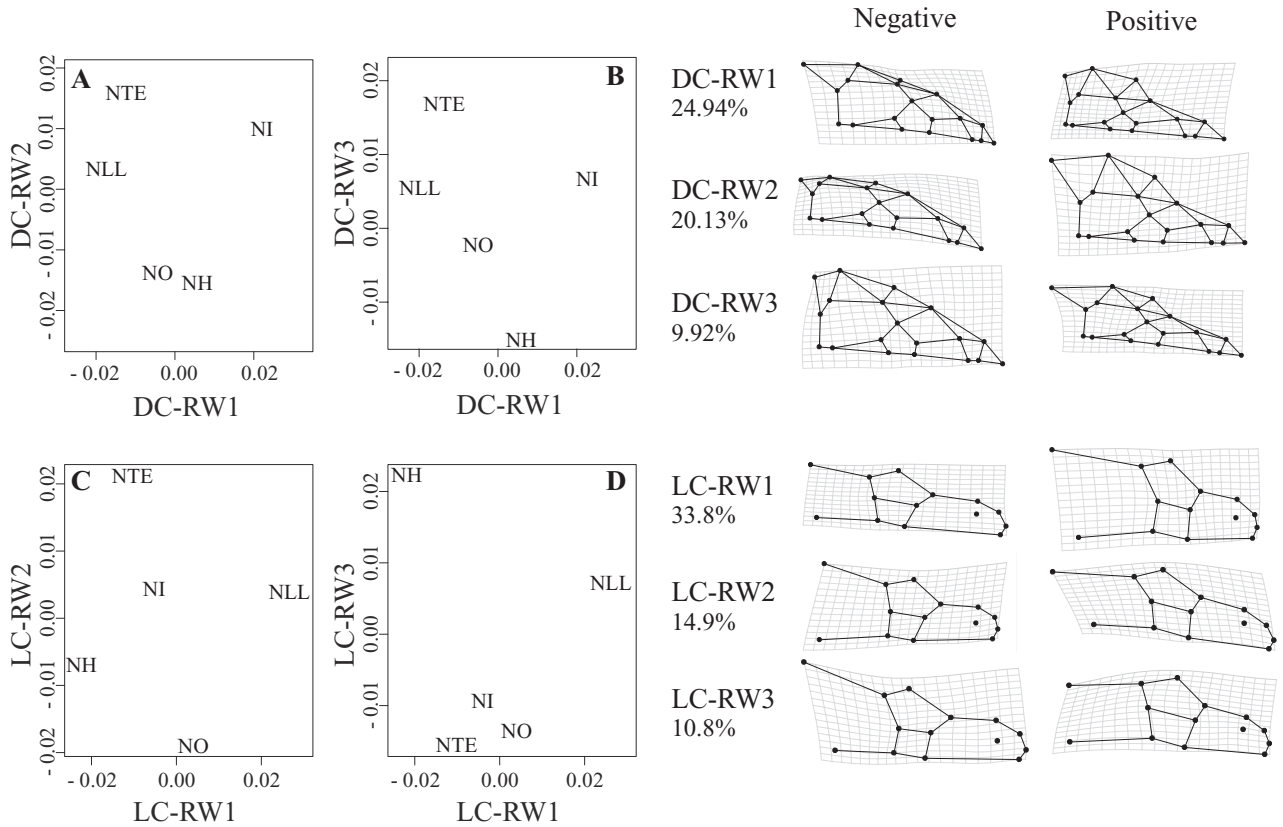


Figure 3. Scatterplots plotting the first three relative warps (RW) components for the dorsal (DC: A, B) and lateral (LC: C, D) views. Deformation grids indicate the cranial shape differences on either the negative or positive ends of the first three relative warp components for the dorsal and lateral views. Percentage of variation explained by each component is shown. Key to species abbreviations in each plot: NH, *Nucras holubi*; NI, *Nucras intertexta*; NLL, *Nucras lalandii*; NO, *Nucras ornata*; NTE, *Nucras tessellata*.

