RESEARCH ARTICLE

Ecological and life-history correlates of erythrocyte size and shape in Lepidosauria ⬡⬢

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Abstract

Blood oxygen-carrying capacity is shaped both by the ambient oxygen availability as well as species-specific oxygen demand. Erythrocytes are a critical part of oxygen transport and both their size and shape can change in relation to species-specific life-history, behavioural or ecological conditions. Here, we test whether components of the environment (altitude), life history (reproductive mode, body temperature) and behaviour (diving, foraging mode) drive erythrocyte size variation in the Lepidosauria (lizards, snakes and rhynchocephalians). We collected data on erythrocyte size (area) and shape (L/W: elongation ratio) from Lepidosauria across the globe ($N = 235$ species). Our analyses show the importance of oxygen requirements as a driver of erythrocyte size. Smaller erythrocytes were associated with the need for faster delivery (active foragers, high-altitude species, warmer body temperatures), whereas species with greater oxygen demands (diving species, viviparous species) had larger erythrocytes. Erythrocyte size shows considerable cross-species variation, with a range of factors linked to the oxygen delivery requirements being major drivers of these differences. A key future aspect for study would include within-individual plasticity and how changing states, for example, pregnancy, perhaps alter the size and shape of erythrocytes in Lepidosaurs.

KEYWORDS
blood oxygen-carrying capacity, erythrocyte, life history, oviparity, viviparity

1 | INTRODUCTION

There is considerable variation in erythrocyte size between- and within-taxa across all vertebrates (Gregory, 2000; Hawkey et al., 1991; Lay & Baldwin, 1999). There are a number of key drivers of erythrocyte size such as a positive relationship between genome size and cell size across vertebrates (Gregory, 2000, 2001, 2002; Mueller et al., 2008). For vertebrates with nucleated erythrocytes, erythrocyte size is directly related to nucleus size (Gregory, 2002). There are also broad differences between ectotherms and endotherms with reptiles, amphibians and fish all having larger cells than mammals and birds (Gregory, 2001; Hawkey et al., 1991). In addition, nucleated avian erythrocytes are larger than those of non-nucleated mammals of an equivalent body mass (Gregory, 2002). The smaller erythrocytes of endotherms (bird, mammals) in comparison to ectotherms (reptiles, fish, amphibia) are thought to derive from lower mass-specific resting metabolic rates (RMR) and lower oxygen demands of ectotherms (Hawkey et al., 1991), something that also contributes to within-taxon patterns (Gregory, 2002; Kozłowski et al., 2020). In contrast, body mass does not necessarily

correlate with erythrocyte size. Positive interspecific relationships between erythrocyte size and body mass have been demonstrated in geckos (Starostová et al., 2005) and birds (Kostelecka-Myrcha & Chołostiakow-Gromek, 2001; Soulsbury et al., 2022). In mammals and teleost fish, there is no relationship between erythrocyte size and body mass or size (mammals: Promislow, 1991; Savage et al., 2007; teleosts: Martins et al., 2021), although for mammals there is a significant positive correlation when analysed with independent contrasts (Promislow, 1991).

Erythrocyte size can also change throughout an organism's life. In some species, erythrocytes can increase in size throughout ontogeny, as seen in monitor lizards (Frýdlová et al., 2013), 10 species of teleost (Lahnsteiner, 2021) and three of five species of eyelid geckos (Starostová et al., 2013). In contrast, erythrocyte size did not change in mole salamanders (Davies, 2008) or two of five species of eyelid geckos (Starostová et al., 2013). Such plasticity is undoubtedly underpinned by between-species differences in oxygen demand as well as changing mass-specific metabolic rate (Pis, 2008).

