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# Comparative analysis of osteoderms across the lizard body

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## Abstract

Osteoderms (ODs) are mineralized tissue embedded within the skin and are particularly common in reptiles. They are generally thought to form a protective layer between the soft tissues of the animal and potential external threats, although other functions have been proposed. The aim of this study was to characterize OD variation across the lizard body. Adults of three lizard species were chosen for this study. After whole body CT scanning of each lizard, single ODs were extracted from 10 different anatomical regions, CT scanned, and characterized using sectioning and nanoindentation. Morphological analysis and material characterization revealed considerable diversity in OD structure across the species investigated. The scincid *Tiliqua gigas* was the only studied species in which ODs had a similar external morphology across the head and body. Greater osteoderm diversity was found in the gerrhosaurid *Broadleysaurus major* and the scincid *Tribolonotus novaeguineae*. Dense capping tissue, like that reported for *Heloderma*, was found in only one of the three species examined, *B. major*. Osteoderm structure can be surprisingly complex and variable, both among related taxa, and across the body of individual animals. This raises many questions about OD function but also about the genetic and developmental factors controlling OD shape.

# 1 | INTRODUCTION

Osteoderms (ODs) are mineralized tissue structures formed 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; Moss, 1969, 1972; Paluh et al., 2017; Vickaryous et al., 2015; Williams et al., 2022). The exact roles of osteoderms are, however, still the subject of scientific debate. Although their main function has been proposed to be for protection from potential threats, such as predation, intraspecific aggression, or environmental hazards (Broeckhoven, 2022; Chen et al., 2015; Liang et al., 2021; Schuett et al., 2023; Yang et al., 2013), they may also contribute to thermoregulation (Farlow et al., 2010; Seidel, 1979), calcium storage (Broeckhoven & du Plessis, 2022), the mechanics of the craniofacial system (Kéver et al., 2022; Marghoub et al., 2022, 2023), and/or courtship displays or other forms of social behavior (Broeckhoven, De Kock, et al., 2017; Broeckhoven, du Plessis, et al., 2017; Lissethe et al., 2022; Losos et al., 2002; Seidel, 1979).

Lizards, with over 7000 living species, show the greatest diversity in OD morphology and distribution. Previous authors have characterized the morphological variation of the ODs across several lizard species (Kirby et al., 2020; Levrat-Calviac & Zylberberg, 1986; Marghoub et al., 2022; Vickaryous et al., 2015), demonstrating that the nano, micro, and macro structure of ODs varies considerably. Lizard ODs can be simple (having a single ossification center and forming a continuous structure) or compound (comprising several independently ossifying plates, or osteodermites, that fuse to form a mosaic structure). Marghoub et al. (2022) recently showed that the ODs from the anguimorphs *Heloderma suspectum* and *Pseudopus apodus* developed a mineral-dense, relatively cell poor capping tissue, whereas many other species lack this specialized layer. Nonetheless, to the best of our knowledge, very few studies have investigated OD variation across the body of a single species (exceptions include Bhullar & Bell, 2008; Levrat-Calviac & Zylberberg, 1986; Marghoub et al., 2022; Vickaryous et al., 2015) and none have carried out a detailed characterization of this variation.

The underlying hypotheses of this study were that across the body of a single lizard species, ODs would have (1) the same basic micro- and macrostructure and (2) that their component tissues would have the same mechanical properties. Therefore, the specific aims of this study were to carry out a detailed morphological and material characterization (using nano-indentation) of the ODs across the body of three species of the lizard clade Scincoidea (Kumar et al., 2022) (Figure 1). Scincoidea is one of the largest and most widely distributed lizard groups, with a diversity of body forms and lifestyles. The majority of its members, other than xantusiids, possess osteoderms on all or part of their bodies. Hence it is an ideal group to further investigate OD variation across the body of lizards and provide a preliminary test of the underlying hypotheses of this study.

# 2 | MATERIALS AND METHODS

## 2.1 | Study species

The scincoid study species included two scincids and one gerrhosaurid, varying in size and ecology. Note that a comprehensive survey of other OD bearing members of Scincoidea (i.e., Cordylidae and a greater range of Scincidae) was beyond the scope of this preliminary study. The White-eyed crocodile skink (*Tribolonotus novaeguineae*, snout vent length [SVL] = 85 mm, head length = 24 mm, female) is an insectivorous lizard found in the island of New Guinea (Papua and Papua New Guinea) (Austin et al., 2010). It is secretive and semi-fossorial and is typically found in/under vegetation close to water. The Indonesian blue-tongued skink (*Tiliqua gigas*, SVL = 260 mm, head length = 58 mm, male) is another scincid lizard from New Guinea and neighboring islands. It can occur in a variety of habitats from forest floor to semi-desert, but it is more typically found in the rainforest (Koppetsch et al., 2021; Shea, 2004). By contrast, the Sudan plated lizard, also known as the Eastern Plated Lizard (*Broadleysaurus major*, SVL = 209 mm, head length = 52 mm, male), belongs in the family Gerrhosauridae, and is native from Ethiopia and the Republic of South Sudan, to South Africa (Bates et al., 2013). It mostly lives around rock piles and crevices, in semi-open areas of woodland or savannah (Schmidt, 1919). Fresh frozen cadavers of adult *T. novaeguineae*, *T. gigas*, and *B. major* (N = 1) were obtained from commercial dealers.

