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Proximate causes of variation in dermal armour: insights from armadillo lizards

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ABSTRACT

Although it is widely assumed that body armour in animals evolved to thwart predator attacks, assessing the role that predators may play in shaping defensive morphologies has proven to be difficult. Recent studies suggest that body armour might be influenced by additional factors besides predation, and/or even by sexual selection. We investigated variation in dermal armour in 13 populations of armadillo lizards Ouroborus cataphractus, spanning the entire distribution range of the species. We obtained thickness measurements of osteoderms – bony plates embedded in dermal layer of the skin – using micro- and nano-computed tomography. Using these data, we examined the effects of predation pressure/risk and climatic variables on dermal armour variation and addressed sexual and ontogenetic influence. Our results show that climate is the only factor affecting variation in dermal armour. Populations inhabiting more arid environments, characterized by low summer precipitation and mild winter temperatures, are relatively more armoured than those present in less arid environments. In contrast to our expectations, predation pressure or perceived predation risk was not associated with osteoderm thickness. The results of our study support the idea that the evolution of defensive traits might not be driven exclusively by predator-prey interactions, but could be moulded by environmental factors. In particular, we highlight the role of dermal armour as a potentially important mechanism to reduce evaporative water loss in arid environments.

1 | INTRODUCTION

Defensive morphologies have evolved throughout the animal kingdom as a way for species to avoid predation (Edmunds 1974, Cloudsley-Thompson 1994, Caro 2005, Stankowich 2012). Some defensive traits, like spines, may hinder prey handling and ingestion and are therefore predominantly involved in predator deterrence. Classic examples hereof include the spines of porcupines and hedgehogs (Stankowich 2012, Stankowich and Campbell 2016) or lizards (Losos et al. 2002, Broeckhoven et al. 2016). Others, like scales, carapaces and osteoderms (i.e., mineralised deposits that form bony structures in the dermal layer of the skin) may provide an effective physical barrier against predator bites (Meyers et al. 2012, Zhu et al. 2013, Broeckhoven et al. 2015, Broeckhoven et al. 2017a).

Body armour is generally assumed to provide prey with an advantage during a predatory attack, yet, attributing variation in body armour to predation risk or pressure remains challenging (e.g. Spence et al. 2013, MacColl and Aucott 2014, Smith et al. 2014). This is further complicated by accumulating bodies of evidence supporting the idea that defensive morphologies are not exclusively the outcome of predator-prey interactions, but instead have a multifunctional nature shaped by functional trade-offs (Rivera and Stayton 2011, Magwene and Socha 2013, Broeckhoven et al. 2017a). For example, body armour might play an important role during thermoregulation (Endo et al. 2009, Krmpotic et al. 2015, Broeckhoven et al. 2017a, Ciancio et al. 2017, Clarac et al. 2017). In addition, besides being a product of natural selection, intrasexual aggression might contribute significantly to variation in the expression of defensive traits (Broeckhoven et al. 2017b, English 2018). Disentangling the contribution of multiple selective pressures to variation in body armour may therefore be pivotal for our understanding of the evolution of defensive traits.

Girdled lizards (Squamata: Cordylinae), sit-and-wait foraging lizards from the southern and eastern parts of Africa (Mouton and van Wyk 1997), provide ample

opportunities to address the effects of predators and other factors on body armour, because considerable variation is present within and among species (Broeckhoven et al. 2015, 2016). One species in particular, the armadillo lizard, *Ouroborus cataphractus* (Boie 1828), is a highly suitable model organism for understanding the proximate causes of variation in body armour (Broeckhoven et al. 2015). Armadillo lizards occur in the western parts of South Africa and their range falls within the semi-arid Succulent Karoo biome (Shuttleworth et al. 2013). They are termite-eating specialists (Shuttleworth et al. 2008, Mouton et al. 2000, Broeckhoven & Mouton 2013, Shuttleworth et al. 2013) that evolved heavy body armour, including long spines and thick imbricating osteoderms, as a defence mechanism against mongoose predators when exploiting termites away from the safety of their shelters (Broeckhoven et al. 2015). Although strongly linked, the relationship between dermal armour and predation is not a straightforward one, because significant variation in osteoderm thickness is present among populations (Broeckhoven et al. 2015).

In this study, we examine the correlates of intraspecific variation in dermal armour in armadillo lizards in an attempt to unravel the selective pressures involved in the evolution and diversification of defensive traits in general. Firstly, dermal armour might be under sexual selection as it could play an important role during intraspecific competition by protecting individuals against aggressive bites or attacks from conspecifics (Song et al. 2011, Broeckhoven et al. 2017b). Secondly, the presence or absence of specific predator species (i.e., predation pressure), or changes in vulnerability to predators (i.e., predation risk) may impose selection on defensive traits, especially if variation in the trait of interest directly relates to the survival and reproductive success of the bearer (Le Rouzic et al. 2011, Broeckhoven et al. 2015, 2016). For example, the presence of predator species with a more powerful bite force or, alternatively, the exploitation of food sources in open, exposed environments, might favour more elaborated body armour (Broeckhoven et al. 2015). Thirdly,

environmental factors might have a greater effect on body armour than biotic factors. For instance, dermal armour and its associated vascularization might be involved in the distribution of absorbed radiant heat (Farlow et al. 2010, Clarac et al. 2017). In a similar fashion, highly vascularized dermal armour could play a role in regulating evaporative water loss (Ruibal and Shoemaker 1984, Toledo and Jared 1993). The possession of thicker osteoderms with higher degree of vascularization, may be associated with more extreme climatic conditions such as high temperatures or low precipitation (Broeckhoven et al. 2017a). These hypotheses are summarized in a conceptual model (Figure 1) illustrating how predation and other factors may interact to generate variation in dermal armour.