In other cases, some behavioural and ecological variables may impact erythrocyte sizes. For example, differences in oxygen availability, for example, altitude, can drive variation in blood oxygen-carrying capacity (González-Morales et al., 2015; Lu et al., 2015; Monge & Leon-Velarde, 1991; Navas & Chauí-Berlinck, 2007). Across-species, erythrocytes of anurans are smaller at higher altitudes (Navas & Chauí-Berlinck, 2007), whereas comparisons within species of lizards show increasing erythrocyte size with higher altitude (González-Morales et al., 2017), largest sizes at intermediate altitudes (González-Morales et al., 2017) or decreasing erythrocyte size with higher altitude (Guadarrama et al., 2020). Similarly, species with seasonal altitudinal migration can show plastic size changes in erythrocyte size; alpine accentors (*Prunella collaris*), for example, have smaller erythrocytes when living at high altitude (Haas & Janiga, 2020; Janiga et al., 2017). Other sources of variation in erythrocyte size are less well studied, but again thought to be related to oxygen demand. For example, in tropical reef teleosts, species with a higher aerobic capacity and greater activity levels have smaller erythrocytes (Well & Baldwin, 1990), but more broadly across teleosts, erythrocyte shape was unrelated to habitat or body size (Martins et al., 2021). Similarly, birds with greater energetic demands (e.g. long distance migration) had smaller erythrocytes, whereas diving birds had larger erythrocytes (Soulsbury et al., 2022). These studies demonstrate that components of species ecology and life history are important for determining erythrocyte size. Finally, erythrocyte shape can also vary, being rounder (smaller length to width ratio) or elongated (greater length to width ratio). Erythrocyte shape is poorly studied, but is known to vary plastically within-species, for example, elongated erythrocytes were reported in alpine accentors breeding at high altitudes (Haas & Janiga, 2020; Janiga et al., 2017), whereas across bird species, erythrocytes were more elongated at higher altitudes and for species migrating long distances (Soulsbury et al., 2022). In contrast, cell shape (PCA of cell shape components) also varies extensively in teleosts, but did not vary with habitat or body size (Martins et al., 2021). To date,

erythrocyte shape is relatively unexplored and key drivers of shape variation are uncertain.

Lepidosauria (lizards, snakes and tuatara) are excellent models to study the relationships between ecology and physiology, because of their widespread global distribution and their diversification in a range of ecological niches (Meiri, 2018). As such, cross-species analyses of Lepidosauria allow the study of key patterns in evolutionary ecology at a global scale (Meiri et al., 2020a; Slavenko et al., 2019). A number of key studies have examined macroecological variation and ecological drivers in key traits in squamates (lizards and snakes). These traits include body size (Slavenko et al., 2019), body plan (Wiens et al., 2006), reproductive mode (Ma et al., 2018; Pincheira-Donoso et al., 2017; Rafferty et al., 2013) and life history (Costa et al., 2008; Meiri et al., 2020a, 2020b; Scharf et al., 2015). Studies that examine environmental physiology on a global scale are generally rarer and have mostly focused on thermal ecology (Andrews & Schwarzkopf, 2012; Garcia-Porta et al., 2019).

In this study, we take a first step in understanding the relationships between ecology and physiology by compiling a unique dataset to analyse whether a variety of life-history and ecological traits have driven selection on erythrocyte size in Lepidosauria. We built on recent analyses in birds (Soulsbury et al., 2022), and predict that components of Lepidosauria ecology and behaviour will play a major role in shaping erythrocyte sizes. Smaller erythrocytes may be predicted to occur where the ability and speed to exchange gases between pulmonary and tissues are critical. Hence, we predict that where tissue-oxygen demands are likely to be increased (higher body temperatures, active foraging) or there is a need for enhanced gas exchange (altitude), species will have smaller erythrocytes. Conversely, where there is a need for greater within-blood oxygen-carriage (viviparity vs. oviparity) and storage (diving), there will be increases in erythrocyte size. Finally, we test the same set of factors in relation to erythrocyte shape. However, too few studies have previously tested this parameter, hence the predictions may be too speculative.

2 | MATERIALS AND METHODS

2.1 | Data collection: Erythrocyte size

We gathered data from the published literature. Specifically, we focussed on erythrocyte area (μm^2), rather than mean corpuscular volume (MCV). MCV is usually derived by multiplying a volume of blood by the proportion of blood that is cellular (the haematocrit), and dividing that product by the number of erythrocytes (red blood cells) in that volume. This misses key information on cellular shape. We instead collected length of the long axis (L, in μm) and width of the short axis (W, in μm), and area, which was calculated as the area based on length x width measurements of an ellipsoid (area = $\pi \cdot L/2 \cdot W/2$ in μm^2). We calculated the elongation ratio of the cell using the length and width of the erythrocytes (L/W). Elongation ratio is a dimensionless measure, but captures whether

the erythrocyte is round (L/W = 1), oval shaped (L/W = 1.4–1.6) or flatter ellipses (L/Q = >1.8). Greater elongation ratios give greater surface areas for a given volume (see example in bacteria: Ojkic et al., 2019), which may impact gas exchange rates.