## 2.2 | Morphological analysis

The body of each lizard was scanned using microcomputed tomography (microCT) using a Nikon XT H 225 ST scanner (Nikon Metrology Ltd., UK).
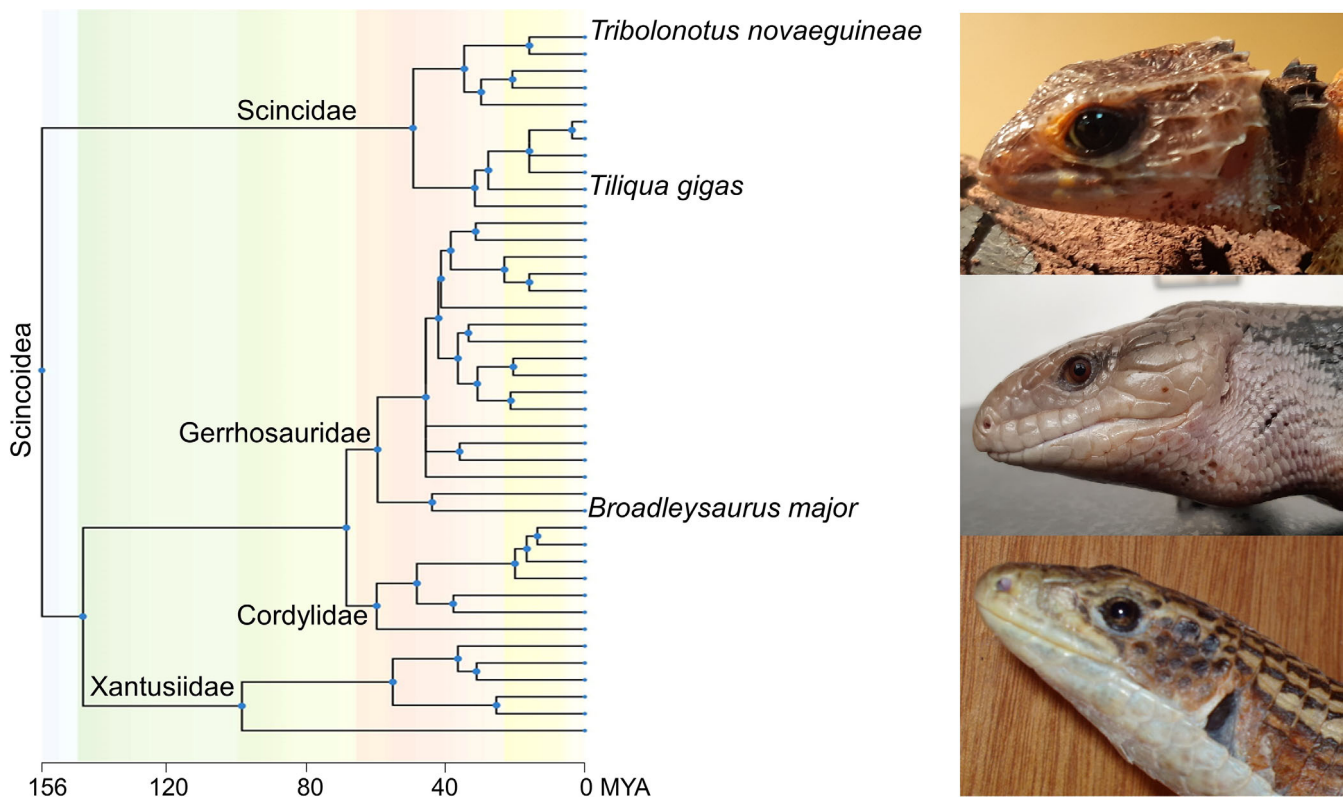
**FIGURE 1** A time-calibrated phylogeny of the species used in this study (based on TimeTree:: The Timescale of Life, Kumar et al., 2022). Photographs by one of the authors (A. H.).

After whole body scanning, single ODs were extracted from the temporal and ventral head zones, the dorsal and ventral sides of the cervical region, central body, tail, and hind limb for all investigated species and lateral side of central body for *B. major* (Figure 1). These 10 (or 11 for *B. major*) ODs were rescanned individually for each species. Image stacks from the CT scans were imported and ODs reconstructed in the Avizo image processing software (Thermo Fisher Scientific, MA, USA). The 3D reconstructions of the ODs were used for basic morphological measurements (i.e., length, width, and height) as summarized in Table 1.

## 2.3 | Internal morphology

Dissected ODs (10 per *T. novaeguineae* and *T. gigas* and 11 per *B. major*, previously scanned) were embedded under vacuum in cold cure epoxy resin (Buehler, Germany). After 24 h, the samples were cut and polished in the longitudinal plane, with a sample also cut transversely. Polishing was performed using silicon carbide paper (320, 600, 1200, and 2500 grit), and aluminum oxide slurries (0.3 and 0.05 µm particle size) on neoprene cloth (Buehler, Germany). The polishing protocol led to a final surface roughness of ca. 0.2 µm and was similar to the previous studies of

Marghoub et al. (2022) and Moazen et al. (2015). All samples were then visualized under a microscope (KEYENCE VHX-7000) prior to nanoindentation.

## 2.4 | Material characterization

Nanoindentation was performed at room temperature using an Anton Paar system (UNHT3, Anton Paar GmbH, Switzerland). A Berkovich diamond tip was employed because it has been widely used for indentation of hard calcified tissues (Ebenstein & Pruitt, 2006; Zysset et al., 1999). Selected regions were indented under a load-control protocol at 100 mN/min with 50 mN, followed by 10 s hold. A minimum spacing of 30 µm between the indents was ensured. The elastic modulus was calculated using the standard Oliver-Pharr method (Marghoub et al., 2022).

## 3 | RESULTS

## 3.1 | External morphology

The external morphology of ODs can vary considerably in shape and size both within individuals and between study species (Figure 2). Each OD was covered by a single scale.