2 | MATERIALS AND METHODS

2.1 | Measurement of dermal armour

Micro- and nano-computed tomography (CT) scanning was used to investigate variation in dermal armour (Fig. 2). Firstly, because the development of osteoderms might vary during different developmental or life stages (Broeckhoven et al. 2017b), we conducted a detailed examination of post-cranial osteoderm development in armadillo lizards. Skin sections measuring approximately 5 x 10 mm were excised from formalin-fixed ethanol-preserved specimens belonging to the Ellerman Collection at Stellenbosch University. These specimens were collected by Mouton et al. (1999) as part of a larger project on the biology of armadillo lizards and represent all ontogenetic stages. Skin sections were excised from 25 males and 20 females and included the entire range of body sizes. All sections were nano-CT scanned at high resolution using a GE Phoenix Nanotom S system (Phoenix X-ray, General Electric Sensing and Technologies, Wunstorf, Germany) located at the CT Scanner Facility, Stellenbosch University (du Plessis et al. 2016). The following settings were used: X-ray tube voltage of 80 kV, beam current of 120 μA and spatial resolution of 5 μm.

Secondly, we examined dermal armour variation among armadillo lizard populations. Morphological measurements were obtained from a larger number of specimens ($n = 166$) collected at various localities ($n = 13$) throughout the range of the armadillo lizard (Fig. 3). Because osteoderms are embedded in the dermis, invasive labour-intensive techniques, such as histology, are often used to obtain meaningful measurements (e.g. Broeckhoven et al., 2015). Recently, Broeckhoven et al. (2017c) developed a protocol for *in vivo* micro-CT imaging of skeletal and extraskeletal (i.e., osteoderms) bones of reptiles and amphibians, which was implemented in this study. In brief, lizards ($n = 103$) were cooled to $\pm 8°C$, restrained between two Styrofoam plates and placed in a Styrofoam holder. The holder was mounted inside a GE Phoenix v|tome|x L240 dual tube CT instrument (Phoenix X-ray, General Electric Sensing and Technologies, Wunstorf, Germany) located at the CT Scanner Facility, Stellenbosch University (du Plessis et al. 2016) and scanned with an X-ray tube voltage of 50 kV, beam current of 180 mA, a 0.1mm Cu filter and spatial resolution of approximately 35 μm (Broeckhoven et al. 2017c). All individuals were released at the point of capture after *in vivo* micro-CT scanning was conducted (Broeckhoven et al. 2017c). To increase sample size, the dataset was supplemented with preserved specimens belonging to the Ellerman Collection at Stellenbosch University ($n = 63$). Broeckhoven et al. (2017c) found no difference in image quality between *in vivo* scans and *post-mortem* (i.e., using preserved specimens) scans when the analysis of anatomical features was limited to the abdominal region. Hence, in order to combine data from *in vivo* and *post-mortem* scans, osteoderm measurements were restricted to the abdominal region.

All three-dimensional reconstructions were performed using the system-supplied software, datos|x (General Electric Sensing and Technologies, Wunstorf, Germany) and CT scans were subsequently analysed using VGStudio Max 3.0 (Volume Graphics GmbH, Heidelberg, Germany). The thickness of osteoderms belonging to a girdle located in the mid-

abdominal region (Fig. 2B) was measured in the transversal slice plane (Fig. 2C, D) at randomly chosen intervals. We aimed to obtain 30 measurements per individual, but this was not always possible for *in vivo* scans in which one side of the body moved during scanning. Nevertheless, in contrast to measurements resulting from histological sections (e.g. Broeckhoven et al. 2015), micro-CT scans allowed us to obtain measurements for each osteoderm at identical spatial localities, resulting in lower intra-individual variation and measurement error. All measurements were averaged to obtain an estimate of osteoderm thickness for each specimen. Measurements were limited to osteoderm thickness as it directly relates to the proposed functionalities (Broeckhoven et al. 2015, 2017) and our hypotheses.

Body length was used as covariate in all analyses. For the interpopulation analyses, we measured body length, i.e. the distance between the posterior ends of the skull and pelvis, directly from the micro-CT scans. For the intrapopulation analyses, we measured body length, i.e. the distance from the posterior end of the head to the cloacal opening, using digital callipers.

2.2 | Environmental and ecological data

For each sampling location, we obtained a number of variables pertaining to environmental and ecological features. Firstly, we extracted values for 14 climatic variables from the WordClim 2 database (Fick & Hijmans 2017) using the R package RASTER version 2.5-8 (Hijmans et al. 2016). Seven climatic variables related to variation in temperature, i.e. annual mean temperature (BIO1), maximum temperature of the warmest month (BIO5), minimum temperature of the warmest month (BIO6), mean temperature of the wettest quarter (BIO8), mean temperature of the driest quarter (BIO9), mean temperature of the warmest quarter (BIO10) and mean temperature of the coldest quarter (BIO11). The other seven variables were associated with precipitation, i.e., annual precipitation (BIO12), precipitation of the wettest month (BIO13), precipitation of the driest month (BIO14), precipitation of the wettest

quarter (BIO16), precipitation of the driest quarter (BIO17), precipitation of the warmest quarter (BIO18) and precipitation of the coldest quarter (BIO19). A principal component analysis performed on the 14 bioclimatic variables revealed two significant axes that explained in total 91% of the variation (Table 1). The first axis, coined PC_{ENVIR1} correlated positively with winter temperatures and negatively with summer precipitation, whereas the second axis, coined PC_{ENVIR2}, correlated negatively with summer temperatures and winter rainfall (Table 1).