2.2 | Data collection: Species ecology and natural history

The data set comes from populations across the five continents even though there is a bias for some reptile biodiversity hotspots, particularly in south eastern Europe (Figure S1). For each species, we extracted the location of capture (where possible). Altitude was included as reported in the articles or as calculated from http://www.twcc.fr/ using the coordinates given in the original publication. The exception was one species of sea snake, which had no capture location but was collected at sea level.

Few studies had any information on study-specific body masses, ages or sexes of sampled animals. To include body mass, we used published datasets to assign body mass (Feldman et al., 2016). We also included the reproductive mode (oviparous or viviparous) (Pyron & Burbrink, 2014), but although there is a continuum of maternal–foetal contact (Albergotti & Guillette, 2011), we lacked enough data to assign these to individual species. Any ovo-viviparous species in our data set were considered viviparous in this context because the mother carries the young until full term.

We also included information on whether the species regularly dives, that is, whether the species was aquatic or semi-aquatic, or not (yes/no). Where information was available, we included information on foraging mode. We used the nomenclature of Meiri (2018) who categorized Sauria (lizards) as ambush predators ('sit and wait'), active foragers ('active foraging') or using a mixed strategy ('mixed'). We extended these data to include snakes, some additional lizards and the tuatara using additional datasets, for example, Lourdais et al. (2014) and Glaudas et al. (2019), and natural history descriptions. Our distribution of foraging strategies had a limited number of 'mixed' strategies. Instead, we simplified categories to those that were active foragers or those using ambush foraging some or all of the time, that is, combining 'ambush' and 'mixed; categories. Finally, we used existing sources to include species-specific mean body temperature (°C) where measured; for ectotherms, most behavioural and physiological processes depend directly on body temperature (see dataset for sources).

2.3 | Data processing and statistical analysis

From our initial data set, we carried out four different analyses covering differing information per study/species (Table S1). Initially, we built the allometric relationships between log_{10}-transformed body size and erythrocyte size and shape. Second, we analysed a model that tested the effects of body mass, altitude, diving behaviour and reproductive mode. Initially, we considered interactions between variables, specifically reproductive mode and altitude. However, interactions were not significant, and the model was simplified. The third model included the additional variable of foraging mode. In this analysis, we note a clear outlier during exploratory plotting – the rock python *Python sebae*. In our data set, the erythrocyte size for this species was taken from a study using small (~2.7kg) snakes (Jegede et al., 2020) and body masses of adults are ~50kg (Feldman et al., 2016). There may be age- or size-specific changes in foraging, so we chose to exclude this single data point as we were not sure how to categorize such small snakes. Our final model tested the additional effect of mean body temperature to the second model. The use of these four models maximized coverage of the data but meant that there were differing numbers of species and samples from each species for each of the four different models.

Analysis was carried using phylogenetic mixed models (PMM) using the MCMCglmm package (Hadfield, 2010), with the inclusion of a phylogenetic covariance matrix, with species retained as a second random effect within the models (random = ~animal). The phylogenetic covariance matrix was created using a time-calibrated Lepidosauria phylogeny (Tonini et al., 2016), and pruned to fit the species in each model subset. For the PMM models, we set uninformative priors (R~inverse.gamma(V = 1, nu = 0.002); G~ inverse.gamma(V = 1, nu = 0.002)), used a single independent chain of 500,000 iterations, with sampling taking place every 500 iterations after a 10,000 burn in. The phylogenetic signal (lambda, λ) was estimated by dividing the variance explained by the phylogeny, by the sum of all variance components. The phylogenetic signal varies between 0 and 1, where 0 represents no evolutionary signal (no covariance in the residuals due to shared ancestry), and 1 indicates that the observed covariance in residuals follows that expected under a Brownian motion model of trait evolution (Freckleton et al., 2002). Convergence of the MCMC chain was checked using Heidelberger and Welch's convergence diagnostic test for stationarity (implemented in the R package *coda 0.19–3*; Plummer et al., 2006), and the level of autocorrelation was checked to ensure adequate (>1,000) effective sample size for each estimated parameter. All models were run in R version 4.0.3 (R Core Team, 2020).