**TABLE 1**    Summary of the quantified parameters of a single OD representative of the study species.

| ODs | Length (mm) | | | Width (mm) | | | Height (thickness) (mm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Tribolonotus novaeguineae* | *Tiliqua gigas* | *Broadleysaurus major* | *Tribolonotus novaeguineae* | *Tiliqua gigas* | *Broadleysaurus major* | *Tribolonotus novaeguineae* | *Tiliqua gigas* | *Broadleysaurus major* |
| **Dorsal** | | | | | | | | | |
| Central body | 7.4 | 7.1 | 8.6 | 4.4 | 9.6 | 4.3 | 1.6 | 0.8 | 0.8 |
| Cervical | 3.2 | 5.8 | 7.3 | 2.6 | 8.2 | 4.2 | 1.7 | 0.7 | 1.0 |
| Tail | 4.2 | 6.2 | 10.0 | 1.7 | 11.6 | 4.0 | 1.8 | 0.7 | 0.8 |
| Limb | 2.3 | 3.1 | 4.8 | 1.7 | 4.2 | 3.7 | 0.5 | 0.3 | 0.7 |
| **Ventral** | | | | | | | | | |
| Central body | 2.3 | 6.2 | 7.1 | 4.0 | 8.1 | 2.9 | 0.1 | 0.4 | 0.4 |
| Cervical | 1.7 | 6.5 | 7.2 | 2.6 | 8.3 | 11.8 | 0.2 | 0.3 | 0.8 |
| Head | 1.8 | 5.5 | 8.3 | 2.1 | 7.1 | 12.9 | 0.2 | 0.3 | 0.6 |
| Tail | 2.7 | 5.6 | 7.2 | 1.6 | 10.0 | 3.5 | 0.2 | 0.3 | 0.8 |
| Limb | 1.8 | 3.0 | 6.3 | 1.7 | 4.3 | 9.8 | 0.1 | 0.2 | 0.5 |
| **Temporal** | | | | | | | | | |
| Head | 3.8 | 4.7 | 3.6 | 4.3 | 8.2 | 2.8 | 0.7 | 0.9 | 0.7 |
| **Lateral** | | | | | | | | | |
| Central body | Not found | Not examined | 9.1 | Not found | Not examined | 4.7 | Not found | Not examined | 0.7 |
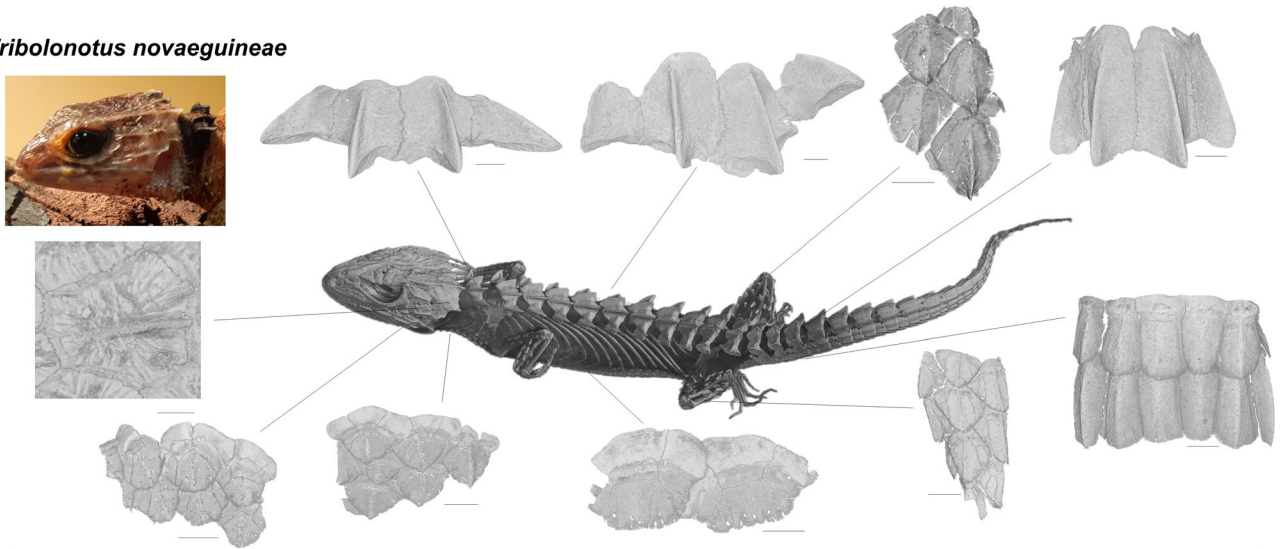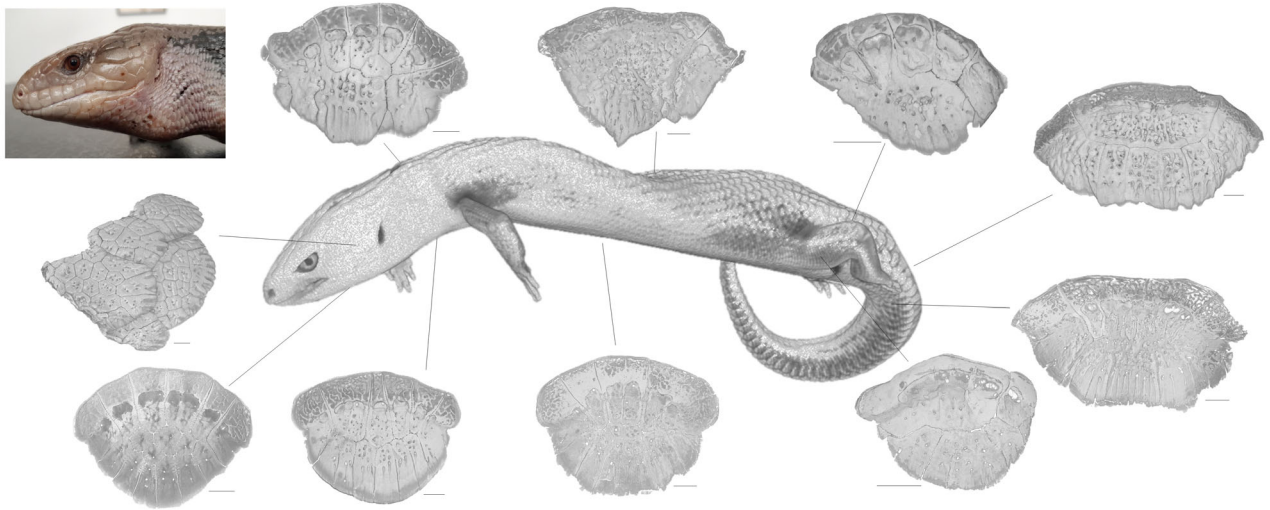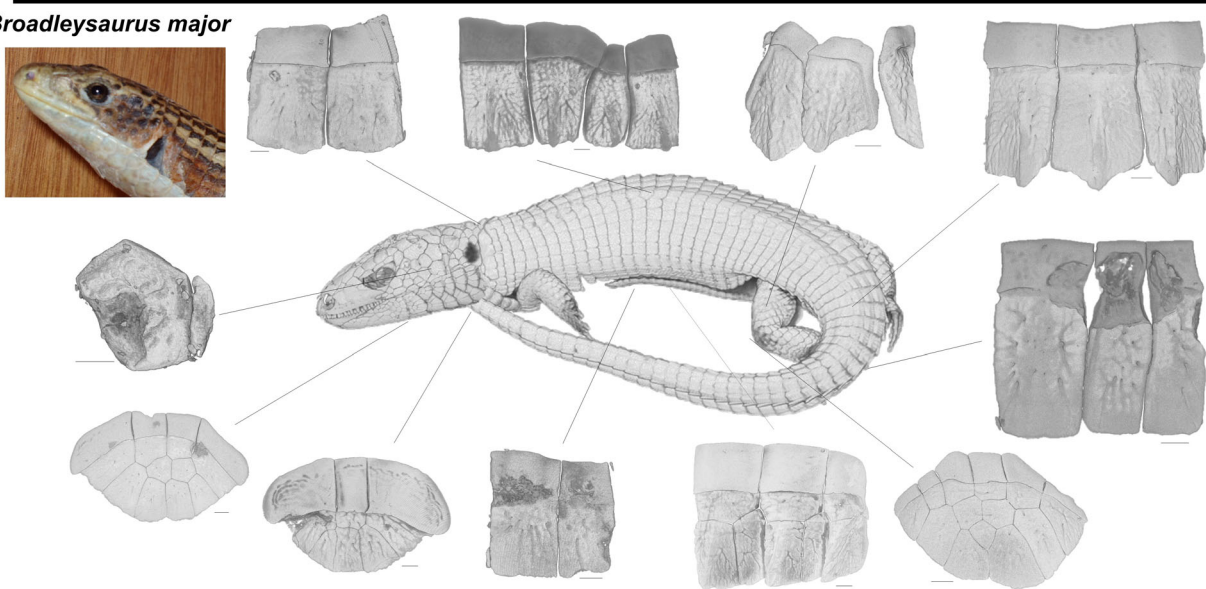
**FIGURE 2** External morphological variation in the ODs of three lizards across the body of *Tribolonotus novaeguineae* (a), *Tiliqua gigas* (b), and (c) *Broadleysaurus major*. Different numbers of ODs figured for each species reflect the degree of variation for each taxon.