Secondly, to obtain an estimate of predation pressure, we used the Red List of Mammals of South Africa (Child et al. 2016) to determine which mongoose species are present at each of the study populations. Predator-prey overlap was approximated on a quarter-degree spatial grid and the *predation pressure* was calculated as follows: if no mongoose predator sightings have been recorded in the area, a score of 0 was allocated. If small grey mongoose (*Galerella pulverulenta*) and/or yellow mongoose (*Cynictis penicillata*) were present, a score of 1 was allocated, whereas a score of 2 was allocated if any of the aforementioned species and/or meerkat (*Suricata suricatta*) were present. Meerkats form a greater threat to armadillo lizards because of their significantly higher bite force than yellow and small grey mongoose (see Broeckhoven et al. 2015). We did not consider the large grey mongoose (*Herpestes ichneumon*), because it has only been recorded close to the south-eastern limit of the distribution range of the armadillo lizard post-1999 (Do Linh San 2016).

Thirdly, habitat openness was calculated for each of the populations and served as a proxy for *predation risk* (Stankowich and Campbell 2016). Armadillo lizards living in more open environments will be more exposed to predators during foraging excursions, and consequently, will be at greater risk of mortality. Because geographical variation in vegetation index values (e.g., normalized difference vegetation) is low to non-existent in semi-arid environments, we adapted a qualitative scoring system to estimate habitat openness.

Vegetation types for each of the sampling localities were determined using Mucina and Rutherford (2006) as reference. We scored habitat openness as the amount of the substrate covered by vegetation: 0, closed habitats dominated by dense tall shrubs (e.g., Sandstone Fynbos); 1, closed + intermediate habitats dominated by tall scattered or dense shrubs (e.g., Strandveld, Sand Fynbos); 2, intermediate habitats dominated by low scattered shrubs (e.g., Renosterveld, Shrubland); 3, intermediate + open habitats dominated by low scattered herbs (e.g., Quartzite Fynbos); and 4, open habitats dominated by low sparse succulent vegetation (e.g., Succulent Shrubland).

2.3 | Statistical analyses

2.4.1 | Ontogenetic development of dermal armour

To address sexual and ontogenetic influence on the degree of dermal armour, we first tested whether the relationship between log-transformed osteoderm thickness and log-transformed body size follows an isometric pattern or a gradually allometric pattern by comparing the fit of linear and quadratic regression models using the likelihood ratio test implemented in the R package lmtest (Hothorn et al. 2017). Next, we compared the fit of a model that includes sex as a grouping factor against a model without grouping factor to determine whether osteoderm thickness is sexually dimorphic. Finally, these results were used to orient us in our choice of specimens for the subsequent interpopulation analyses.

2.4.2 | Ecological correlates of dermal armour

To identify the combination of variables that could most likely explain intraspecific variation in degree of dermal armour, a spatial autoregressive (SAR) model was used with predation pressure, predation risk and the scores of the principal component analysis (i.e., PC_{ENVIR1} and PC_{ENVIR2}) as the explanatory variables. The interaction effects predation risk*PC_{ENVIR1} and predation risk*PC_{ENVIR2} were also included in the model. The SAR model supplements the

ordinary least squares regression model and assumes that the value of the response variable is not only a function of all explanatory variables, but also of values of the response variables at neighbouring localities (Kissling and Carl 2008). Hence, it takes into account the tendency for closely spaced locations to have more similar values than widely spaced locations (i.e. positive spatial autocorrelation). SAR analyses were conducted using the function "spautolm" implemented in R package SPDEP (Bivand 2017). Prior to running the SAR, a spatial weights matrix was defined to assign weights to the localities that are linked using the function "nb2listw" implemented in R package SPDEP (Bivand 2017). SAR models with single and multiple explanatory variables were conducted to investigate the relationships between osteoderm thickness and the environmental/ecological variables. In the latter case, the combination of variables that could most likely explain intraspecific variation in degree of dermal armour was determined using an information-theoretic approach based on the Akaike information criterion corrected for small sample size (AICc) (Burnham and Anderson 2002).

3 | RESULTS

3.1 | Ontogenetic scaling of dermal armour

Expression of osteoderms occurs early in the development of armadillo lizards (Fig. 4). The expression of post-cranial osteoderms appears to start at around 50 mm body size, before individuals reach one year of age (Fig. 4). The relationship between osteoderm thickness and body size followed a gradually allometric pattern rather than an isometric pattern (likelihood ratio test: $\chi^2 = 33.96$, $P < 0.0001$). During development, the bone tissue radiates in a radial fashion from a prominent keel (Fig. 4 A, B). When individuals reach a body size of approximately 63 mm, a plate-like appearance is achieved and osteoderm thickness starts to increase (Fig. 4 C). A model that included sex as a grouping factor did not provide a better fit than the model without the grouping factor (likelihood ratio test: $\chi^2 = 1.59$, $P = 0.66$), indicating that osteoderm thickness is not a sexually dimorphic trait in armadillo lizards.

Based on the aforementioned results, we excluded individuals with a body size smaller than 65 mm ($n = 23$) from subsequent interpopulation analyses and pooled data for both sexes.

3.2 | Influence of environmental/ecological variables on variation in body armour

Armadillo lizards showed substantial geographic variation in dermal armour (ANOVA: $F_{12,127}$ = 2.20, $P = 0.015$), with individuals from locality F (Lambertsbaai) in Figure 2 possessing relatively the thickest osteoderms (Table 2). SAR models with single and multiple explanatory variables demonstrated a statistically significant association between PC_{ENVIR1} and relative osteoderm thickness (Table 3). Populations inhabiting arid environments, characterized by mild winters and low summer precipitation, had relatively thicker osteoderms than those inhabiting less arid environments (Figure 5). Model selection confirmed these findings and indicated that PC_{ENVIR1} was the most important predictor of relative osteoderm thickness (Table 4).

4 | DISCUSSION

Although it is widely accepted that predation is an important source of selection for body armour, few studies have investigated the contribution of other factors to variation in these allegedly defensive traits (Broeckhoven et al. 2015, 2017a, b). Despite the antipredator advantages that body armour may have (Broeckhoven et al. 2015), our results indicate that osteoderm thickness appears to be associated with climatic conditions, with lizards inhabiting more arid environments possessing relatively thicker osteoderms than those found elsewhere.

