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Lizard osteoderms – Morphological characterisation, biomimetic design and manufacturing based on three species

To cite this article: Ce Liang et al 2021 Bioinspir. Biomim. 16 066011

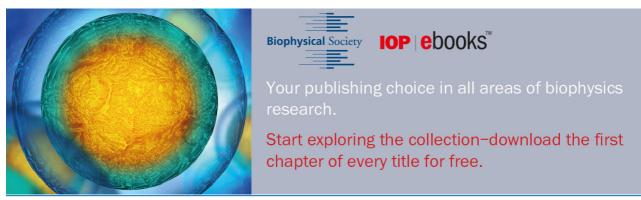
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RECEIVED 16 April 2021

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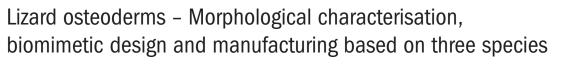
ACCEPTED FOR PUBLICATION 15 September 2021

PUBLISHED 21 October 2021

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Keywords: lizard, osteoderms, morphological, characterisations, biomimetics, design, biomechanics

Abstract

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Osteoderms (OD) are mineralised dermal structures consisting mainly of calcium phosphate and collagen. The sheer diversity of OD morphologies and their distribution within the skin of lizards makes these reptiles an ideal group in which to study ODs. Nonetheless, our understanding of the structure, development, and function of lizard ODs remains limited. The specific aims of this study were: (1) to carry out a detailed morphological characterisation of ODs in three lizard species; (2) to design and manufacture biomimetic sheets of ODs corresponding to the OD arrangement in each species; and (3) to evaluate the impact resistance of the manufactured biomimetic sheets under a drop weight test. Skin samples of the anguimorphs H. suspectum and O. ventralis, and the skink C. zebrata were obtained from frozen lab specimens. Following a series of imaging and image characterisations, 3D biomimetic models of the ODs were developed. 3D models were then printed using additive manufacturing techniques and subjected to drop weight impact tests. The results suggest that a 3D printed compound of overlapping ODs as observed in Corucia can potentially offers a higher energy absorption by comparison with the overlapping ODs of *Ophisaurus* and the non-overlapping ODs of Heloderma. Compound overlapping ODs need to be further tested and explored as a biomimetic concept to increase the shock absorption capabilities of devices and structures.

1. Introduction

Osteoderms (ODs) are mineralised structures that form directly within the skin in a wide range of tetrapods, including some frogs, many reptiles, and some mammals (Bever *et al* 2005, Mead *et al* 2012, Vickaryous *et al* 2015, Paluh *et al* 2017). These dermal elements consist mainly of calcium phosphate and collagen, but vary in their histological structure, their individual shape, and their distribution across the head and body (e.g. Moss 1969, Zylberberg and Castanet 1985, Vickaryous and Hall 2006, Laver *et al* 2020). ODs show particular diversity in lizards, where they may be completely absent; restricted to certain areas like the head; or distributed all over the body as non-overlapping clusters, as a complete covering of overlapping plates, or as spicular mineralisations that thicken with age.

Considering the structural design classification proposed to capture the diversity found in the materials of a wide variety of animal taxa (i.e. fibers, helices, gradients, layers, tubules, cellular structures, sutures,

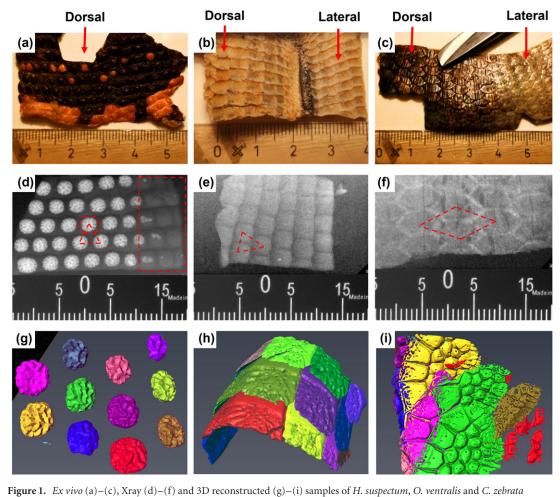


Figure 1. Ex vivo (a)–(c), Xray (d)–(f) and 3D reconstructed (g)–(i) samples of H. suspectum, O. ventralis and C. zebrata respectively.