*T. novaeguineae* bears keeled ODs across the body with varying keel heights. The most pronounced ridges were observed across the dorsal part of the tail (ca. 1.8 mm) while the lowest ridges were present across the ventral part of the mid-body (ca. 0.1 mm—see Table 1). ODs across the dorsal cervical and cranial portion of the body are sutured together in transverse arrangements of four elements, a median pair of sharply ridged elements flanked by wing-like lateral elements. Together these four ODs form a transverse bar that is repeated along the vertebral column from neck to tail tip. Cranial ODs consist of single hexagonal components that are tightly connected by sutures in a mosaic-like arrangement (Figure 2a). Ventral head, cervical, body, and hind limb osteoderms are flattened overlapping structures of an irregular shape. Each OD is compound and composed of several smaller elements (osteodermites) that are linked via fibrous joints. Ventral caudal ODs overlap slightly but are not compound (Figure 2a).

*T. gigas* differs from *Tribolonotus* in that its ODs are of similar external morphology across the dorsal and ventral parts of the body (Figure 2b). All ODs are compound, plate-like, and overlapping. *T. gigas* was the only species in this study in which the temporal ODs of the head were observed to be compound and slightly overlapping. Dorsal ODs bear an external vermiculate ornamentation of pits and grooves (Figure 2b). Ventral ODs are thinner, smooth, and contain many internal cavities. They are also smaller than dorsal ODs (see Table 1). The largest ODs were found on the dorsal part of the tail (ca. 11.6 mm in width). Temporal ODs were thicker than those in other body regions and also thicker than those in the same region of other study species (ca. 0.9 mm) (Table 1).

*B. major* showed a remarkable diversity of OD morphology across its body (Figure 2c). Temporal ODs were non-compound, with juxtaposed polygonal ODs creating a mosaic-like arrangement. Like the temporal ODs, all dorsal ODs were simple, but were imbricate and slightly cambered. The largest dorsal ODs were those on the tail (length: 10.0 mm, width: 11.6 mm) whereas the thickest were on the neck (depth: 1.0 mm, see Table 1). In contrast, ventral ODs on the head, neck, and hind limb were plate-like, compound, and overlapping, whereas the ventral osteoderms of the mid-body and tail appeared simple, rectangular, and flat. Lateral body ODs, examined only in this species, combine features of dorsal and ventral ODs (Figure 2c).

## 3.2 | Internal morphology

Previous work on squamate osteoderm microstructure (e.g., Kirby et al., 2020; Marghoub et al., 2022; Moss, 1969; Vickaryous & Sire, 2009; Williams et al., 2022) has highlighted the heterogeneity across ontogeny and phylogeny of ODs which can include soft tissue elements like neurovascular structures and marrow, as well as mineralized and unmineralized collagen. The most commonly distributed mineralized components are: woven-fibered bone; Sharpey-fibered bone (i.e., bone rich in Sharpey's fibers, sometimes referred to as structural fibers sensu Scheyer & Sander, 2004); organized bone and capping tissue. Organized bone includes both lamellar bone and parallel-fibered bone (Davesne et al., 2020; Stein & Prondvai, 2014). Both these fibrillar matrices are composed of closely packed parallel collagen fibers and embedded osteocytes/lacunae that are generally compressed or flattened (Francillon-Vieillot et al., 1990). Hence, they are often challenging to discretely identify using light microscopy (Davesne et al., 2020; Francillon-Vieillot et al., 1990).

In addition to bone, ODs of some lizard species also develop a second type of mineralized tissue type (e.g., de Buffrénil et al., 2011; Kéver et al., 2022; Kirby et al., 2020; Marghoub et al., 2022; Vickaryous et al., 2015). This capping tissue (referred to in some species as osteodermine; de Buffrénil et al., 2011), is described as lacking intrinsic collagen, having relatively few cells, and an elastic modulus similar to enamel (de Buffrénil et al., 2010, 2011; Marghoub et al., 2022; Vickaryous et al., 2015; Williams et al., 2022). Further, capping tissue is only formed across the superficial surface of the OD.