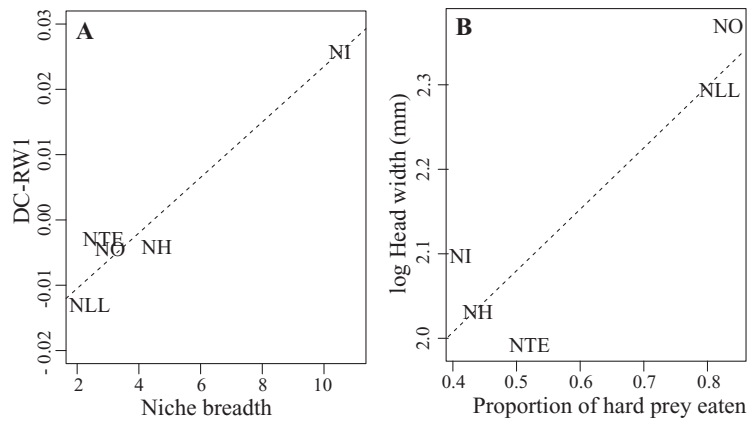


Figure 4. Scatterplots of the means of the significant correlations for the nonphylogenetic correlation analyses (Tables 3, 4), with the slope of the correlations shown by a dashed line within plots. Variables plotted are: niche breadth against the first dorsal relative warp component (A) and proportion of hard prey eaten against \log_{10} -transformed absolute head width (B). Key to the species abbreviations is as provided in Fig. 3.

small number of species included in the present study, however, the results involving bite force need to be treated as preliminary, and increasing sample sizes may clarify this relationship with more confidence. Thus, further studies correlating individual prey hardness with bite force are needed to better understand the factors driving the evolution of head shape in *Nucras* lizards.

Sprint speed was related to body size and limb morphology in absolute terms, although neither of these was related to the proportion of evasive prey taken. This lack of a relationship was also found for other lacertid lizards (Vanhooydonck *et al.*, 2007). As suggested previously (Vanhooydonck *et al.*, 2007), maximal sprint speed may not be as important as fast acceleration for the capture of evasive prey. Once the prey takes flight, it is essentially out of reach of the lizards and no amount of running at top speed will enable the lizard to capture the prey. Thus, the ability to capture the evasive prey immediately once sighted before it escapes would be crucial. In comparisons of dietary and functional capacities, measures of acceleration in addition to sprint speed and stamina may turn out to be more informative in understanding a lizards' ability to capture elusive prey.

In conclusion, the PGLS analyses retrieved significant relationships between niche breadth and the first relative warp score of the head in dorsal view, as well as between limb morphology and the proportion of evasive prey eaten. The proportion of hard prey taken did not show any relationship with head shape descriptors when phylogeny was accounted for, suggesting an important role of shared ancestry in the observed co-variation between head shape, diet and bite force. By contrast, the proportion of elusive prey eaten was shown to co-evolve with forelimb dimensions in the species included in the present study. Future analyses incorporating a larger number of species and incorporating data on both prey size as well as functional properties are needed to better understand the evolution of body proportions in relation to diet in this genus. Despite these limitations, our data do suggest interesting co-variation between morphology, niche breadth, prey type, and performance that would be worth exploring further.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Map of the distributions within the African continent of all *Nucras* species used in the phylogenetic analyses. The key to the coloration (for genetic clades) and patterns (for morphological clusters) within each species distribution is shown to the right of the map. Countries are labelled and each species is labelled in italic font. Distributions for the species were adapted from Branch (1998) and Spawls *et al.* (2006).

Figure S2. Phylogenetic tree of the genus *Nucras* based on the combined partial 16S, ND4, RAG1 and KIAA-2018 gene regions and inferred by BI and ML (Bayesian topology shown). Sample numbers are indicated at terminal tips, and species names are given. Nodes are considered supported if posterior probabilities > 0.95 (estimated using Bayesian inference) and/or bootstrap values > 75% (using maximum likelihood analyses).