4.1 | Ecological and environmental correlates of variation in body armour

The most unexpected finding of our study was the lack of association between the degree of dermal armour and predation pressure. The inability to detect a predator effect may have resulted from the coarse way in which we measured predation pressure, i.e. by calculating the number of predators present in the environment. Although predator density might have been a

better predictor variable, mongoose home range sizes are generally large (Skinner and Chimimba 2005) with only a single individual (e.g., *G. pulverulenta*) or group of individuals (e.g., *S. suricatta*) present at a specific locality (Broeckhoven and Mouton 2015). Nevertheless, differential exposure to predators, rather than predator species or densities, might have contributed, albeit weakly, to variation in body armour. Armadillo lizards rely on harvester termites (*Microhodotermes viator*) as a food source, especially during the dry season (Shuttleworth et al. 2008, Mouton 2011, Shuttleworth et al. 2013). Populations that inhabit more arid environments might have an increased dependency on termites and might be more frequently exposed to mammalian predators during foraging excursions. Additionally, lizards in arid environments might simply be more exposed to predators due to the low vegetative cover. The exploitation of clumped food sources (e.g., termites, ants) in arid, open environments appears to be associated with a heavily armoured phenotype in several other lizard species, including the Australian thorny devil (*Moloch horridus*) and North American horned lizards (*Phrynosoma*) (Pianka 1970, Pianka and Parker 1975). Likewise, in mammals, medium-sized insectivorous taxa that inhabit open habitats (e.g., pangolins and armadillos) are more likely to possess extreme body armour (Stankowich and Campbell 2016). However, we did not find a relationship between degree of dermal armour and habitat openness (~ predation risk), with the latter being explained by PC_{ENVIR2} (linear regression: $\beta = -0.61$, $P = 0.03$).

Environmental factors and their relation to physiology may more directly contribute to variation in body armour. Broeckhoven et al. (2017a) found that the high degree of vascularization present in the osteoderms of armadillo lizards might provide a significant thermal advantage in arid environments. Given that a higher degree of vascularization lowers the puncture resistance of osteoderms, selection might favour relatively thicker osteoderms in populations inhabiting arid environments to support a higher degree of vascularization

without comprising strength (Broeckhoven et al. 2017a). Nonetheless, temperature during summer (PC_{ENVIR2}) did not have a significant effect on osteoderm thickness (Table 3). In contrast, populations inhabiting locations characterized by low winter temperatures (PC_{ENVIR1}) appear to have a relatively thinner osteoderms (Table 3). This could be attributed to the fact that the possession of elaborated body armour might impede effective thermoregulation in colder environments.

Alternatively, that presence of dermal armour might play a role in reducing cutaneous evaporative water loss (Toledo and Jared 1993). Given the strong association between aridity and evaporative water loss (Cox and Cox 2015), adaptations that lead to the minimization of evaporative water loss are might be important to species inhabiting (semi-) arid environments, such as the armadillo lizard. Osteoderms, for instance, have been proposed to reduce water loss in frogs (Ruibal and Shoemaker 1984; Toledo and Jared 1993). In our example, thicker osteoderms might reduce more water loss from the skin. Experimental data, however, are required to verify both the thermoregulation and water loss hypothesis.

4.2 | Ontogenetic development and sexual dimorphism in body armour

Recently, Broeckhoven et al. (2017b) demonstrated sexual dimorphism in osteoderm expression in the Cape cliff lizard (*Hemicordylus capensis*) and suggested a potential role of osteoderms during agonistic interactions between males. In contrast to their findings, our results provide no evidence for a role of sexual selection in determining dermal armour variation in armadillo lizards. The possession of dermal armour clearly provides an antipredatory benefit to armadillo lizards as osteoderms are sufficiently strong to withstand a mongoose bite (Broeckhoven et al. 2015). Hence, additional investment in dermal armour (i.e., increased thickness) might not necessarily confer any advantage during male-male combat. The osteoderms of Cape cliff lizard, on the contrary, are isolated disc-like elements

that do not have any obvious anti-predatory advantage, but could reinforce the skin during male-male combat and minimise the risk of skin tearing.

CONCLUSIONS

In summary, our results suggest that climate is associated with the degree of dermal armour, most likely through its effect on animal's physiology (i.e., water balance regulation). In contrast, although mongoose predation has played a key role in the evolution of armour in armadillo lizards (Broeckhoven and Mouton 2015, Broeckhoven et al. 2015), differences in predation risk or pressure among study locations do not appear to have a major influence on variation in dermal armour. Rigorous phylogenetic comparative analyses should, however, be conducted to investigate whether this trend can be extrapolated to the diversification of body armour in general, particularly that of dermal armour.

DECLARATIONS

- Conflicts of interest – The authors have no conflicts of interest to declare.

- Permit(s) – Armadillo lizards were collected in the Northern Cape Province (permit number: FAUNA 1541/2015) and Western Cape Province (permit number: AAA007-00158-0056), South Africa. All land owners have given their permission to collect specimens on their property. The *in vivo* micro-CT scanning protocol was approved by the Ethical Committee of Stellenbosch University (SU-ACUD15-00044).