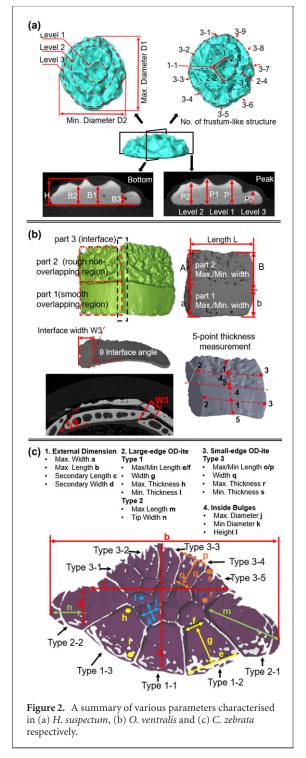
and overlapping structures), perhaps the majority of single ODs fall under the gradients and layers classification (see e.g. Naleway *et al* 2015, San Ha and Lu 2020, Ingrole *et al* 2021). However, there are ODs that display characteristics of cellular structures and some compound ODs that display elements of suture structures. Moreover, depending on the species under consideration, OD can be arranged on the body in overlapping or non-overlapping sheets.

The sheer diversity of OD morphologies and their distribution makes lizards an ideal group in which to study ODs (see e.g. Williams et al 2021). Nonetheless, our understanding of lizard ODs is still very limited. Whereas several authors have carried out detailed histological analysis of ODs in lizards to try to understand their developmental origin and biological constituents (e.g. Moss 1969, Levrat-Calviac and Zylberberg 1986, de Buffrénil et al 2010, Vickaryous et al 2015, Kirby et al 2020), only a few studies have characterised their overall morphology (e.g. Costantini et al 2010, Maisano et al 2019). Even fewer studies have characterised their mechanical properties (i.e. elastic modulus and toughness) and biomechanics (e.g. Broeckhoven et al 2015, 2017, Iacoviello et al 2020) despite a significant body of literature on such characterisation for other groups e.g. alligators, armadillos,

leatherback turtles, and a number of fishes (e.g. Chen *et al* 2011, 2015, Sun and Chen 2013, Zhu *et al* 2013). Moreover, a better understanding of these structures in lizards has the potential to lead to the development of bioinspired and biomimetic structures and devices (e.g. Yang *et al* 2013, Wen *et al* 2014, Chintapalli *et al* 2014, Chen *et al* 2018).

To the best of our knowledge there is no biomimetic/bioinspired study based on lizard ODs. However, there have been several studies on other groups (see review by Yang et al 2013). Given the common view that ODs are a natural armour protecting the internal organs, several authors have explored a range of bio-inspired and biomimetic applications based on ODs (and scales [e.g. Wen et al 2014, Chintapalli et al 2014]). Perhaps the earliest classical examples are the armour used by Scythian, Roman and Japanese warriors, where individual OD/scale like elements were sewn or laced to a backing to make a protective sheet (Yang et al 2013). See also recent reviews of various bio-inspired structures and materials for energy absorption application (e.g. San Ha and Lu 2020, Ingrole et al 2021).

The specific aims of this study were: (1) to carry out a detail morphological characterisation of ODs in



three lizard taxa in which the ODs are arranged differently; (2) to design and manufacture biomimetic sheets of ODs corresponding to each species; and (3) to evaluate the impact resistance of the manufactured biomimetic sheets under a drop weight test. This is a preliminary study considering the diversity that exists in the structure and arrangement of lizard ODs and their potential.