Table S1. List of specimens used for the phylogenetic analyses. Genus, species, museum, and field accession numbers are given, as well as EMBL-Bank accession numbers, for the two mitochondrial (16S, ND4) and two nuclear (RAG1, KIAA-2018) gene fragments sequenced.

Table S2. List of specimens used for the morphometric analyses. Genus, species, museum, and field accession numbers are given, as well as an indication of whether the specimen was used in the linear morphometric and geometric morphometric analyses.

Table S3. List of specimens used for the performance analyses (all specimens were caught in the field). Species, sample size for performance analyses, and field accession numbers are given.

SUPPLEMENTARY MATERIAL:

**Is dietary niche breadth is linked to morphology and performance in Sandveld lizards
Nucras (Sauria: Lacertidae)?**

Shelley Edwards, Krystal A. Tolley, Bieke Vanhooydonck, G. John Measey, Anthony Herrel

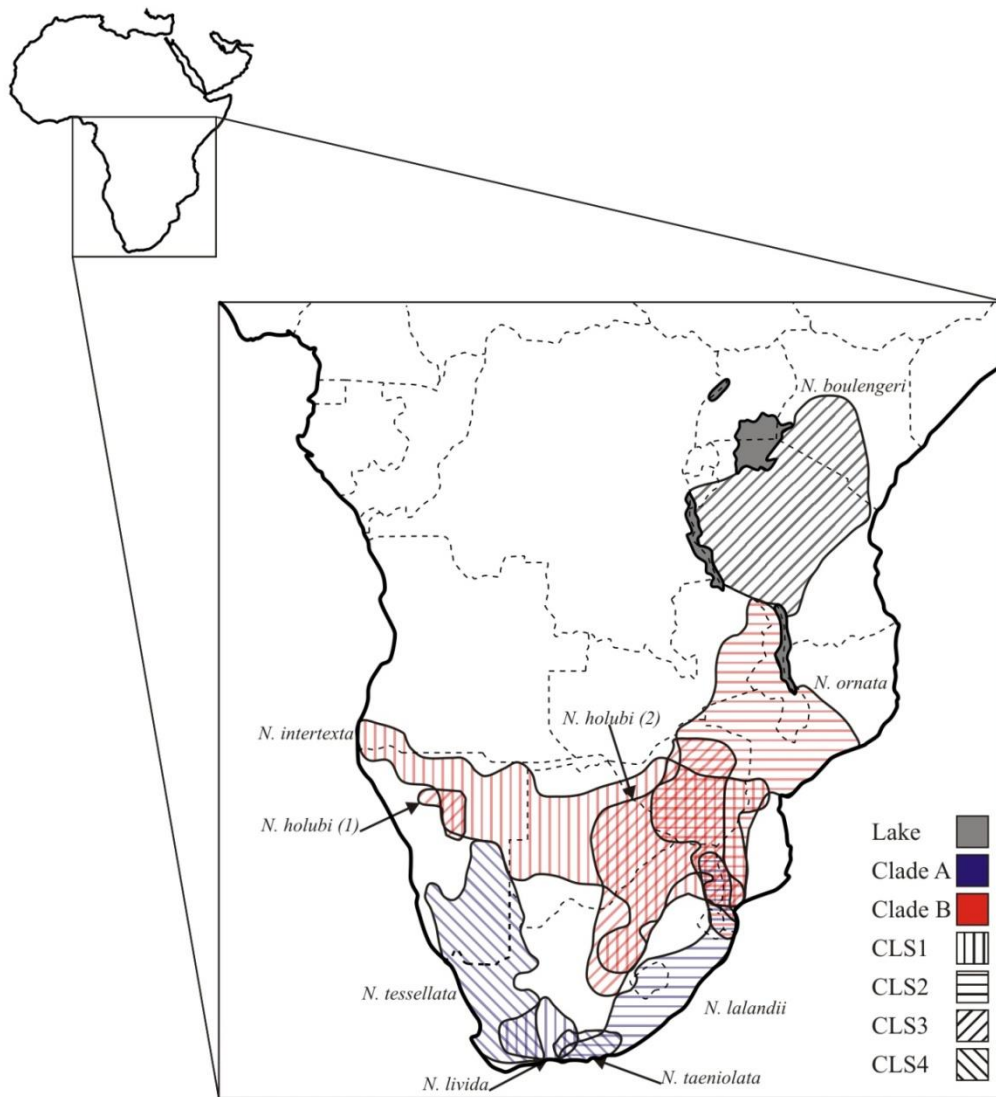


Figure S1: Map of the distributions within the African continent of all *Nucras* species used in the phylogenetic analyses. The key to the colouration (for genetic clades) and patterns (for morphological clusters) within each species distribution is shown to the right of the map. Countries are labelled and each species is labelled in italic font. Distributions for the species were adapted from Branch (1998) and Spawls *et al.* (2006).