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Figure Legends

Figure 1. Conceptual framework showing the hypothesised proximate causes of body armour variation. The degree of dermal armour might be associated with (1) the presence or absence of specific predators (= predation pressure), (2) vulnerability to predators mediated by the habitat (= predation risk), (3) climatic conditions such as ambient temperature and precipitation, and/or (4) male combat resulting in sexual dimorphism. The central image shows the extent of dermal armour in the armadillo lizard.
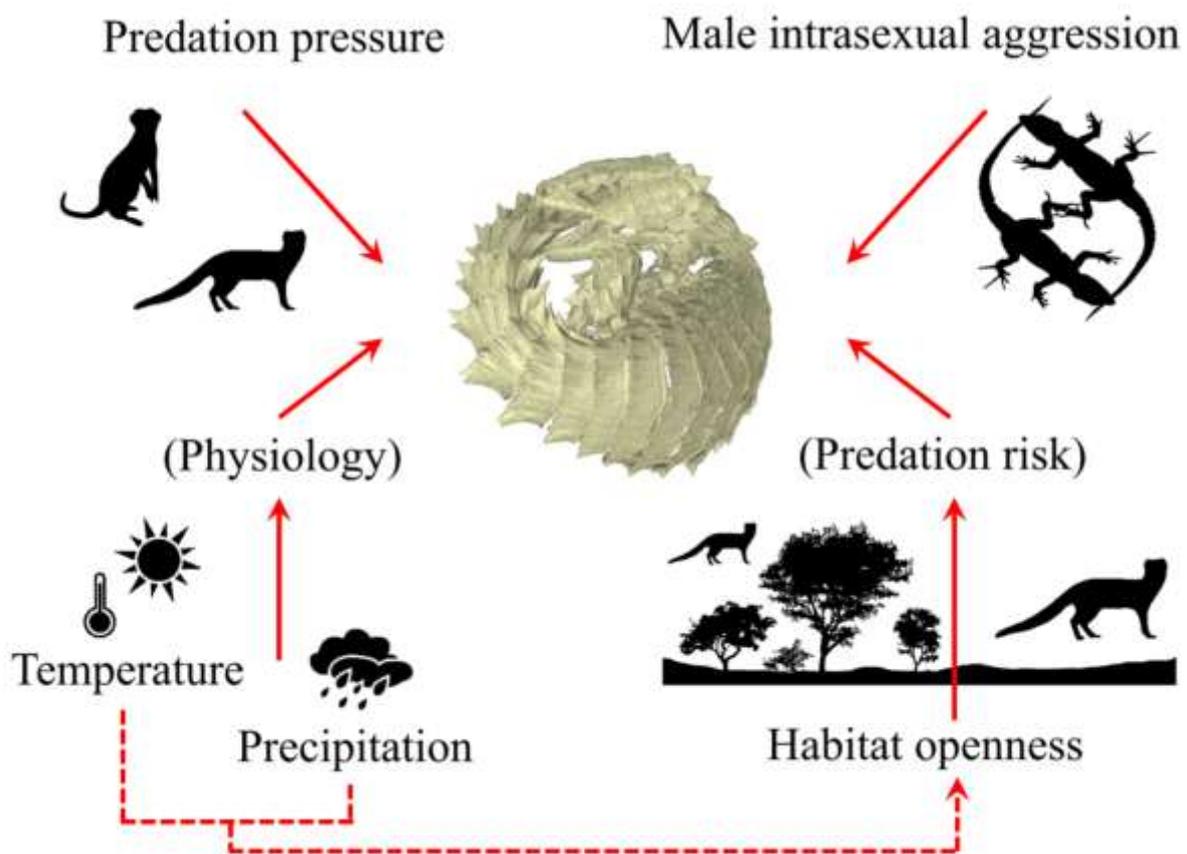

Predation pressure

Male intrasexual aggression

(Physiology)

(Predation risk)