2. Materials and methods

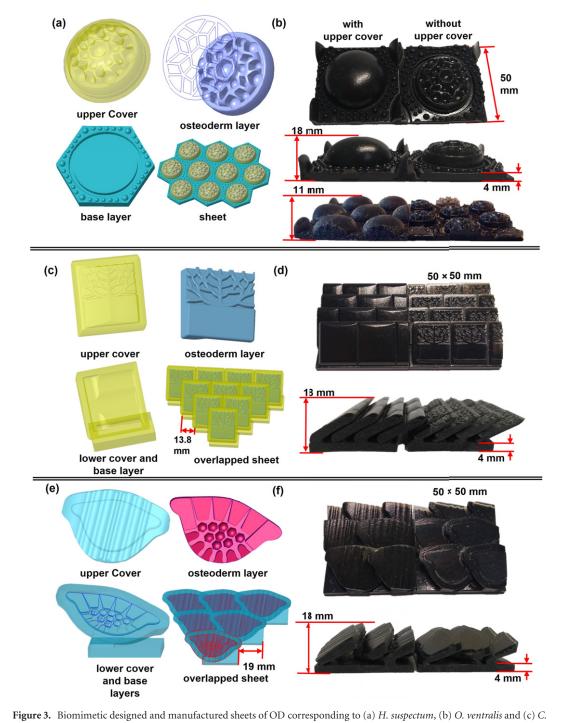
Skin samples of *H. suspectum*, *O. ventralis* and *C. zebrata* were obtained from cadaveric tissues donated by the Pathology Laboratory of London Zoo (cause of death unknown but showing no outward signs of injury or disease). Following a series of imaging and image characterisations, 3D biomimetic models of ODs were developed. 3D models were then 3D printed using additive manufacturing techniques and were then subjected to a drop weight impact test.

Imaging and characterisation. Dissected skin samples (figures 1(a)-(c)) were washed five times in distilled water, and then left to dry. An initial 2D x-ray was carried out to identify the ODs using a Nomad Pro 2 x-ray system (Kavo Kerr Group Corp., USA). The samples were then imaged using micro-computed tomography (microCT) Nikon XT H 225 scanner (Nikon Metrology Ltd., UK) at the University of Cambridge Zoology museum CT lab. Voxel resolution was 10, 47 and 11 (micron) for *Heloderma, Ophisaurus* and *Corucia* respectively.

MicroCT images were imported into an image processing software and were manually segmented (Avizo 9.2.0, Thermo Fisher Scientific Inc., USA). 3D solid models of single OD were developed for *Heloderma* (n = 8), *Ophisaurus* (n = 4) and *Corucia* (n = 8). The 3D models were exported to a computer aided design (CAD) software (Solidwork 2018, Dassault Systèmes SOLIDWORKS Corp., USA) for further measurements. Various measurements were carried out to characterise the morphology of the ODs. See figure 2 for details of the measured parameters.

Biomimetic development and manufacturing. Biomimetic 3D CAD models of ODs were developed based on the results of structural analysis mimicking the main morphological features of the OD sheets. For manufacturing accuracy, the complex morphology of the ODs was simplified while maintaining the overall features and patterns. Two sheets of ODs were designed per species i.e. with or without a protective upper cover. The protecting cover mimicked the stratum corneum, the keratinized outer layer of the lizard skin. Aside from the absence or presence of the protecting cover, each sheet consisted of a hard component mimicking the mineralized osteoderm, and a soft base mimicking the stratum compactum of the skin in which the OD is embedded.

3D models of the OD sheets were manufactured using a multi-material printer Objet Connex500 (Stratasys, MN, USA). The plane resolution (XY) was 600×600 DPI with minimum printable thickness of 0.5 mm. The superficial and deep covering layers

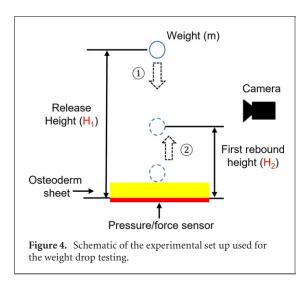


zebrata respectively.