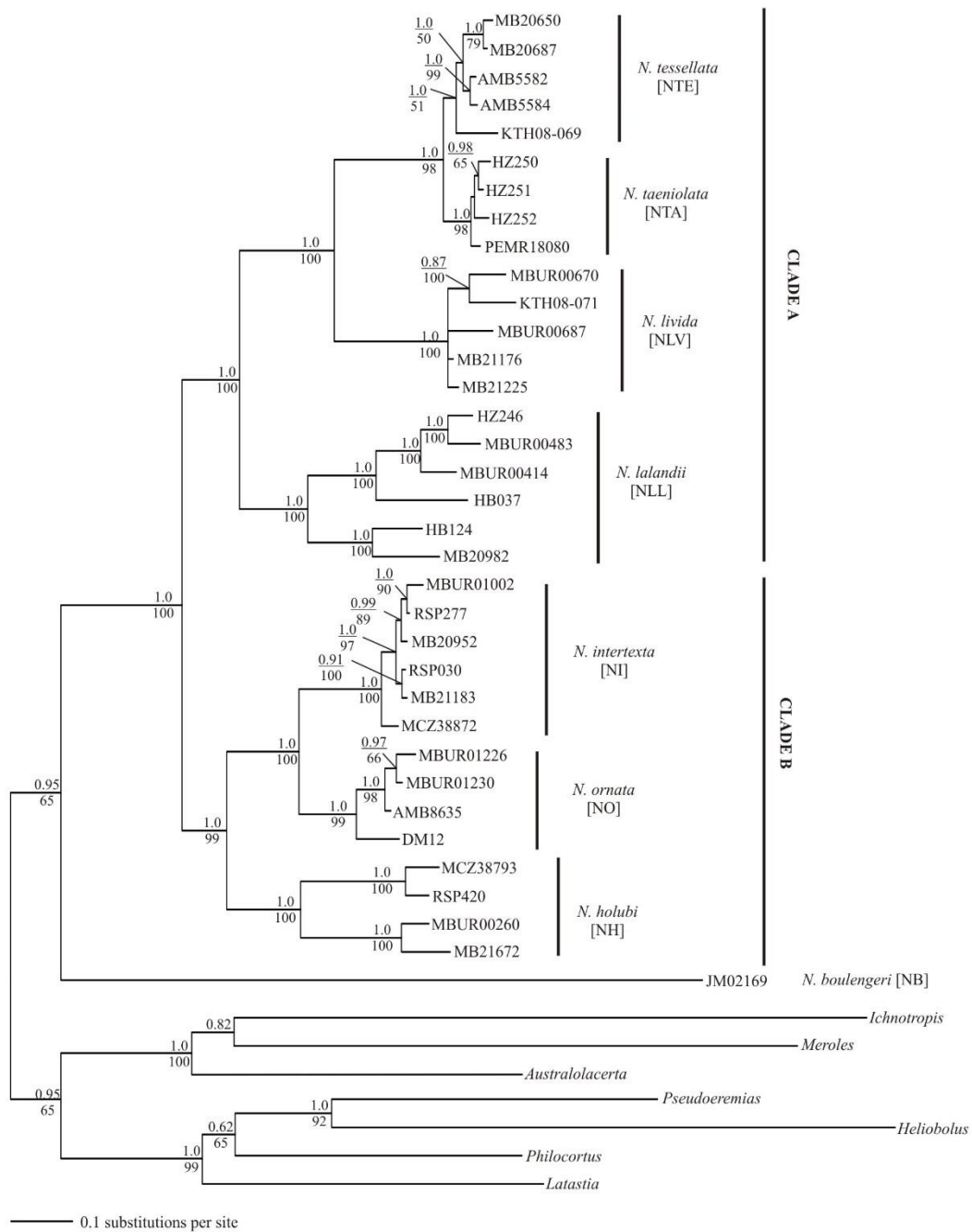


Figure S2. Phylogenetic tree of the genus *Nucras* based on the combined partial 16S, ND4, RAG1 and KIAA-2018 gene regions and inferred by BI and ML (Bayesian topology shown). Sample numbers are indicated at terminal tips, and species names are given. Nodes are considered supported if posterior probabilities > 0.95 (estimated using Bayesian inference) and/or bootstrap values > 75% (using maximum likelihood analyses).

Table S1: List of specimens used for the phylogenetic analyses. Genus, species, museum and field accession numbers given, and EMBL-Bank accession numbers for the two mitochondrial (16S, ND4) and two nuclear (RAG1, KIAA-2018) gene fragments sequenced.