3 | RESULTS

3.1 | General patterns of erythrocyte size

Erythrocyte size varied considerably between species (Figure 1). The lizard with the smallest erythrocyte areas was the eastern smooth-throated lizard *Liolaemus wiegmannii* (47.12 μm^2) and the snake with the smallest erythrocyte was the Egyptian cobra *Naja haje* (52.77 μm^2). The species with the three largest erythrocyte areas were marine file snake *Acrochordus granulatus* (287.11 μm^2), the Komodo dragon *Varanus komodoensis* (252.90 μm^2) and the tuatara *Sphenodon punctatus* (257.62 μm^2; Figure 1). Most species had
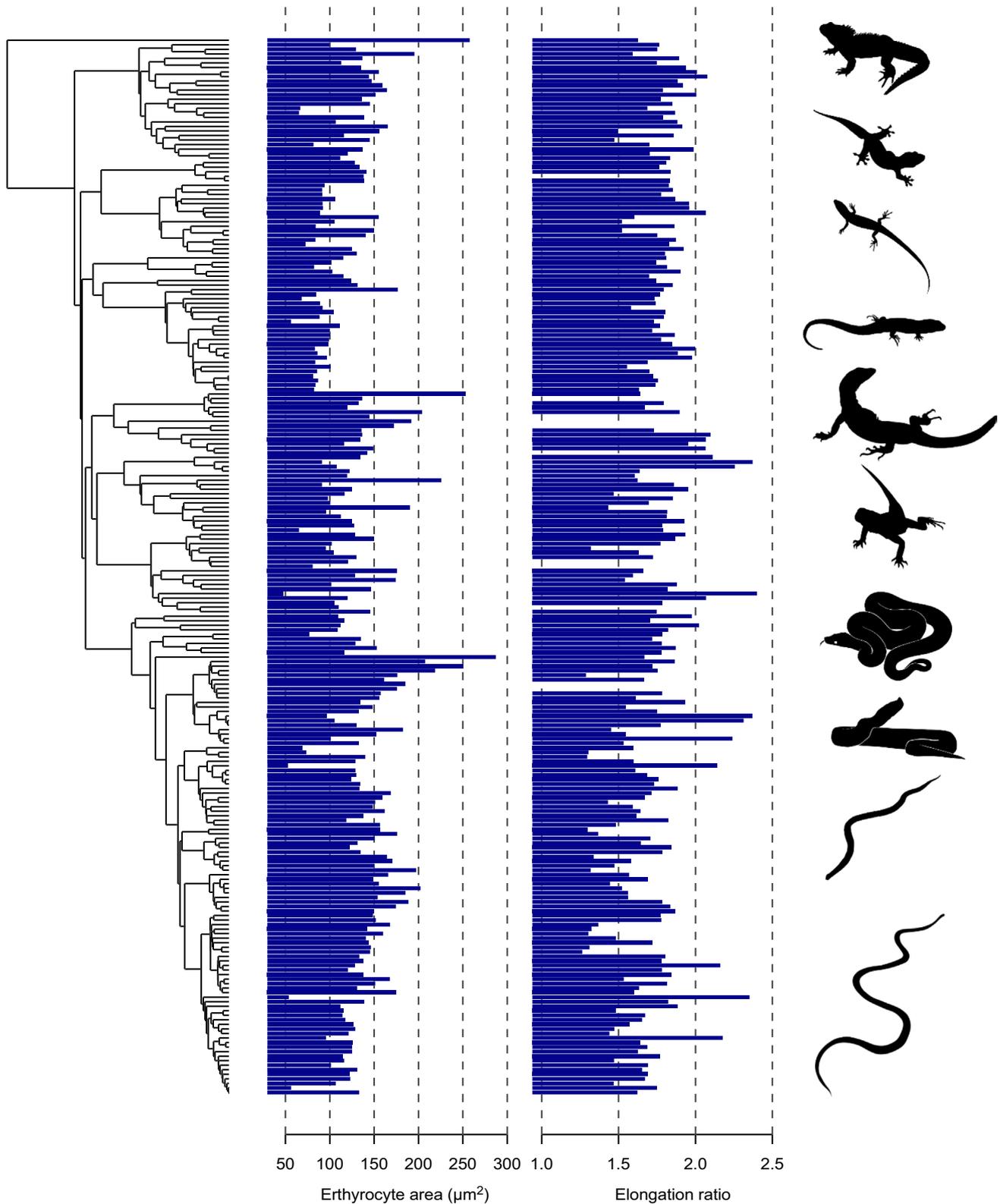
FIGURE 1 Mean erythrocyte area (μm²) and mean elongation ratio for individual species across the phylogenetic relationship of Lepidosauria. For illustration, the approximate location of representative taxa (top to bottom: *Sphenodon punctatus*; *Gehyra mutilata*; *Eumeces fasciatus*; *Lacerta agilis*; *Varanus komodoensis*; *Stellagama stellio*; *Boa constrictor*; *Vipera latastei*; *Notoechis ater*; *Elaphe quadrivirgata*) are included as tip labels were too small to be visible. Species that are underlined are not in the phylogeny but represent closely related species. All images are taken from Phylopics (http://phylopic.org) under Public Domain Dedication 1.0 license(http://creativecommons.org/publi cdomain/zero/1.0/)