In Figure 3 all ventral ODs are shown inverted (external side uppermost) to facilitate the comparison with the dorsal ODs. Dorsal ODs of *T. novaeguineae* are triangular in parasagittal section, corresponding to the keeled pattern observed at macro scale. Each OD is primarily composed of woven-fibered bone around one or more marrow cavities, with organized bone forming a thin layer on the superficial and deep (ventral) margins of the ODs (Figure 3a). In some ODs, the deep layer of bone was separated from the central woven-fibered core by a series of rounded hollows, likely corresponding to marrow cavities. Large Sharpey-fibers are invested within the bone of the deep surface, anchoring each OD to the dermis. The histological structure of the temporal (cranial) ODs is similar to those from the dorsal body, with woven-fibered bone centrally, surrounded by organized bone (Figure 3a). Temporal ODs have fewer, smaller, marrow cavities compared to those across the dorsal surface of the body. Capping tissue was absent from all *T. novaeguineae* ODs.

In section, the dorsal ODs of *T. gigas* are mainly composed of woven-fibered and Sharpey-fibered bone, with many large irregularly-shaped marrow cavities. Organized bone is present across the superficial OD surface, penetrated in places by Sharpey's fibers (Figure 3b). Sharpey-fibered bone is present along the deep OD surface, and between adjacent osteodermites. Ventral body ODs have fewer marrow cavities compared to the dorsal
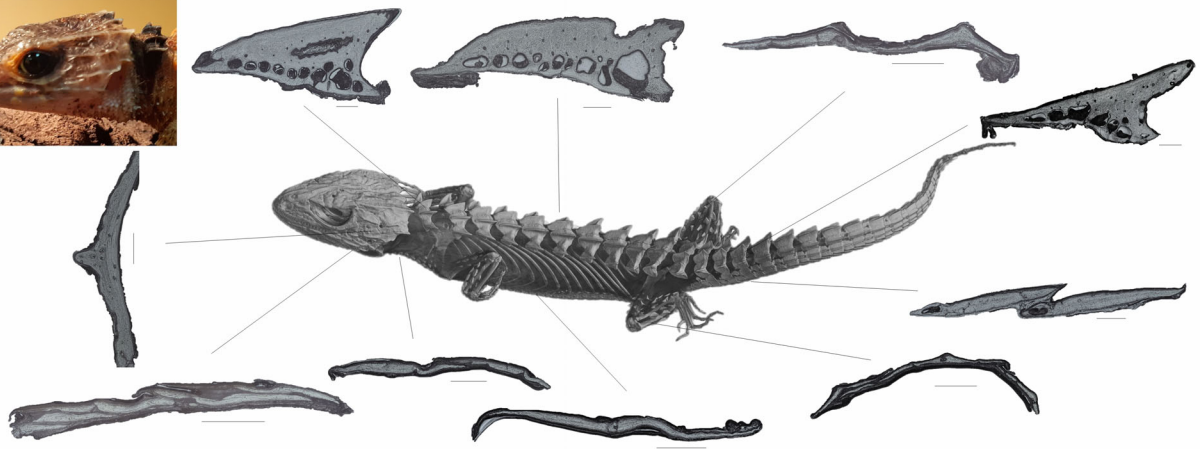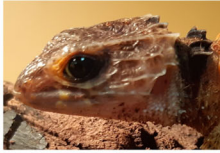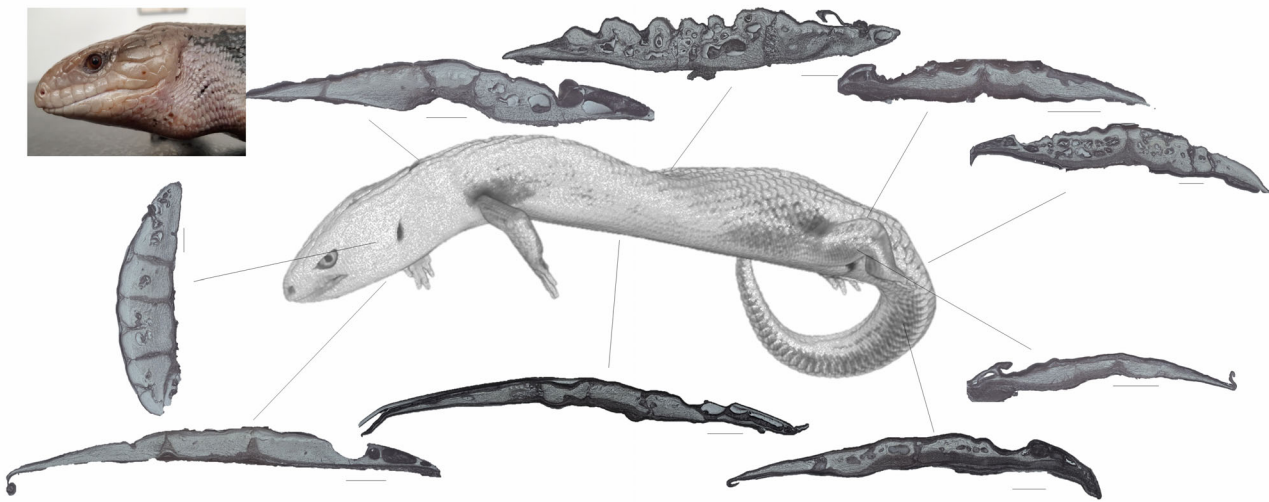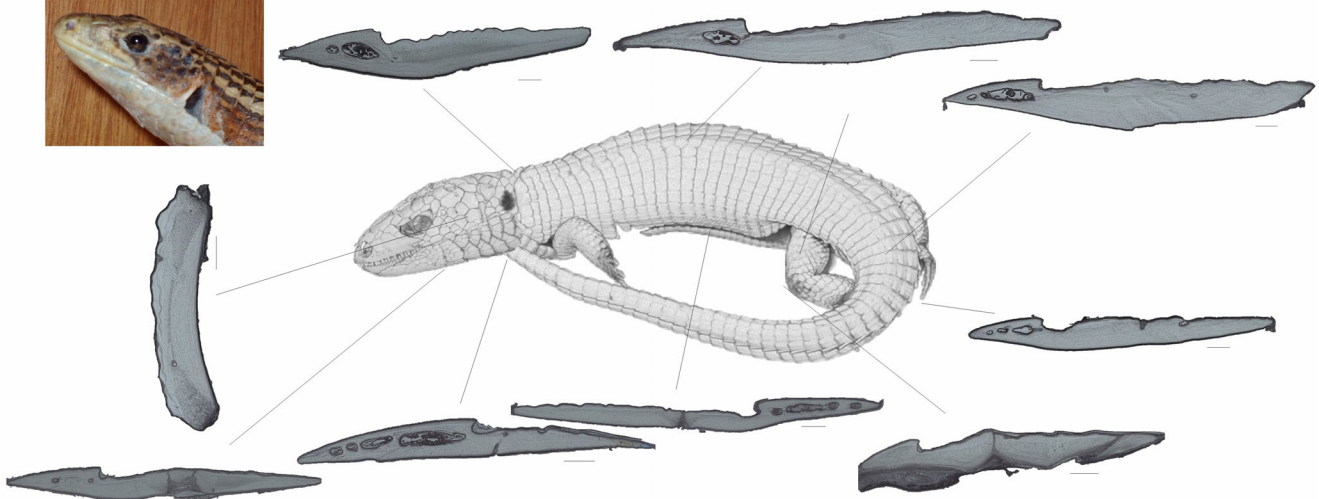
**(a)** *Tribolonotus novaeguineae*