Genus	Species	Field accession ID	Museum accession number	EMBL-Bank accession for 16S	EMBL-Bank accession for ND4	EMBL-Bank accession for RAG1	EMBL-Bank accession for KIAA
<i>Philocortus</i>	<i>spinalis</i>			—	—	EF632238	—
<i>Pseuderemias</i>	<i>smithii</i>			—	—	EF632243	—
<i>Latastia</i>	<i>longicaudata</i>			AF080358	—	EF632229	—
<i>Heliobolus</i>	<i>lugubris</i>			—	—	EF632216	—
<i>Australolacerta</i>	<i>australis</i>	MH0531		DQ871152	FR751398	DG871208	HF547652
<i>Australolacerta</i>	<i>australis</i>	GW08		HF547772	HF547725	HF54769	HF547651
<i>Ichnotropis</i>	<i>capensis</i>	AMB6001	NMNW	DQ871148	HF547732	DQ871206	HF547657
<i>Ichnotropis</i>	<i>capensis</i>	AMB6067	CAS 209602	DQ871149	HF547733	DQ871207	HF547658
<i>Meroles</i>	<i>ctenodactylus</i>	JM03611		—	HF547742	HF547705	HF547665
<i>Meroles</i>	<i>suborbitalis</i>	SVN049	PEMR18376	HF547800	HF547759	HF547718	HF547678
<i>Nucras</i>	<i>boulengeri</i>	JM02169		HG005184	HG005212	HG005233	HG005258
<i>Nucras</i>	<i>holubi</i>	DM12		HG005185	HG005213	HG005234	HG005259
<i>Nucras</i>	<i>holubi</i>	MCZ38793		HG005186	HG005214	HG005235	HG005260
<i>Nucras</i>	<i>holubi</i>	RSP420		HG005187	HG005215	HG005236	HG005261
<i>Nucras</i>	<i>holubi</i>	MBUR00260		HG005188	HG005216	HG005237	HG005262
<i>Nucras</i>	<i>holubi</i>	MBUR01002		HG005189	HG005217	HG005238	HG005263
<i>Nucras</i>	<i>intertexta</i>	RSP277		HG005190	HG005218	HG005239	HG005264
<i>Nucras</i>	<i>intertexta</i>	RSP030	PEMR18257	HG005191	HG005219	HG005240	HG005265
<i>Nucras</i>	<i>intertexta</i>	MCZ38872		HG005192	HG005220	HG005241	HG005266
<i>Nucras</i>	<i>intertexta</i>	MB20952		HG005193	HG005221	HG005242	HG005267
<i>Nucras</i>	<i>intertexta</i>	MB21183		HG005194	HG005222	—	HG005268
<i>Nucras</i>	<i>lalandii</i>	HB124		HF951553	HF951532	HF951537	—
<i>Nucras</i>	<i>lalandii</i>	HB037		HF951554	HF951533	HF951538	HF951548