Temperature

Precipitation

Habitat openness

Figure 2. Armadillo lizard (*Ouroborus cataphractus*). (a) Full view of the body depicting the heavily armoured morphology. (b) Three-dimensionally rendered image' of the trunk region obtained using micro-CT with a spatial resolution of 35 μm. (c) Two-dimensional slice view of an abdominal girdle used to calculate osteoderm thickness. (d) Enlarged view of osteoderms.
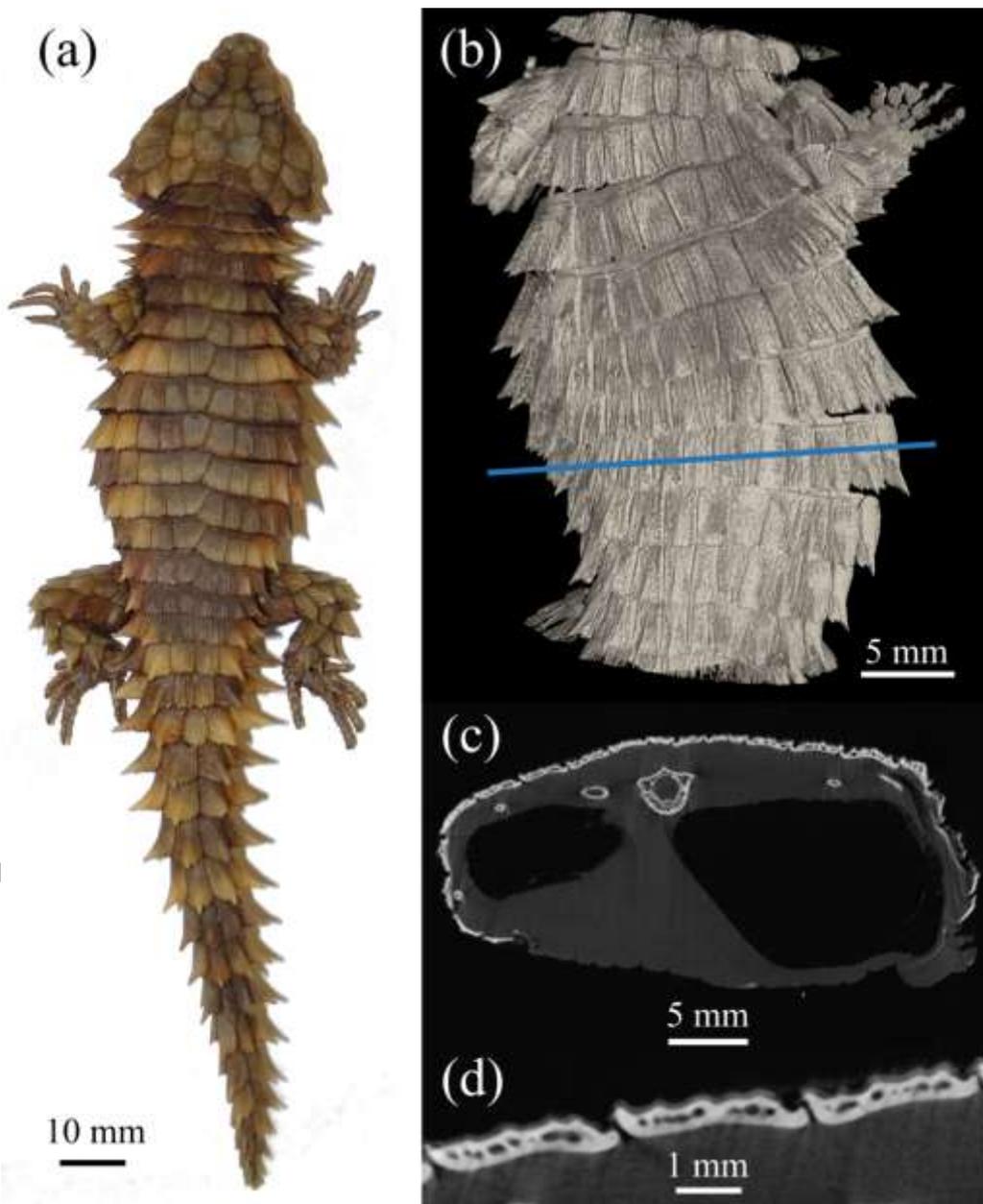
Figure 3. Sampling localities for all 13 armadillo lizard populations. The colour gradient represents precipitation of driest quarter (in mm) and serves to illustrate aridity.
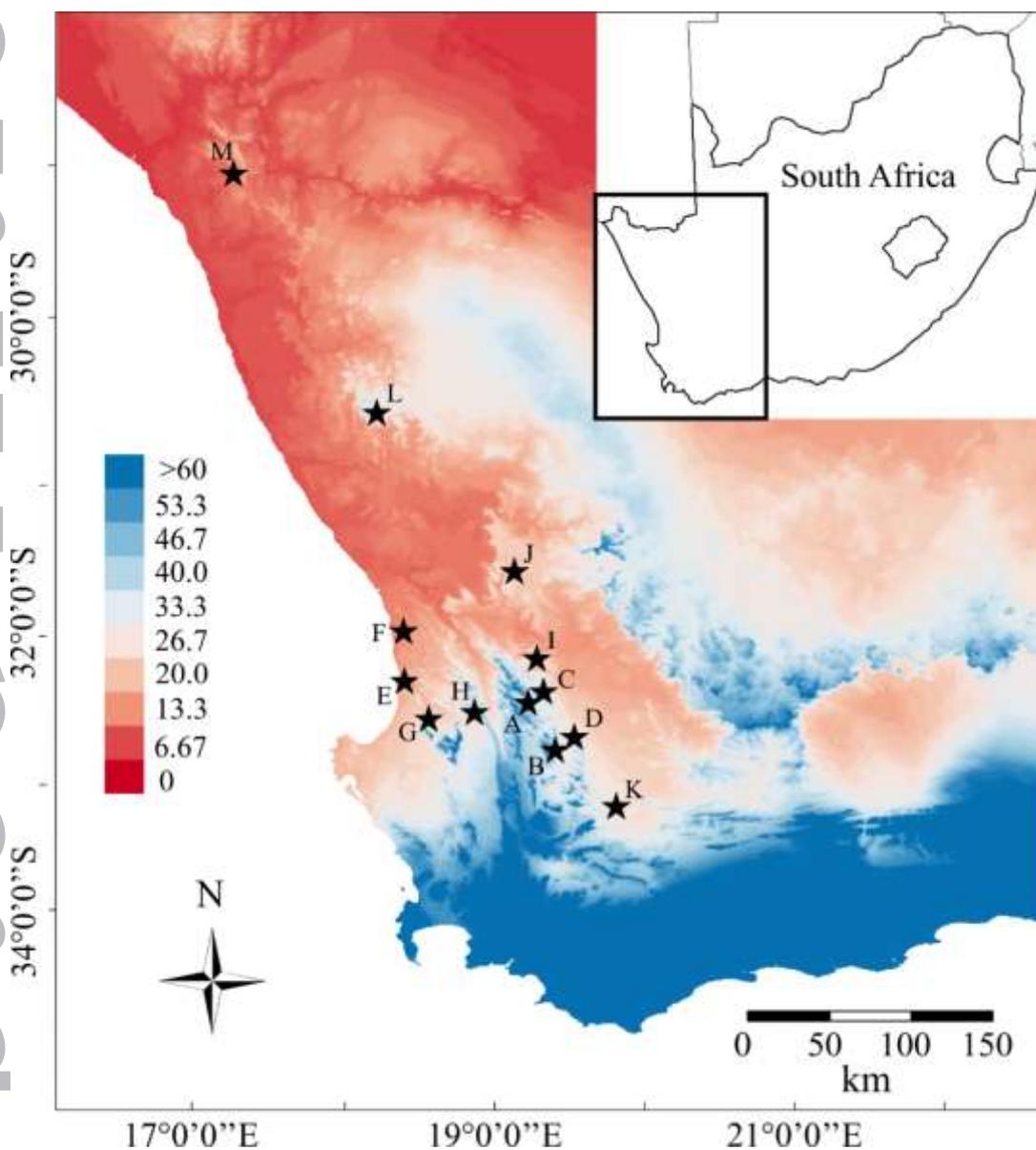
Figure 4. Relationship between body size and osteoderm thickness in male (blue) and female (red) armadillo lizards. The inner bands are the pointwise 95% confidence intervals around the curves, the