**(b)** *Tiliqua gigas*

**(c)** *Broadleysaurus major*
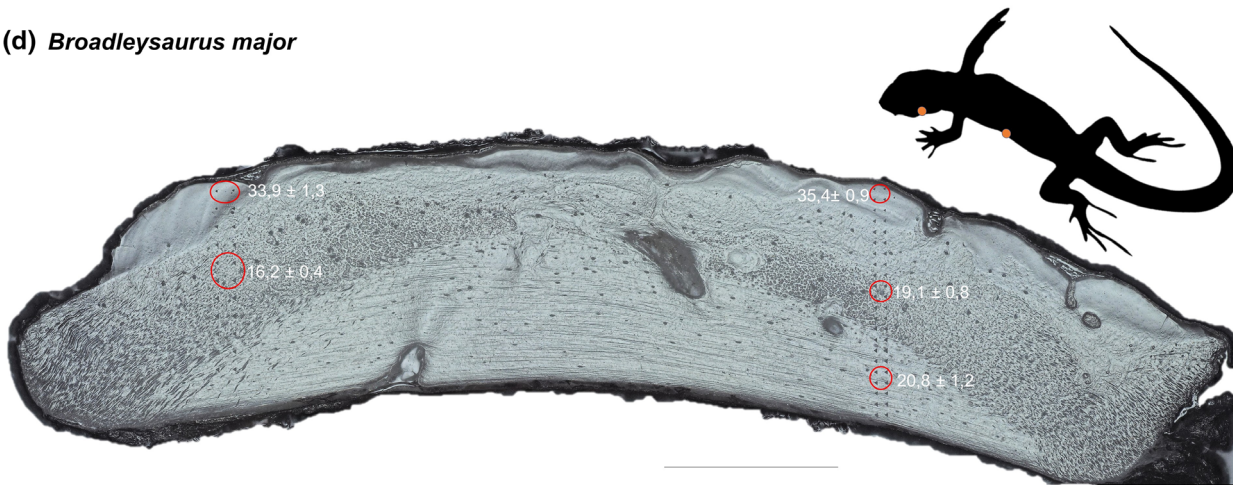


**FIGURE 3** Internal morphological variation seen in polished cross-section of the ODs of three lizards across the body of *Tribolonotus novaeguineae* (a), *Tiliqua gigas* (b), and (c) *Broadleysaurus major*.
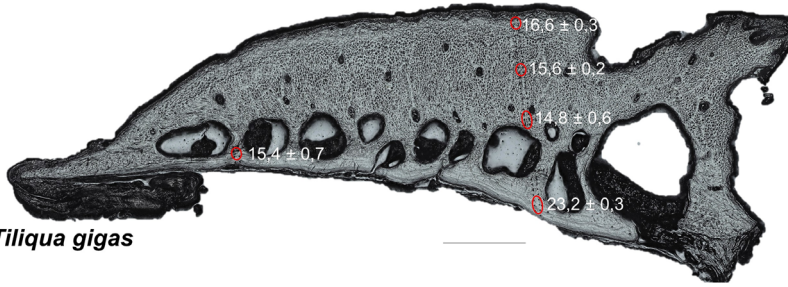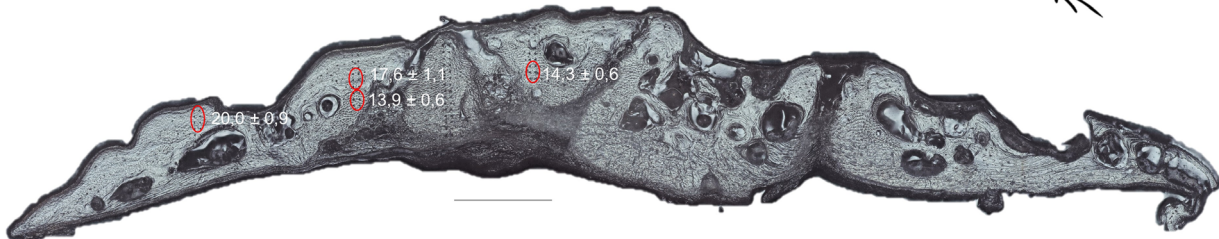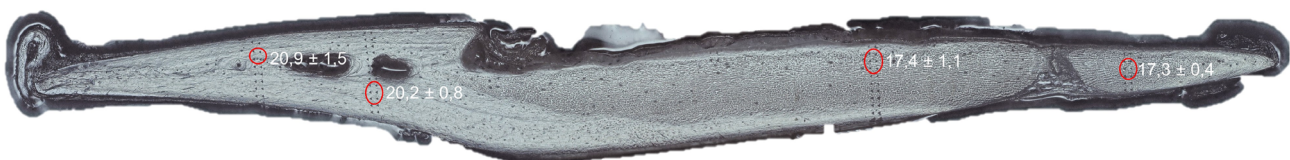
**FIGURE 4** Polished cross-section of ODs of the study species showing the mechanical properties of different regions of ODs in different regions of the body. Areas of nanoindentation are shown (red circles) and were used to provide a mean ± standard deviation for each circled area of the section. (a–c) Dorsal body sections of *Tribolonotus novaeguineae*, *Tiliqua gigas*, and *Broadleysaurus major*. (d and e) Temporal and lateral body sections of *Broadleysaurus major*. The silhouette was taken from Free Clip Art Collection.

