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

SHELLEY EDWARDS[1,2]*, KRYSTAL A. TOLLEY[1,2], BIEKE VANHOOYDONCK[3], G. JOHN MEASEY[4] and ANTHONY HERREL[5]

[1]*Applied Biodiversity Research Division, South African National Biodiversity Institute, Claremont 7735, Cape Town, South Africa*
[2]*Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*
[3]*Department of Biology, University of Antwerp, Antwerp, Belgium*
[4]*Department of Zoology, Nelson Mandela Metropolitan University, PO Box 77000, Port Elizabeth 6031, South Africa*
[5]*Département d'Ecologie et de Gestion de la Biodiversité, UMR 7179 CNRS/MNHN, 57 rue Cuvier, Case postale 55, 75231, Paris, Cedex 5, France*

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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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*Corresponding author. E-mail: s.edwards@sanbi.org.za method (Huey & Pianka, 1981; Huey *et al*., 1984; Verwaijen & Van Damme, 2007a, b, 2008; McBrayer & Wylie, 2009), and prey availability (Herrel *et al*., 2001; Verwaijen, Van Damme & Herrel, 2002). The dietary composition, particularly the type of prey taken, may influence the head morphology of lizards (Herrel *et al*., 2001; Verwaijen *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*., 1999*a*) 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 *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 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-μL PCR mixes contained approximately 50 ng of genomic DNA, 1 × SuperTherm reaction buffer, 1.5 mM $MgCl_2$, 0.2 μM of each primer, 200 μM dNTPs, and 0.025 U/μL 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 20×10^6 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 (1×10^6 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 reached stationarity; and (3) the effective sample sizes of all parameters were ≥ 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 ≥ 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):

$$B = 1 \Big/ \sum_{i=1}^{N} p_{\mathrm{i}}^{2}$$

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; Verwaijen *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	*Nucras holubi*	*Nucras intertexta*	*Nucras lalandii*	*Nucras ornata*	*Nucras tessellata*
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.

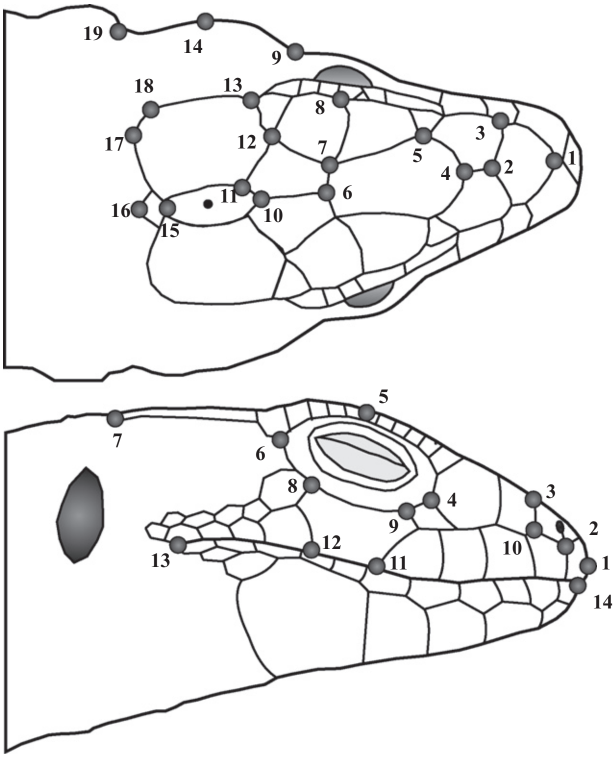

Figure 1. Diagram depicting the homologous landmarks that were digitized for the geometric morphometric analyses for the dorsal (top) and lateral (bottom) views of the *Nucras* crania.