outer bands are the simultaneous 95% confidence intervals. Osteoderm expression occurs early in the development before the advent of sexual maturity (indicated by the dotted line). High-resolution nano-CT images show that during the development, bone tissue radiates from a prominent keel (a, b) until a plate-like appearance is achieved (c) after which thickness increases. Data were visualized using the R package ggplot2 (Wickham and Chang 2016).
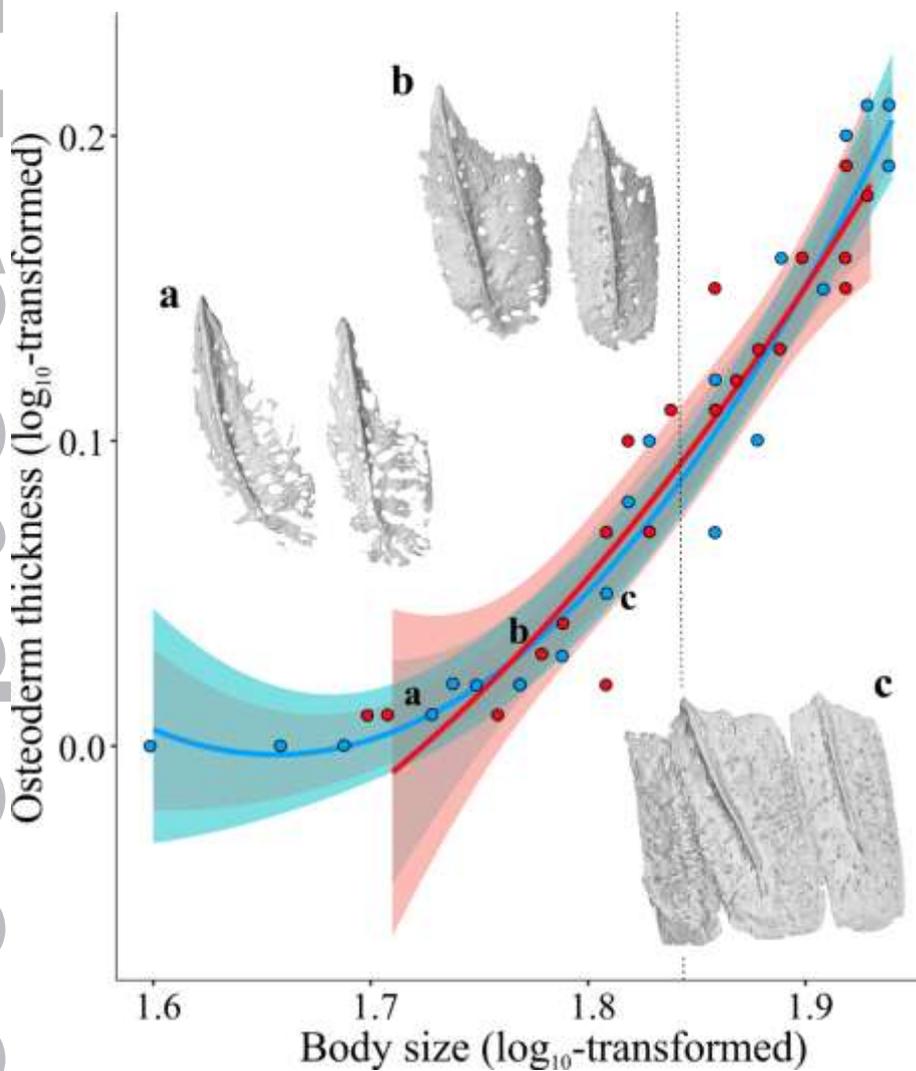
Figure 5. Relationships between ecological/environmental variables and relative osteoderm thickness. Armadillo lizards inhabiting more arid environments, characterized by low summer precipitation, are relatively more armoured than those inhabiting less arid environments. Letters correspond to the sampling localities in Fig. 3.
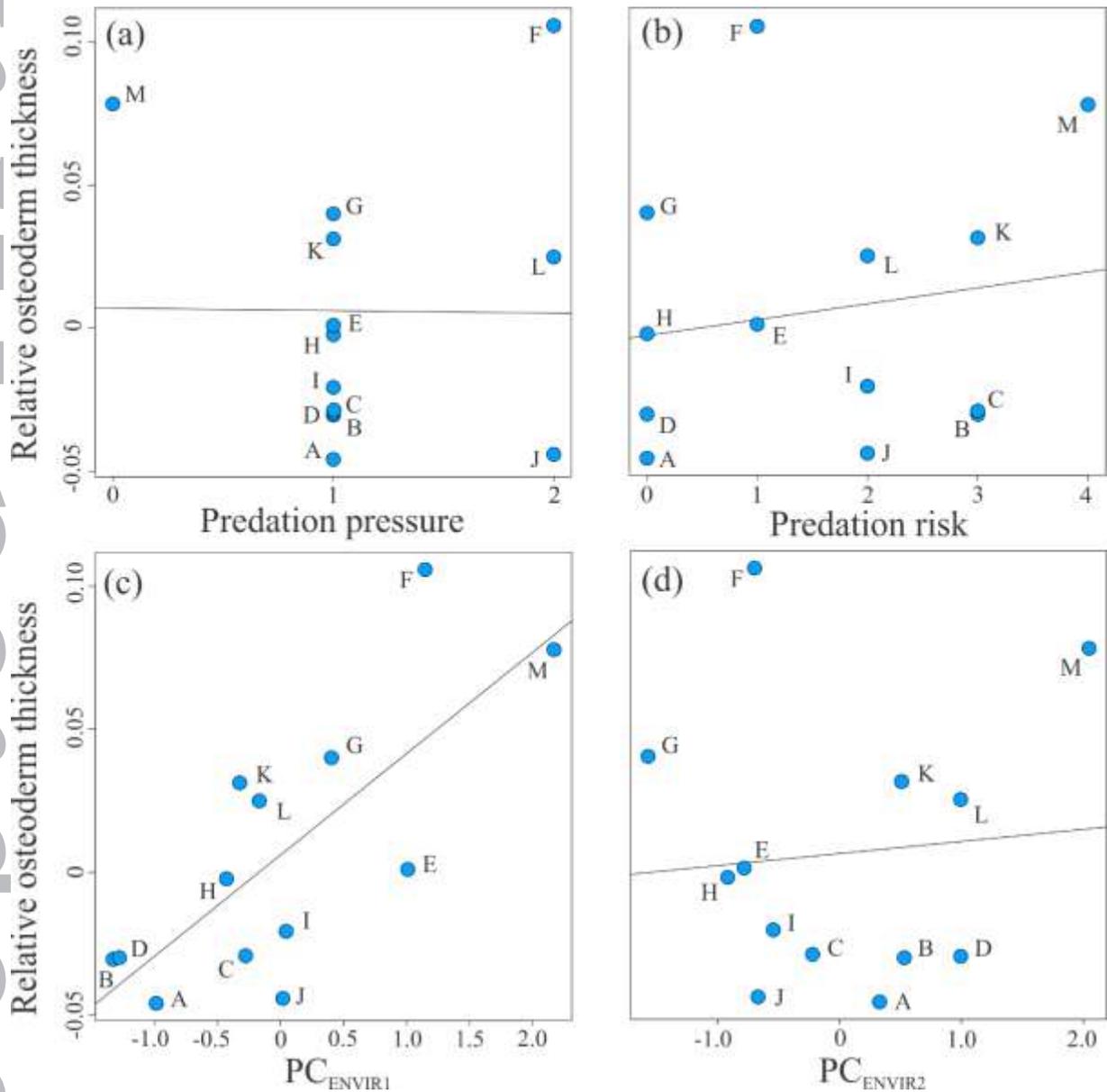
Table Legends