an erythrocyte area lying within the range of 50–200 μm^2 (95.4%, 272/285 species). For elongation ratios, the species with round-est cells were snakes, such as the eastern ribbon snake *Thamnophis saurita* [*sauritus*] (ratio = 1.22) and the Montpellier snake *Malpolon monspessulanus* (ratio = 1.23; Figure 1), with the most elongated cells found in eastern smooth-throated lizard *Liolaemus wiegmannii* (ratio = 2.40) and the coastal viper *Montivipera xanthina* (ratio = 2.37; Figure 1).

Irrespective of species, larger Lepidosauria had significantly larger erythrocytes in terms of area (Posterior mean = 11.43, upper and lower bounds of the 95% Credible Intervals (CI) = 5.48–17.43, pMCMC <0.001; λ = 0.70 (0.54/0.83)), Figure 2; N = 285 from 235 species). In contrast, there was no effect of body mass on elongation ratio (Posterior mean = 0.01, 95% CI = −0.03–0.04, pMCMC = 0.926; λ = 0.42 (0.14/0.67); N = 266 from 227 species).

3.2 | Effect of diving, altitude and reproductive mode on erythrocyte size

Species that dived had significantly larger erythrocytes (Table 1a; Figure 3a), but no change in elongation ratio (Table 1a). Viviparous species also had erythrocytes with a larger area (Figure 3b), but elongation ratio did not differ between oviparous and viviparous species (Table 1a). Finally, altitude had a significant negative effect on erythrocyte area (Table 1a; Figure 3c), and had a positive relation-ship with elongation ratio, that is, more elongated cells (Table 1a; Figure 4). Interestingly, viviparous species had larger erythrocytes than oviparous species irrespective of altitude (Figure 3c).
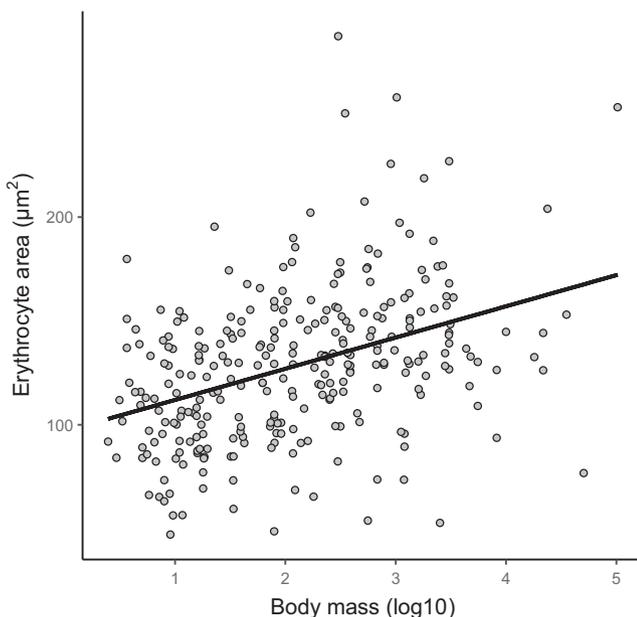

FIGURE 2 Relationships between body mass (log10, g) and erythrocyte area (μm^2). Raw data points are shown, with regression line (+95CI) drawn through predicted values derived from the phylogenetic MCMC model

3.3 | Foraging, body temperature and erythrocyte size

Across species, those that were active foragers as opposed to those using mixed/sit-and-wait strategies had significantly smaller erythro-cytes (Table 1c; Figure 5a). Similarly, those species with higher mean body temperatures had smaller erythrocytes (Table 1c; Figure 5b). In contrast, neither foraging strategy or mean body temperature af-fected elongation ratio (Table 1c).