of OD sheets, mimicking the soft tissues, were manufactured using a 'rubber-like' material (FLX9060_DM, E = 0.78 MPa) while the ODs were manufactured using a 'hard' material (RGD8715_DM, E = 2.3 GPa). All sheets were printed in 50 mm × 50 mm × 18 mm (length × width × max. height) and had the same base thickness of 4 mm. Considering the relationships that exist between the various morphological features of the three chosen species, two alternative sheets of ODs were developed for *Heloderma*. In one of these, a

single OD was developed matching the overall height (18 mm) of the OD sheet for other species. The second *Heloderma* sheet had multiple ODs but the overall height of the sheet was less than that of the other two species (see figure 3). Note the sheets were manufactured in their entirety in one step and it is possible that support materials are trapped between the layers. However, we consider this should be minimal. In addition, the lacunae of the *Ophisaurus* ODs were not included in the biomimetic designed and manufactured sheets of this species.





Testing. A drop weight impact test was carried out on the manufactured sheets, see schematic representations in figure 4. In brief, the manufactured sheets were placed on a hard surface. Weights of 50 g, 100 g and 200 g were then dropped onto the sheets from an initial height of H1; the weights collided with the sheets and bounced back. The bounce back height was recorded using a high-speed camera (Phantom Miro M320S). Total energy absorbed during the impact test was calculated and divided by the initial potential energy of the weights to obtain the total energy absorption ratio using the following equation

Energy Absorption Ratio (EAR)

$$=\frac{mgH_1-mgH_2}{mgH_1}=\frac{H_1-H_2}{H_1}.$$

The transmitted impact load that the OD sheets and underlying hard surface were subject to during the drop weight test was measured for the weights. This was carried out using a thin film force sensor RP-S40-ST with 40×40 active area (Shenzhen Film Sensor Technology Co., Ltd., China) connected with an ELEGOO UNO R3 Board (ELEGOO Inc., China) and placed under the 3D printed samples and over a hard surface during the drop weight test. Detailed functions for data acquisition were programmed and verified in Arduino IDE (Arduino Software Inc., Italy).

3. Results

Morphological characterisation. Tables 1-3 summarises the morphological measurements made for the ODs considered in this study.

The ODs of *H. suspectum* had a 'tile'-like arrangement without any overlapping. Each OD had a nearcircular base with outer diameters of ca 2.4-2.7 mm depending on the exact measurement points (see D1 and D2 in table 1 and figure 2(a)). Three radiating levels were identified. The center of the OD had the highest point with a single frustrum-like morphology. A similar morphology was repeated in a circular pattern at two lower levels of slightly lower thickness. The OD heights measured at levels 1-3 (see P1–P3 in figure 2(a)) were 0.79 ± 0.06 mm, 0.69 ± 0.07 mm and 0.41 ± 0.02 mm respectively.

O. ventralis ODs had an overlapping arrangement. Each single OD had a 'smooth'gliding surface (where it is overlapped by an adjacent OD) and a sculptured free (overlapping) surface (see figure 2(b)). Both the smooth and sculptured parts of the OD were comparable in length, width and thickness. For example, the average width of the smooth and sculptured parts was 2.09 \pm 0.12 mm and 2.45 \pm 0.16 mm respectively (table 2). The overall length of each OD (i.e. the combined length of the smooth and sculptured parts) was ca 8 mm. Their thickness varied and was dependent on the point of measurement e.g. varying in the range of 0.28 \pm 0.03 mm (at measurement point 5 in the smooth part) to 0.73 ± 0.07 mm (at measurement point 2 in the smooth part). ODs overlapped at $44.45^{\circ} \pm 2.16^{\circ}$ (see figure 2(b)).

C. zebrata ODs are compound, in that they are composed of several elements (osteodermites) forming a distinctive morphology. These 'fan' shaped ODs consisted of several 'rectangular' peripheral osteodermites (here categorised into three types see figure 2(c) tightly connected to one another and surrounding a varied number of more 'rounded' central osteodermites. The maximum diameter of the rounded osteodermites was 0.9 ± 0.08 mm with the overall maximum length and width of the compound OD ((a) and (b) as outlined in figure 2(c)) in the range of 8.77–9.82 mm and 4.74–5.07 mm (table 3). The thickness of each OD was highest at its center (i.e. where the rounded osteodermites were located) in the range of 0.19-0.24 mm (table 3). The thickness gradually decreased toward the edges of the compound OD (i.e. the outer edges of the 'rectangular osteodermites) in the range of 0.09 ± 0.01 (see e.g. measurement point S in figure 2(c)).