Table 1. Loading scores of a principal component analysis conducted on 14 bioclimatic variables. Values in bold represent loading scores greater than 0.70.

Variables	PC$_{ENVIR1}$	PC$_{ENVIR2}$
BIO1	**0.852**	-0.512
BIO5	0.056	**-0.805**
BIO6	**0.932**	-0.098
BIO8	**0.945**	-0.181
BIO9	0.414	**-0.777**
BIO10	0.360	**-0.870**
BIO11	**0.951**	-0.228
BIO12	-0.678	**-0.710**
BIO13	-0.515	**-0.825**
BIO14	**-0.982**	0.039
BIO16	-0.475	**-0.851**
BIO17	**-0.989**	0.022
BIO18	**-0.969**	0.069
BIO19	-0.486	**-0.847**
Standard deviation	7.766	4.997
Proportion of variance	0.555	0.357
Cumulative proportion of variance	0.555	0.911

Table 2. Summary of body size and dermal armour variation in armadillo lizards. Geographical coordinates of the sampling localities can be provided upon request.

	Locality	*n*	Body size (mean in mm ± SD)	Absolute osteoderm thickness (mean in mm ± SD)	Relative osteoderm thickness (mean ± SD)
A	Cederberg 1	17	70.93 ± 4.38	0.281 ± 0.096	-0.046 ± 0.126
B	Cederberg 2	5	71.08 ± 1.19	0.289 ± 0.098	-0.030 ± 0.143
C	Cederberg 3	16	72.92 ± 3.51	0.311 ± 0.090	-0.029 ± 0.176
D	Cederberg 4	17	72.67 ± 3.84	0.304 ± 0.079	-0.030 ± 0.111
E	Elandsbaai	14	76.15 ± 4.95	0.364 ± 0.072	0.080 ± 0.001
F	Lambertsbaai	14	74.20 ± 5.12	0.438 ± 0.108	0.106 ± 0.084
G	Redelinghuys	6	76.34 ± 4.01	0.397 ± 0.045	0.040 ± 0.042
H	Citrusdal	7	71.33 ± 3.29	0.310 ± 0.089	-0.002 ± 0.094
I	Wupperthal	4	71.39 ± 3.67	0.295 ± 0.086	-0.021 ± 0.081
J	Nieuwoudtville	14	78.65 ± 3.92	0.358 ± 0.062	-0.044 ± 0.066
K	Ceres	9	73.36 ± 3.48	0.360 ± 0.089	0.031 ± 0.088
L	Leliefontein	12	71.88 ± 3.22	0.332 ± 0.083	0.025 ± 0.081
M	Port Nolloth	4	68.58 ± 4.05	0.329 ± 0.099	0.078 ± 0.051

Legend: *n*, sample size

Table 3. Results of spatial autoregressive models examining the effect of predation pressure, predation risk and climate (i.e., PC-scores from a principal component analysis conducted on 14 bioclimatic variables) on relative osteoderm thickness in 13 populations of armadillo lizards.

Variable	Estimate	SE	*Z*-score	*P*-value	R^2
Predation risk	0.006	0.009	0.629	0.529	0.027
Predation pressure	-0.002	0.023	-0.087	0.931	0.002
PC_{ENVIR1}	0.039	0.009	4.515	<0.0001	0.573
PC_{ENVIR2}	0.007	0.013	0.566	0.571	0.011

Legend: SE, standard error; R^2, regression coefficient adjusted according to Nagelkerke (1991).

Table 4. Results of spatial autoregressive models examining the relationships between environmental/ecological variables and osteoderm thickness. Models with ΔAICc < 2 are presented and the best-fitting model is indicated in bold. The statistical significance of a likelihood ratio test comparing the model with lowest AICc value to the alternative models is also presented.

Model	N	AICc	Δ	ω	ln L
PC$_{ENVIR1}$	**1**	**-44.78**	**0**	**0.37**	**27.20**
PC$_{ENVIR1}$ + PC$_{ENVIR2}$	2	-42.93	1.85	0.15	27.61

Legend: N, number of explanatory variables; ln L, log-likelihood; Δ, difference in mean AICc value among models; and ω, Akaike weights.