<i>Nucras</i>	<i>lalandii</i>	HZ246		HF951555	HF951534	HF951539	HF951549
<i>Nucras</i>	<i>lalandii</i>	MBUR00414		HG005195	HG005223	HG005243	HG005269
<i>Nucras</i>	<i>lalandii</i>	MBUR00483		HG005196	HG005224	HG005244	HG005270
<i>Nucras</i>	<i>lalandii</i>	MB20982		HG005197	HG005225	HG005245	HG005271
<i>Nucras</i>	<i>livida</i>	MBUR00670		HG005198	—	HG005246	HG005272
<i>Nucras</i>	<i>livida</i>	MBUR00687		HG005199	HG005226	—	—
<i>Nucras</i>	<i>livida</i>	KTH08-071		HG005200	HG005227	HG005247	HG005273
<i>Nucras</i>	<i>livida</i>	MB21176		HG005201	HG005228	HG005248	HG005274
<i>Nucras</i>	<i>livida</i>	MB21225		HG005202	HG005229	HG005249	—
<i>Nucras</i>	<i>ornata</i>	MBUR01226		HG005203	—	HG005250	—
<i>Nucras</i>	<i>ornata</i>	MBUR01230		HG005204	—	HG005251	HG005275
<i>Nucras</i>	<i>ornata</i>	MB21672		HG005205	—	—	HG005276
<i>Nucras</i>	<i>ornata</i>	AMB8635		HG005206	—	HG005252	HG005277
<i>Nucras</i>	<i>taeniolata</i>	HZ250		HG005207	—	HG005253	HG005278
<i>Nucras</i>	<i>taeniolata</i>	HZ251		HG005208	HG005230	HG005254	HG005279
<i>Nucras</i>	<i>taeniolata</i>	HZ252		HG005209	—	HG005255	HG005280
<i>Nucras</i>	<i>taeniolata</i>	PEMR18080		HG005210	HG005231	HG005256	HG005281
<i>Nucras</i>	<i>tessellata</i>	MB20650		HF951556	HF951535	HF951540	HF951550
<i>Nucras</i>	<i>tessellata</i>	MB20687		HF951557	HF951536	HF951541	HF951551
<i>Nucras</i>	<i>tessellata</i>	AMB5582	CAS 206723	DQ871143	—	DQ871201	—
<i>Nucras</i>	<i>tessellata</i>	AMB5584		HG005211	HG005232	HG005257	HG005282
<i>Nucras</i>	<i>tessellata</i>	KTH08-069		HF951559	—	HF951543	—

Table S2: List of specimens used for the morphometric analyses. Genus, species, museum and field accession numbers given, and an indication of whether the specimen was used in the linear morphometric, and geometric morphometric analyses.