Manufacturing and testing. Designed and manufactured individual ODs and sheets of ODs based on *H. suspectum, O. ventralis* and *C. zebrata* are shown in figure 3. Testing these sheets with a soft cover demonstrated that their energy absorption can be load dependent (figure 5(a)). Moreover, the sheets mimicking *H. suspectum* showed a reduction in the energy absorption, regardless of the number of ODs, when increasing the drop weight load from 50 to 200 g. The opposite result was obtained for the sheets mimicking *O. ventralis* and *C. zebrata* ODs (for the loads considered in this study) with the sheets mimicking the *Corucia* ODs showing the highest energy absorption ratio at 200 g (figure 5(a)).

When considering the impact of the soft cover placed over the OD sheets, all manufactured sheets showed a reduction in their energy absorption ratio without this cover (figure 5(b)). The differently

Table 1.	A summar	y of morphologica	l characterisation	of the H. sus	pectum, OD ^{a,b} .
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External parameter (Unit: mm)	Average max. diameter (D1)	Average min. diameter (D2)	Average max. peak height (H)
Value	2.74 ± 0.09	2.41 ± 0.13	0.84 ± 0.07
Internal parameter (Unit: mm)	Level 1	Level 2	Level 3
Average peak height (P)	0.79 ± 0.06	0.69 ± 0.07	0.41 ± 0.02
Average bottom thickness (B)	0.55 ± 0.04	0.52 ± 0.05	0.34 ± 0.03
Ratio of peak heights of three levels (P1:P2:P3)		1:0.88:0.53	
Ratio of bottom thicknesses of three levels (B1:B2:B3)		1:0.95:0.61	
Average max. Area (L*W) of frustum-like structure (FLS) (mm ²)	0.77 ± 0.04	0.4 ± 0.03	0.18 ± 0.03
Average number of frustum-like structure	1.00	6 ± 0.82	10.00

^aThe average values are based on the eight three-level *Heloderma* OD samples.

^bThe average area of FLS is defined as the max. length * max. width where the geometry of FLS is roughly regarded as parallelogram or rectangle.

Table 2.	A summary of morphol	ological characterisation of the	О.
ventralis	OD ^a .		

Unit: mm	Parameter	Range or Value
	Max. Width (a)	2.09-2.36
	Min. Width (b)	1.71-2.12
	Average width (W1)	2.09 ± 0.12
	Average length (L)	3.99 ± 0.24
Smooth part 1	Width/length ratio (R1)	0.52
	Average thickness 1	0.51 ± 0.03
	Average thickness 2	0.73 ± 0.07
	Average thickness 3	0.29 ± 0.03
	Average thickness 4	0.63 ± 0.06
	Average thickness 5	0.28 ± 0.03
	Max. Width (A)	2.17-2.94
	Min. Width (B)	2.01-2.69
	Average width (W2)	2.45 ± 0.16
	Average length (L)	3.99 ± 0.24
Rough part 2	Width/Length ratio (R2)	0.61
	Average thickness 1	0.52 ± 0.05
	Average thickness 2	0.63 ± 0.03
	Average thickness 3	0.30 ± 0.02
	Average thickness 4	0.61 ± 0.09
	Average thickness 5	0.29 ± 0.02
Interface part 3	Average width (W3)	0.47 ± 0.03
	Average degree (θ)	44.45 ± 2.16

^aThe average values are based on the eight *Ophisaurus* OD samples.

designed sheets showed varying levels of reduction in their energy absorption ratio, with the multiple OD *H. suspectum* sheet showing the highest drop in its energy absorption ratio when the soft cover was absent (ca 7.5% see figure 5(b)).