4 | DISCUSSION

In Lepidosauria, there were significant positive relationships be-tween body mass and erythrocyte size but no significant effect on shape, as measured by elongation ratio. Our analysis found that while controlling for body mass there were complex patterns of changes in Lepidosaurian erythrocyte size and shape. Some drivers were linked to environmental conditions, including altitude and climate, whereas others were related to life-history and behaviour factors such as re-productive mode, body temperature, foraging or diving.

4.1 | Body mass effects

We found a significant positive interspecific relationship between body mass and erythrocyte size in Lepidosauria, which has also been found within species (Frýdlová et al., 2013; Starostová et al., 2005). Increased body size is also associated with larger erythro-cytes in amphibians (Gregory, 2001; Hawkey et al., 1991) and birds (Kostelecka-Myrcha & Chołostiakow-Gromek, 2001; Soulsbury et al., 2022). The increase in genome size with body size (Gregory, 2000, 2001, 2002; Mueller et al., 2008), leads to larger nucleus sizes; larger erythrocytes are, therefore, needed to house the larger nu-cleus. Hence, the anucleate erythrocytes of mammals do not exhibit any relationship with body size, although many nucleated cell types do exhibit such a relationship (Savage et al., 2007). Erythrocyte size is larger in fish than in amphibians, which are larger than in reptiles (Hartman & Lesser, 1964), and this reflects broad changes in the pulmonary-circulatory system (Snyder & Sheafor, 1999). Having demonstrated that body size is important in Lepidosauria, further analysis is required to investigate how other blood parameters that are important for oxygen transport are affected by environmental and life-history variables.

4.2 | Altitude and climatic effects on erythrocyte size

Vertebrates that live at high altitudes (above 2000 m) are subjected to hypoxic conditions that challenge aerobic metabolism. They usu-ally exhibit functional and structural modifications that allow them to cope with the concomitant decrease in O_2 tension that potentially

TABLE 1 Phylogenetic mixed model analysis outputs of erythrocyte area and elongation ratio in relation to (a) body mass, altitude, diving behaviour and reproductive mode and (b) foraging mode (mixed or active foraging) and (c) maximum body temperature

	Variable	Posterior mean	lower 95% CI	upper 95% CI	pMCMC[a]
(a) Altitude, diving and reproductive mode					
Area	Body mass	10.45	3.01	17.26	0.006
$\lambda = 0.24\ (0.00/0.58)$	Altitude	−0.01	−0.02	−0.01	0.001
$N = 151$ (129 species)	Diving	32.31	10.66	55.78	0.006
	Reproductive mode	21.44	6.41	37.64	0.005
Elongation ratio	Altitude	8.21×10^5	2.45×10^5	1.43×10^4	0.005
$\lambda = 0.38\ (0.03/0.72)$	Diving	−0.04	−0.13	0.20	0.621
$N = 132$ (118 species)	Reproductive mode	0.04	−0.07	0.16	0.478
(b) Foraging mode					
Area	Body mass	7.51	−0.90	15.25	0.065
$\lambda = 0.70\ (0.50/0.90)$	Diving	38.85	19.33	57.58	0.001
$N = 177$ (143 species)	Reproductive mode	14.43	−0.72	30.17	0.065
	Foraging mode	16.98	2.70	32.66	0.031
Elongation ratio	Diving	−0.01	−0.14	0.11	0.802
$\lambda = 0.44\ (0.13/0.73)$	Reproductive mode	−0.04	−0.14	0.07	0.523
$N = 168$ (138 species)	Foraging mode	0.02	−0.08	0.13	0.634
(c) Body temperature					
Area	Body mass	13.46	7.50	20.02	0.001
$\lambda = 0.41\ (0.00/0.65)$	Diving	15.63	−2.81	33.43	0.094
$N = 162$ (124 species)	Reproductive mode	7.73	−3.87	21.73	0.220
	Mean body temp	−1.48	−2.79	−0.02	0.037
Elongation ratio	Diving	0.05	−0.08	0.18	0.455
$\lambda = 0.54\ (0.26/0.78)$	Reproductive mode	−0.02	−0.13	0.07	0.684
$N = 143$ (116 species)	Mean body temp	0.006	−0.004	0.02	0.244