Species	Linear morphometrics	Geometric morphometrics – dorsal cranial view	Geometric morphometrics – lateral cranial view
<i>Nucras boulengeri</i>	N=7 PEMR7147, PEMR10017, PEMR14030, PEMR16773, PEMR16780, PEMR16790, TM11913		
<i>N. caesicaudata</i>	N=8 TM28819, TM28894, TM28895, TM28954, TM28955, TM29279, TM29317, TM29467		
<i>N. holubi</i>	N=28 PEMR5079, PEMR10426, PEMR10427, PEMR10428, PEMR10430, PEMR10440, PEMR10441, PEMR10444, PEMR10445, PEMR10446, PEMR10447, PEMR10448, PEMR10449, PEMR10450, PEMR10451, PEMR10452, PEMR17430, PEMR18239 (RSP007), PEMR18240 (RSP008), PEMR18285, PEMR18290 (RSP122), PEMR18293 (RSP123), PEMR18296 (RSP121), PEMR18299 (RSP133), RSP420, WP128, WP134, WP137	N=19 PEMR5079, PEMR10427, PEMR10428, PEMR10430, PEMR10440, PEMR10441, PEMR10444, PEMR10446, PEMR10447, PEMR10448, PEMR10449, PEMR10450, PEMR10451, PEMR18239 (RSP007), PEMR18240 (RSP008), PEMR18290 (RSP122), PEMR18293 (RSP123), PEMR18296 (RSP121), PEMR18299 (RSP133)	N=20 PEMR5079, PEMR10427, PEMR10428, PEMR10430, PEMR10440, PEMR10444, PEMR10445, PEMR10446, PEMR10447, PEMR10448, PEMR10449, PEMR10450, PEMR17430, PEMR18239 (RSP007), PEMR18240 (RSP008), PEMR18285, PEMR18290 (RSP122), PEMR18293 (RSP123), PEMR18296 (RSP121), PEMR18299 (RSP133)
<i>N. intertexta</i>	N=29 PEMR8427, PEMR15970, PEMR18257 (RSP030), PEMR18258 (RSP031), TM14538, TM14958, TM28229, TM28820, TM44762, TM49438, TM57832, TM63058, TM67345, TM68838, TM68839, TM68840, TM78705, TM78706, TM78708, TM83339, TM83564, TM83566, RSP277, WP123, WP133, WP139, WP140, WP141, WP143	N=21 PEMR8427, PEMR15970, PEMR18257 (RSP030), PEMR18258 (RSP031), TM14538, TM14958, TM28229, TM28820, TM44762, TM49438, TM57832, TM63058, TM67345, TM68838, TM68839, TM68840, TM78706, TM78708, TM83339, TM83564, TM83566	N=19 PEMR8427, PEMR15970, PEMR18257 (RSP030), PEMR18258 (RSP031), TM14538, TM14958, TM28229, TM28820, TM57832, TM67345, TM68838, TM68839, TM68840, TM78705, TM78706, TM78708, TM83339, TM83564, TM83566
<i>N. lalandii</i>	N=34 PEMR1939, PEMR2693, PEMR3043, PEMR3053, PEMR4576, PEMR7247, PEMR8055, PEMR8164, PEMR8168, PEMR13357, PEMR13358, PEMR16002, PEMR16003, PEMR16005, PEMR16007, PEMR16008, PEMR16012, PEMR16015, PEMR16016, PEMR16022, PEMR16023, PEMR16026, PEMR16027, PEMR16029, PEMR16032, PEMR16035, PEMR16036, PEMR16038, PEMR16039, PEMR16042, PEMR16492, PEMR16493, PEMR17435, HZ246	N=27 PEMR2693, PEMR3043, PEMR3053, PEMR4576, PEMR7247, PEMR8055, PEMR8164, PEMR8168, PEMR13357, PEMR13358, PEMR16002, PEMR16007, PEMR16012, PEMR16015, PEMR16016, PEMR16022, PEMR16023, PEMR16025, PEMR16029, PEMR16032, PEMR16035, PEMR16036, PEMR16038, PEMR16039, PEMR16042, PEMR16493, HZ246	N=26 PEMR3043, PEMR3053, PEMR4576, PEMR7247, PEMR8055, PEMR8164, PEMR8168, PEMR13357, PEMR13358, PEMR16002, PEMR16003, PEMR16007, PEMR16012, PEMR16015, PEMR16016, PEMR16022, PEMR16025, PEMR16026, PEMR16029, PEMR16032, PEMR16035, PEMR16036, PEMR16038, PEMR16039, PEMR16492, PEMR16493

Table S2: cont.