The forces recorded under each of the sheets during the weight drop test showed an increase with each increase in the weight dropped (figure 5(c)). For example, the forces recorded under the single *H. suspectum* sheet ranged from 1.3N at 50 g to 13.3 at 200 g. At the 200 g weight drop, the forces measured under the single *H. suspectum* sheet were highest, followed by similar forces under the *O. ventralis* and *C. zebrata* sheets, with the lowest recorded forces being for the multiple OD *H. suspectum* sheet (figure 5(c)).

4. Discussion

This preliminary investigation highlights the morphological variation that exists between the ODs of three lizard species. Biomimetic ODs were designed and manufactured as 50*50 mm sheets of ODs using additive manufacturing technique. These were then subjected to a simple weight drop test. Although the findings are preliminary, the study as a whole demonstrates how natural and physical sciences can come together to design and develop new biomimetic sheets, in this case based on ODs in lizards.

Morphology. There is a remarkable diversity in the morphology and arrangement of ODs in lizards (Vickaryous and Sire 2009, Williams et al 2021). What underlies this variation is largely unknown, but is likely the result of a combination of natural selection and developmental constraints. The three species investigated in this study have very different lifestyles: H. suspectum is a large, slow moving, ground-living lizard; O. ventralis is a limbless lizard that moves both above and below ground; and C. zebrata is a large, slow-moving herbivorous lizard that lives in the tree canopy and rarely comes to the ground. If OD are largely protective, the slow-moving, ground living Heloderma might be predicted to have the most extensive armour of the three, rather than the reverse. However, Heloderma spends up to 90% of the day under cover and generally avoids open areas. Moreover, it has a venomous bite, a feature it advertises to potential predators (e.g. coyotes, badgers, raptorial birds) through its black and pink/red warning coloration (e.g. Beck 2005). Predation pressure is therefore likely not high. However, Heloderma does engage in aggressive male-male combat, involving a mixture of biting, clawing, and wrestling (Beck and Ramirez-Bautista 1991), and this may help to explain its more flexible osteodermal covering. Ophisaurus and Corucia on the other hand both have a dense covering of overlapping ODs, but the morphological difference

Unit: mm	Parameter	Range or Value
	Length (<i>b</i>)	8.77-9.82
	Width (a)	4.74-5.07
External dimension	Average length/width ratio (<i>R</i> 1)	1.89 ± 0.04
	Secondary length (<i>c</i>)	5.37-7.26
	Secondary width (<i>d</i>)	1.66-1.67
	Type 1	
	Max. length (e)	1.45-2.00
	Min. length (f)	0.64-1.10
Large-edge osteodermite	Average width (<i>g</i>)	1.8 ± 0.05
	Average area $(A1)$ (mm ²)	2.33 ± 0.02
	Average max. thickness (h)	0.19 ± 0.01
	Average min. thickness (i)	0.08 ± 0.01
	Type 2	
	Max. length (m)	2.56 - 2.90
	Tip width (<i>n</i>)	1.19-1.48
	Туре 3	
	Max. Length (<i>o</i>)	0.98
	Min. Length (<i>p</i>)	0.69
	Average width (q)	1.14 ± 0.07
Small-edge osteodermite	Average area (A2) (mm ²)	0.95 ± 0.04
	Average max. thickness (r)	0.2 ± 0.00
	Average min. thickness (s)	0.09 ± 0.01
	Average max. diameter (j)	0.9 ± 0.08
	Average min. diameter (k)	0.74 ± 0.11
Inside bulges	Average area (A3) (mm ²)	0.53 ± 0.02
	Height (<i>l</i>)	0.19-0.24

Table 3. A summary of morphological characterisation of the *C. zebrata* OD^{a,b}.

^aThe average values are based on the four size 1 Corucia OD samples.