[a]Particle Markov chain Monte Carlo (pMCMC) is the proportion of coefficients in the posterior distribution estimates that are =/0, multiplied by two (for a two-tailed test), and is analogous to a frequentist *p*-value.

constrains aerobic life in such environments (González-Morales et al., 2015, 2017). Some of these adaptations include changes in the size or morphology of respiratory surfaces, increased heart size, elevated haemoglobin concentration, higher haematocrit, increased capillary density and elevated myoglobin levels (Monge & Leon-Velarde, 1991; Samaja et al., 2003; Weber, 2007). We show that at higher altitudes, Lepidosauria in general had smaller erythrocytes. Similarly, across several species of anurans, species from higher altitudes also had smaller erythrocytes (Navas & Chauí-Berlinck, 2007), whereas in lizards, within-species comparisons showed mixed effects of altitude on erythrocyte size (González-Morales et al., 2015, 2017; Guadarrama et al., 2020). Size and shape affect surface area to volume ratio, meaning that small erythrocytes will exchange oxygen more efficiently than larger cells, and ellipsoid cells are more efficient than round cells (Hartman & Lesser, 1963). Another haematological parameter, haematocrit, that is, the ratio of the volume of red blood cells to the total volume of blood, tends to be increased at higher altitudes (Lu et al., 2015). Increased haematocrit allows greater oxygen-carrying capacity, but at the same time increases blood viscosity (Mehri et al., 2018). Smaller erythrocytes allow more rapid oxygenation and deoxygenation of haemoglobin (Holland &

Forster, 1966; Jones, 1979) without perhaps the deleterious increase in viscosity. Although not part of the current study, an understanding of the relationship between cell size and haematocrit would help determine whether high altitude, especially in species where erythrocytes size is larger at higher altitudes (e.g. González-Morales et al., 2015).

Altitude was also associated with a change in shape with cells becoming more elongated with increasing altitudinal hypoxia. In birds, those found at higher altitudes also had more elongated cells (Soulsbury et al., 2022), suggesting a general benefit of elongated cells in low-oxygen environments. Narrower, more elongated cells may pass through capillary beds more easily and so maximize the rate of blood flow directly or through a reduction in blood viscosity (e.g. alpine accentors: Janiga et al., 2017), although the exact benefits deserve further investigation.