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 *Nucras* 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 *Nucras* 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

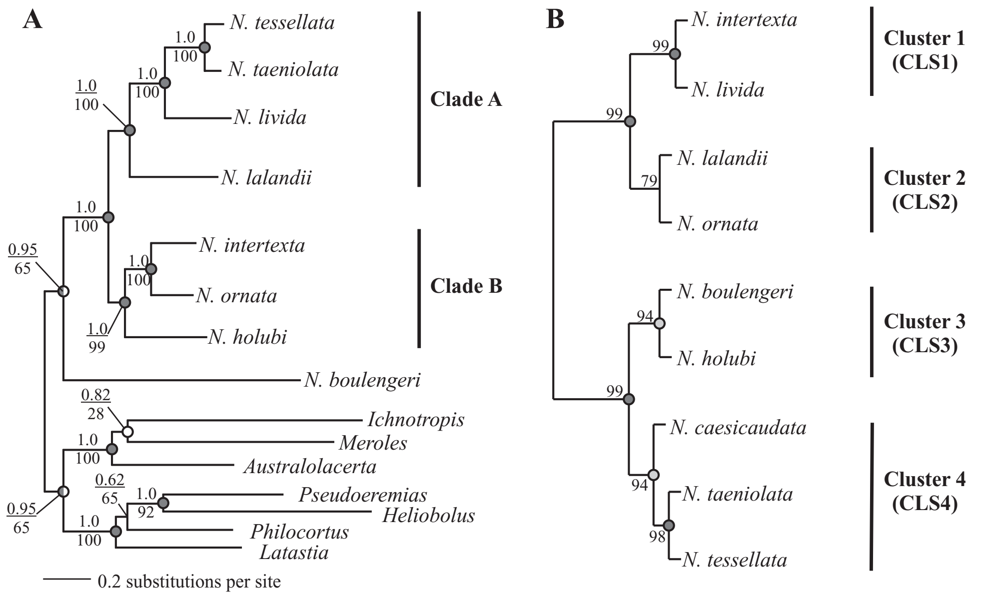

Figure 2. Phylogenetic tree shown (A) inferred from Bayesian analyses (BI) and likelihood methods (ML) using a combined dataset of mtDNA (16S, ND4) and nuclear DNA (RAG1, KIAA-2018) (topology from BI shown). Support values shown at the nodes and indicated by the circles at the nodes: Bayesian posterior 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 ± 2.47%; ND4: 13.31 ± 1.12%; RAG1: 1.07 ± 0.51%; KIAA: 0.58 ± 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 infromation, Fig. S1). The sequence divergences between *N. boulengeri* and the other *Nucras* (16S: 5.98 ± 1.44%; ND4: 16.95 ± 1.03%; RAG1: 5.41 ± 0.84%; KIAA: 1.25 ± 0.41%) approximated the level of sequence divergence between other genera in this study (16S: 10.10 ± 1.79%; ND4: 16.58 ± 1.01%; RAG1: 5.59 ± 0.80%; KIAA: 2.61 ± 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 ± SD of the linear morphometric measurements (mm) for the species used in the dietary analyses

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

		Nonphylogenetic				Phylogenetic		
Independent	Dependent*	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.

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)

		Nonphylogenetic				Phylogenetic		
Independent	Dependent	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. intertexta*) 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; Verwaijen *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