Species	Linear morphometrics	Geometric morphometrics – dorsal cranial view	Geometric morphometrics – lateral cranial view
<i>N. livida</i>	N=16 PEMR542, PEMR4300, PEMR4382, PEMR4401, PEMR6547, PEMR6714, PEMR8186, PEMR8726, PEMR15531, PEMR15968, PEMR15969, TM20129, TM29997, TM36133, TM63817, TM70631		
<i>N. ornata</i>	N=25 PEMR5906, PEMR8421, PEMR8438, PEMR8439, PEMR8450, PEMR8478, PEMR8483, PEMR10425, PEMR10442, PEMR10453, PEMR10454, PEMR10458, PEMR10459, PEMR10463, PEMR10464, PEMR10466, PEMR10469, PEMR10470, PEMR10480, PEMR12000, PEMR12161, PEMR12162, PEMR17591, PEMR17595, PEMR17596	N=21 NANR25, PEMR5906, PEMR8421, PEMR8438, PEMR8439, PEMR8478, PEMR8483, PEMR10442, PEMR10453, PEMR10454, PEMR10458, PEMR10459, PEMR10463, PEMR10464, PEMR10466, PEMR10469, PEMR10470, PEMR10480, PEMR12000, PEMR17591, PEMR17596	N=21 NANR25, PEMR5906, PEMR8421, PEMR8438, PEMR8439, PEMR8478, PEMR8483, PEMR10425, PEMR10442, PEMR10453, PEMR10454, PEMR10458, PEMR10459, PEMR10463, PEMR10464, PEMR10466, PEMR10470, PEMR10480, PEMR12000, PEMR17591, PEMR17596
<i>N. taeniolata</i>	N=18 FP257, HZ250, HZ251, HZ252, HZ254, HZ256, HZ257, HZ259, PEMR4875, PEMR5075, PEMR10135, PEMR15974, PEMR15980, PEMR15983, PEMR15986, PEMR15988, PEMR17628, TM877		
<i>N. tessellata</i>	N=22 PEMR4763, PEMR4857, PEMR7070, PEMR7155, PEMR7590, PEMR7629, PEMR7681, PEMR8147, PEMR8719, PEMR11111, PEMR12410, PEMR13355, PEMR15990, PEMR15992, PEMR15993, PEMR15994, PEMR15997, PEMR16000, PEMR16872, PEMR16873, H5659, H6040	N=17 PEMR4857, PEMR7070, PEMR7629, PEMR8147, PEMR11111, PEMR12410, PEMR13355, PEMR15990, PEMR15991, PEMR15993, PEMR15994, PEMR15997, PEMR16000, PEMR16872, PEMR16873, H5659, H6040	N=19 PEMR4763, PEMR7070, PEMR7155, PEMR7590, PEMR7629, PEMR7681, PEMR8147, PEMR12410, PEMR13355, PEMR15990, PEMR15991, PEMR15993, PEMR15994, PEMR15997, PEMR16000, PEMR16872, PEMR16873, H5659, H6040

* Key to accession numbers: PEMR = Port Elizabeth Museum; TM = Ditsong Museum (formerly the Transvaal Museum); RSP, HZ, FP, WP = field numbers for individuals collected by authors; H = field numbers for individuals collected by Prof. P. L. Mouton.

Table S3: List of specimens used for the performance analyses (all specimens were caught in the field). Species, sample size for performance analyses and field accession numbers given.

Species	Bite	Sprint	Individual field ID numbers
<i>Nucas holubi</i>	5	5	RSP420, GF107, GF108, GF113, HZ603
<i>N. intertexta</i>	19	19	RSP277, 998, 999, GF154, GF176, GF202, GF218, GF221, GF253, GF279, GF286, GF287, HZ604, HZ613, HZ615, HZ619, HZ623, HZ635, 996, 997
<i>N. lalandii</i>	-	1	HZ246
<i>N. tessellata</i>	2	2	NI, 437

* Key to field ID numbers: RSP, GF, HZ, NI = field numbers for individuals collected by authors

REFERENCES FOR THE SUPPLEMENTARY MATERIAL:

Branch WR. 1998. *Field guide to the snakes and other reptiles of southern Africa. Revised edition.*

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Spawls S, Howell K, Drewes RC. 2006. *Reptiles and Amphibians of East Africa.* USA: Princeton

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