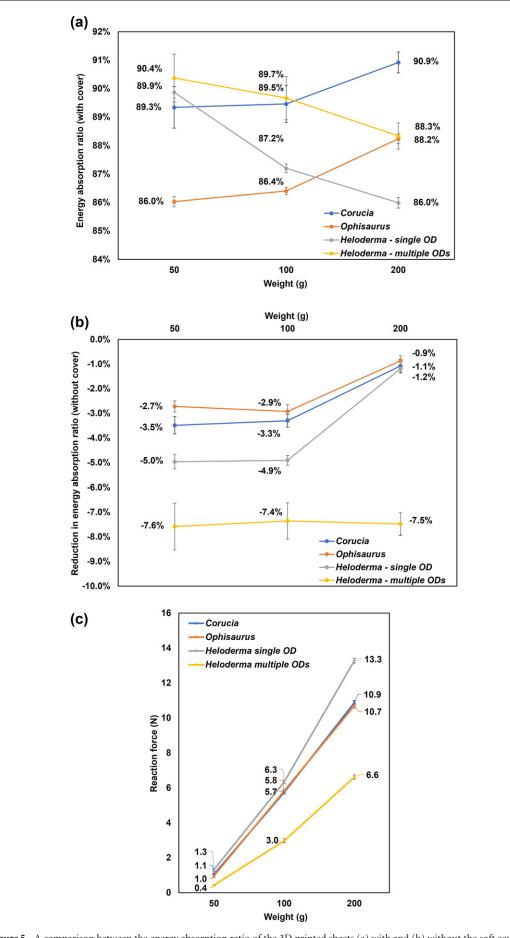
^bThe calculation method for the area of edge OD-ites (*A*1 and *A*2) are same with that for trapezoid, e.g. large-edge OD-ite, the $A1 = (e + f)^*g/2$; the *A*3 is the product of max. diameter (*j*) and min. diameter (*k*).

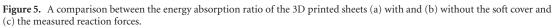
between these ODs of Ophisaurus and Corucia is striking, those of Corucia being compound with potentially higher energy absorption. O. ventralis has a long, slender flexible body and can move much faster than Corucia (and Heloderma). Nonetheless, it has a wide range of mammalian (e.g. pig, bobcat, racoon), avian (e.g. hawks, wading birds) and reptilian (e.g. elapid and viperid snakes) predators (e.g. Durso and Middleton 2019). This raises questions as to the function of its osteodermal cover that can probably only be answered by field observations of predation events. The same applies to Corucia. There is unfortunately relatively little information on Corucia in the wild (e.g. Hagen 2011). This arboreal lizard rarely comes to the ground and is well camouflaged among the foliage of the canopy. Moreover, it is mainly active at night and remains in refugia within the trees by day, an activity pattern that would give it some protection against the raptorial birds that are recorded as being among its major predators (along with snakes and introduced arboreal rats). How well the ODs might protect Corucia from raptor talons or a bite from a large rat is unclear, and nothing has been written about agonistic encounters with conspecifics. In the present study we did not investigate the potential variability of the aforementioned ODs at the nano-scale i.e. in terms of their biochemical composition that could have been adapted in response to the external loading environment imposed on these species.

Manufacturing and testing. A unique contribution of this study was the biomimetic design and development of 3D printed sheets of ODs informed by the morphological characterisation of ODs in the three species considered. Although this was a preliminary study, there are two points that are worth highlighting.

First, two sheets of ODs were developed based on the structure and arrangement of Heloderma ODs. Although the multiple OD sheet was naturally more comparable with the sheets for the other two lizards, keeping the original morphology of Heloderma skin meant that this sheet was thinner overall than the other two sheets. This led to a greater flexibility of the sheet compared to the other two thicker sheets. On the other hand, the overlapping nature of the OD components within the Ophisaurus and Corucia sheets meant that during the impact testing, the gaps between overlapping ODs gave these sheets their flexibility. It is also possible that the compound ODs of Corucia can indeed provide greater flexibility than those of Ophisaurus. However, despite the results (figure 5(a)) showing a higher energy absorption for Corucia over Ophisaurus, this is provisional, given that the printed sheet did not model the collagen fibers that connect the osteodermites within these compound ODs in life.