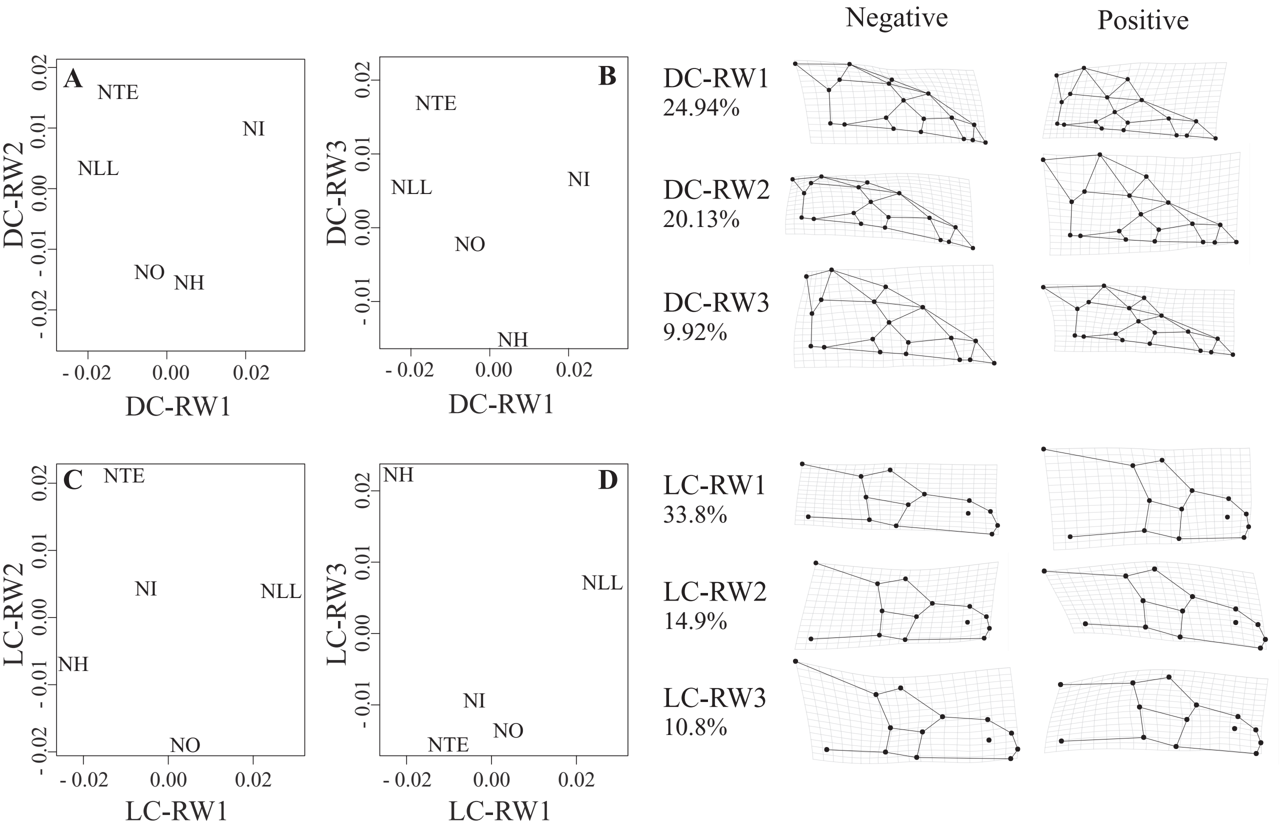

Figure 3. Scatterplots plotting the first three relative warps (RW) components for the dorsal (DC: A, B) and lateral (LC: C, D) views. Deformation grids indicate the cranial shape differences on either the negative or positive ends of the first three relative warp components for the dorsal and lateral 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*.

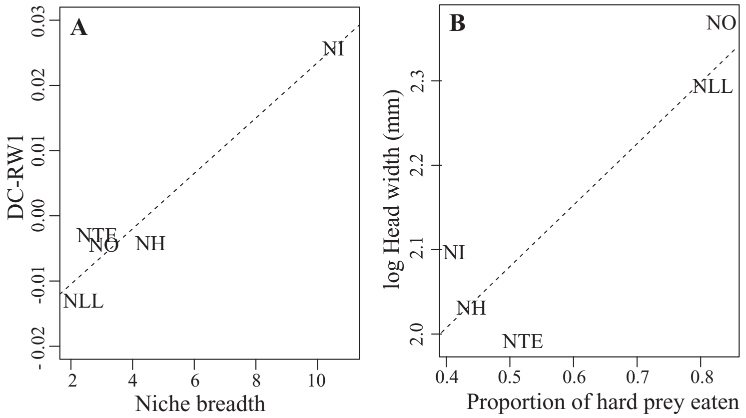

Figure 4. Scatterplots of the means of the significant correlations for the nonphylogenetic correlation analyses (Tables 3, 4), with the slope of the correlations shown by a dashed line within plots. Variables plotted are: niche breadth against the first dorsal relative warp component (A) and proportion 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.