**TABLE 2**  A summary of the quantified elastic modulus (of all indentations carried out) of different regions of the ODs.

| OD | *Tribolonotus novaeguineae* | | *Tiliqua gigas* | | | *Broadleysaurus major* | | |
|---|---|---|---|---|---|---|---|---|
| | **Woven-fibered bone** | **Organized bone** | **Woven-fibered bone** | **Organized bone** | **Capping tissue** | **Woven-fibered bone** | **Organized bone** | **Capping tissue** |
| Dorsal | 12.3–17.7 | 19.1–23.6 | 10.3–17.2 | 15.8–21.0 | 19.5–21.0 | 16.6–19.5 | 16.7–22.1 | 34.6–35.1 |
| Ventral | 13.7–16.1 | 15.9–18.8 | 9.7–14.7 | 14.6–15.5 | Not found | 13.3–18.1 | 16.6–25.6 | Not found |
| Temporal | 14.9–15.2 | 16.3–22.5 | 10.4–17.6 | 16.2–24.0 | 17.3–24.0 | 13.9–18.5 | 17.0–23.9 | 32.6–36.4 |

*Note*: Data are shown as ranges and in GPa (gigapascal). No capping tissue was present in *Tribolonotus novaeguineae*.

body elements and are primarily composed of woven-fibered bone and Sharpey-fibered bone, with some organized bone at the ODs extremities. Across parts of the superficial OD surface, a thin layer of a capping tissue is present. As for ODs across the body, temporal ODs are compound elements, and each osteodermite has a centrally located marrow cavity. Further, temporal ODs also develop a thin layer of capping tissue.

Unlike *Tiliqua* and *Tribolonotus*, dorsal and ventral ODs of *B. major* are mostly composed of organized bone. Further, there are microstructural differences along the length of each element. For example, marrow cavities are restricted to the smooth cranial (underlapping) portion of each OD, while Sharpey's fibers are more abundant within the ornamented caudal (overlapping) portion. There are also structural differences between dorsal and ventral ODs. Collagen bundles within the organized bone of the dorsal ODs are mostly orientated longitudinally (i.e., cranio-caudally). Among ventral ODs, collagen bundle orientation changes from longitudinal in the articulating (underlapping) region of the OD to transverse in the overlapping region. The superficial surfaces of both dorsal and temporal ODs are covered by a collagen-poor capping tissue (Marghoub et al., 2022) that resembles osteodermine (de Buffrénil et al., 2010, 2011; Iacoviello et al., 2020; Kirby et al., 2020; Marghoub et al., 2022; Moss, 1969; Vickaryous et al., 2015), a dense, avascular, cell-poor, enamel-like layer (Figure 3c), first reported in the anguimorph lizard *Heloderma* (Moss, 1969; see also Vickaryous & Sire, 2009).

## 3.3 | Material characterization

Examination of the mechanical properties of the ODs from *Tr. novaeguineae* found a relationship between bone type (i.e., organized vs. woven-fibered bone) and elastic modulus. Woven-fibered bone tissue of the dorsal ODs was in the range of 12.3–17.7 GPa whereas that of organized bone had an elastic modulus in the range of 19.1–23.6 GPa (Figure 4a, Table 2). Ventral ODs had an elastic modulus in the range of 13.7–19.1 GPa, similar to the woven-fibered bone tissue of the dorsal ODs, whereas the organized bone

of temporal ODs had an elastic modulus in the range of 16.3–22.5 GPa.

*T. gigas* ODs had the lowest elastic modulus among the ODs of the species studied. The elastic modulus values for woven-fibered bone tissue of the dorsal ODs were 10.3–17.2 GPa (average 12.5 GPa) and for organized bone were 15.8–21.0 GPa (average 19.2 GPa) (Figure 4b, Table 2). Capping tissue on the dorsal ODs had an elastic modulus similar to that of organized bone ($20.0 \pm 0.8$). Ventral ODs had an elastic modulus in the range of 9.7–15.5 (average 13.2 GPa) perhaps associated with the greater proportion of woven-fibered bone tissue in these elements. The elastic modulus for temporal ODs was similar to that of the dorsal elements, with a range of 10.4–17.6 GPa (average 13.8 GPa) for woven-fibered bone, and 16.2–24.0 GPa (average 18.5 GPa) for organized bone. The capping layer on the temporal osteoderms has a similar value (24.0 GPa) to the organized bone.

In *B. major* all ODs across the body were mostly composed of organized bone (range of 16.6–22.1 GPa), with a layer of capping tissue. The capping tissue had an elastic modulus in the range of 34.6–35.1 GPa in the mid-dorsal ODs and 32.6–36.4 GPa—in the temporal ODs (Figure 4c–e, Table 2).