Secondly, we manufactured the ODs out of a rather 'hard' material with a uniform elastic modulus higher than that of their superficial and deep covers





(see figure 3). Our ongoing histological investigations show that single ODs are a composite structure with non-homogeneous material properties (e.g. Moss 1969, Zylberberg and Castanet 1985 Vickaryous and Hall 2006, Iacoviello *et al* 2020). Manufacturing such functionally graded structures is possible with advanced additive manufacturing techniques, and would be a future step in investigating the contribution of different tissues to impact resistance in sheets of ODs.

Limitations. There were several limitations within this study, notably: (1) the morphological characterization was focused on ODs from a specific region of the body (dorsal neck). There is some variability in the morphology of ODs in different regions of the body. Nonetheless, as far as possible we characterized the OD from comparable body regions in the three species studied. (2) Only one sheet of ODs was manufactured and only one experiment (weight drop) was carried out. A key follow-up experiment would include additional sheets to characterise any variability between printed sheets. (3) Other experiments such as puncture testing should be run. (4) The lacunae of the Ophisaurus OD, as well as the joints between osteodermites in Corucia, and other internal structures that might be present within the single ODs (see e.g. Iacoviello et al 2020) were not included in the biomimetic designs and manufactured sheets considered in this study. These might impact the energy absorptions of individual ODs and can be further investigated in future studies.

In summary this study presented a macroscopic morphological characterisation of ODs in three lizard species and focused on the design and testing of three biomimetic OD sheets. Given the preliminary nature of the testing carried out, caution is needed when interpreting the findings of this study. Nonetheless it suggests that compound overlapping ODs as found in *Corucia* permit a higher energy absorption by comparison with overlapping simple ODs as observed in

Ophisaurus. This concept can be further tested and explored where shock absorption is required.

Acknowledgments

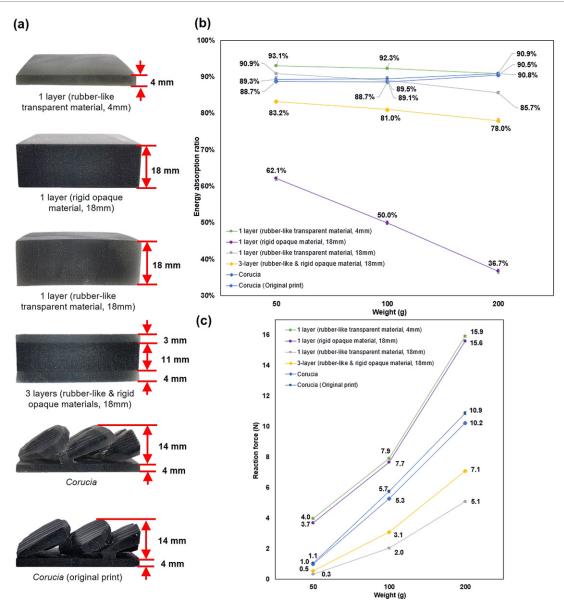
This work was supported by Human Frontier Science Program (RGP0039/2019). We thank the Pathology Department (Zoological Society of London) for cadaveric material of the lizards used in this study.

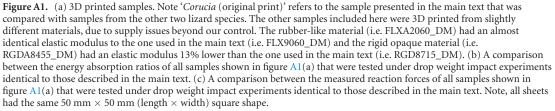
Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Appendix

A series of experiments identical to the drop weight experiment described in the main text was carried out on additional baseline manufactured sheets as shown in figure A1(a). Due to supply issues beyond our control we had to manufacture the additional sheets from slightly different materials. For comparison, we manufactured a Corucia sheet as described in the main text from the new materials, and compared the results with those from the original print, here referred to as 'Corucia (original print)'. The measurements obtained from the 'Corucia (original sheet)' and the re-printed 'Corucia' sheet were very similar (compare the blue lines in figures A1(b) and (c)). The results of these additional tests confirmed that the biomimetic designed Corucia sheets had higher energy absorption characteristics by comparison with a three-layered sheet, a one layer rigid sheet (18 mm) and one layer rubber-like (18 mm) sheet (see figures A1(a)-(c)).





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