## 4 | DISCUSSION

This study has highlighted the remarkable diversity of lizard ODs both between species and across the body of a single species. In combination with other recent studies on lizard ODs (Broeckhoven et al., 2018; Cherepanov et al., 2023; Kéver et al., 2022; Kirby et al., 2020; Laver et al., 2020; Maisano et al., 2019; Marghoub et al., 2022; Schucht et al., 2020), these observations provide insights into the functional and developmental factors driving the OD differences at both inter- and intra-species level. The microstructural analysis has demonstrated that the same basic tissues—mostly organized bone, woven-fibered bone, and Sharpey-fibered bone—are found in all ODs examined in this study. However, the proportions and distribution of each bone type varied among the

species investigated. Similarly, the presence of capping tissue was also variable. Kéver et al. (2022) reported that capping tissue was present in *B. major*, *Corucia zebrata*, *Heloderma horridum*, *H. suspectum*, *P. apodus* and two species of *Tiliqua* (*T. rugosa, T. scincoides*); *T. gigas* was not part of that study. Our histological sections confirmed the presence of capping tissue in *B. major* and a third species of *Tiliqua*, *T. gigas*. Unexpectedly, the material characterization of capping tissues from *B. major* and *T. gigas* yielded different results. Whereas *B. major* had an elastic modulus above 30 GPa, comparable to that of *H. suspectum* (27.1–50.8 GPa, Marghoub et al., 2022), that of *T. gigas* was less than 25 GPa. Although currently untested, this biomechanical variability may relate to species-specific differences in the development and elemental composition of capping tissue. Ongoing efforts to explore capping tissue at the molecular and nano-scale may provide mechanistic insight.

The shape and histological composition of ODs may also provide insight into their function. For example, compared to ventral osteoderms, those from the dorsal regions of the body are generally thicker and (at least in *B. major* and *T. gigas*) have a layer of capping tissue. Previous investigations have shown that the dorsal body surface is the most common location targeted during conspecific (Broeckhoven, 2022) or predatory (e.g., Abramjan et al., 2015; Schmidt, 1919) attacks. Related to this, the midline keels of dorsal ODs of *Tr. novaeguineae* may also reduce vertical stress (Clarac et al., 2020; Kéver et al., 2022). It has also been suggested that ODs may participate in thermoregulation and/or reducing evaporative water loss (Clarac et al., 2020; Seidel, 1979). Related to this, the large size of the marrow cavities, particularly in *T. gigas*, may be linked to thermoregulation. *T. gigas* basks to gain heat (heliotherm) and it has been suggested that the dorsal integument of larger lizards like *Tiliqua* shields the body from radiative heat gain to prevent overheating (Garrick, 2008). Having blood vessels running within the ODs would aid heat transfer between the body and the integument.

Ventral body ODs from all three study species were morphologically similar, being compound and overlapping and relatively thinner than ODs from the dorsal body surface. We suggest that the presence of ventrally positioned compound ODs provides increased flexibility where a purely protective role is less essential, as well as aiding heat transfer from the substrate (e.g., in *Tr. novaeguineae* which are secretive and semi-fossorial). The same thinner flexible compound ODs were seen on the limbs. In the case of *T. gigas*, however, the thinner, potentially less compression resistant, ventral ODs are a little surprising, given that this large lizard is a 'belly dragger' (Dickinson et al., 2022), using a slow gait in which the ventral surface of the body is in contact with the substrate and is subject to friction. However, the ventral body scales may have a role in dealing with this abrasion, given they can be regenerated more easily.

Temporal ODs were generally similar in structure to dorsal ODs, though somewhat less invested with marrow cavities, contributing to protection against conspecific bites to the head but also, as shown by Marghoub et al. (2023), having a role in dissipating feeding strains across the skull. Kéver et al. (2022) examined the biomechanics of temporal ODs and measured their deformation (stiffness) in a range of different lizards, including *B. major* and *Tr. novaeguineae*. *T. gigas* was not included in that study, but two other species of *Tiliqua*, *T. rugosa* and *T. scincoides*, were. Kéver et al. (2022) found differences in strain between OD surfaces, such that the external surfaces largely experienced compressive strain, whereas the inner (deep) surfaces suffered tensile strain, although tensile strain was also recorded in ODs adjacent to the one under loading. The difference in microanatomy between the superficial and deep surfaces of the temporal ODs, may reflect differences in strain type and direction, as may the different fiber orientations seen in dorsal and ventral ODs in *Broadleysaurus*.

Overall, therefore, the structural variation of the ODs across the body suggests that ODs are plastic and that the differences between them, at both macro- and microstructural levels are a response to functional demands (see also Clarac et al., 2020; Kéver et al., 2022; Marghoub et al., 2023; Scheyer & Sander, 2004). Further histological comparisons between dorsal and ventral ODs in other osteoderm-bearing taxa could be revealing, particularly the 3D reconstruction of collagen fiber orientation.

## 5 | CONCLUSIONS

The findings of this study did not support the initial hypotheses regarding uniformity of osteoderm (OD) macro- and microstructure, or mechanical properties, in a single individual, or between broadly related taxa. Although ventral ODs were generally similar within and between the individuals studied, dorsal, and temporal ODs varied to a greater extent. Apart from capping tissue, the same basic tissue types were present in ODs across the three species examined and had similar mechanical properties, but the proportions and distribution of those tissues varied across the body. Moreover, the presence and material properties of capping tissue were distinctly non-uniform. Capping tissue was absent in the ventral ODs of all three species, and in the dorsal ODs of *Tribolonotus*. There was also a clear difference in the elastic modulus of the capping tissue between *B. major* and *T. gigas*, highlighting the need

for further work on the function, development, and nanostructure of this enigmatic material.

The results presented here, though preliminary, demonstrate the need for a more nuanced understanding of osteoderm variation within and between lizard species, and the factors (ecological, functional, and evolutionary) driving that variation. Additionally, it highlights the importance of considering regional differences when investigating the anatomical and biomechanical features of integumentary structures in reptiles.

## AUTHOR CONTRIBUTIONS
**Anastasiia Maliuk:** Conceptualization; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Arsalan Marghoub:** Conceptualization; formal analysis; methodology; validation; writing – review and editing; visualization. **Catherine J.A. Williams:** Conceptualization; methodology; validation; writing – review and editing. **Edward L. Stanley:** Conceptualization; methodology; validation; writing – review and editing. **Loic Kever:** Methodology; validation; writing – review and editing. **Matthew K Vickaryous:** Conceptualization; methodology; funding acquisition; validation; writing – review and editing. **Anthony Herrel:** Conceptualization; investigation; methodology; resources; funding acquisition; validation; writing – review and editing. **Susan Evans:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – review and editing; writing – original draft. **Mehran Moazen:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – review and editing; writing – original draft.

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## CONFLICT OF INTEREST STATEMENT
We declare we have no competing interests.

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