4.3 | Life history and behaviour

Our results had three clear patterns for factors associated with life history and behaviour. First, energetic activities, that is, diving and
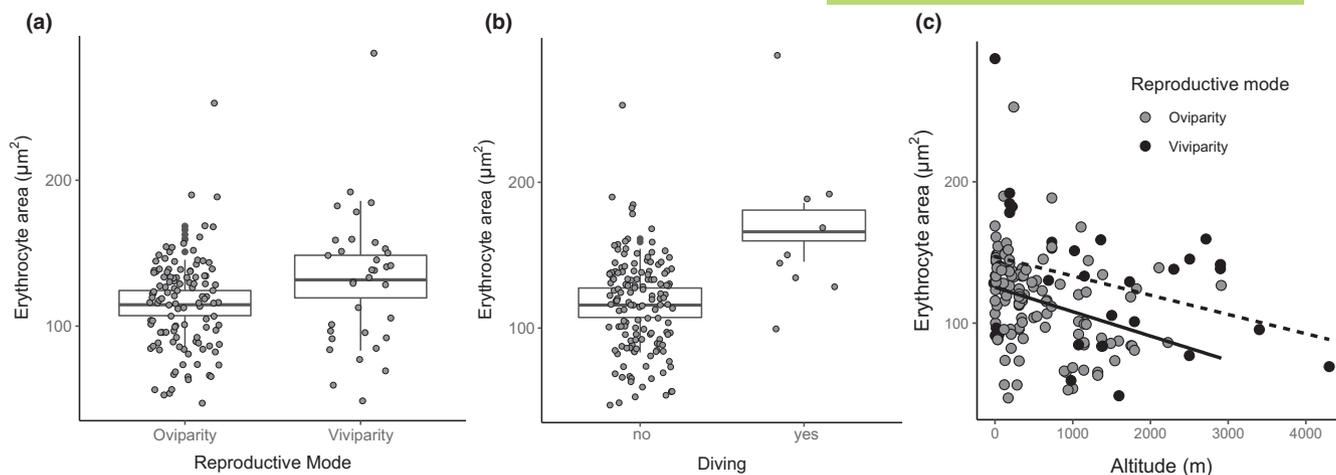
FIGURE 3 Boxplots showing the effect of (a) diving and (b) reproductive mode on erythrocyte area (μm²) and scatterplot showing the effect of (c) altitude and reproductive mode (oviparity = grey circles, viviparity = black circles) on erythrocyte area (μm²). Raw data points are shown and both boxplots and regression lines (+95CI) are drawn through predicted values derived from the phylogenetic MCMC models
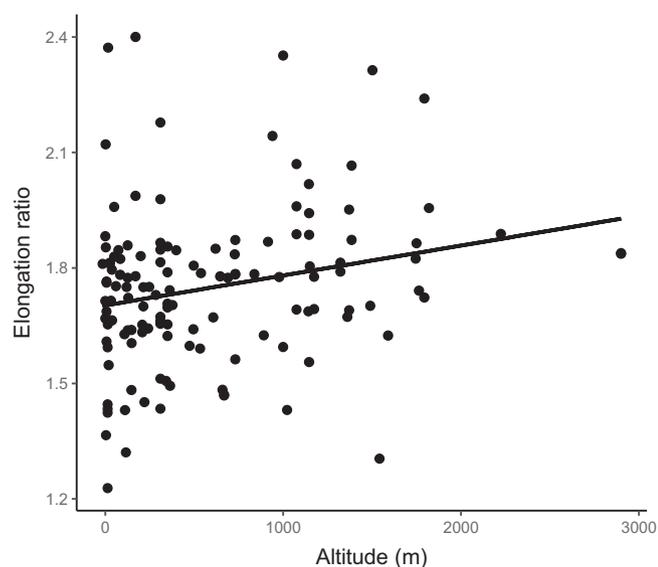

FIGURE 4 The relationship between elongation ratio of erythrocytes and altitude (m). Raw data points are shown, with regression line (+95CI) drawn through predicted values derived from the phylogenetic MCMC model

active foraging in our analysis, had contrasting effects on erythrocyte size. During diving in birds and mammals, the blood is an important oxygen store and haemoglobin concentration is elevated (Minias, 2020). Larger erythrocytes may contribute significantly to binding more oxygen and is, therefore, critical for oxygen storage capacity (Soulsbury et al., 2022). For example, in diving mammals, MCV and erythrocyte sizes are all significantly larger than in non-diving counterparts (Davis, 2014; Wickham et al., 1989). Similarly, reptiles use a range of physiological mechanisms, including bradycardia, prolonged apnoea, cardiac shunts and Bohr effects, to support diving behaviour (Anderson, 1966; Millard & Johansen, 1974; Seymour & Webster, 1975). In diving turtles, oxygen is stored both in the lungs and blood (Arango et al., 2021; Lutz & Bentley, 1985), and

blood oxygen stores are thought to be important in some squamates (Ferguson & Thornton, 1984). Marine snakes have significantly higher haematocrit levels (Brischoux et al., 2011; Feder, 1980), with the file snake *Acrochordus granulatus*, the species with the largest recorded erythrocytes, having the largest blood oxygen stores of any reptile (Feder, 1980). Our work demonstrates that along with other blood oxygen components, diving species have larger erythrocytes which allow greater quantities of oxygen to be stored in the blood. Larger erythrocytes are also found in diving birds (Soulsbury et al., 2022), suggesting a widespread adaptation to diving across vertebrates.

Our second result was the finding that Lepidosauria that gave birth to live young (viviparous and ovo-viviparous) had larger erythrocytes. Producing live offspring places a metabolic cost on the gravid females, leading to higher oxygen consumption especially towards the end of pregnancy (Beuchat & Vleck, 1990; Jackson et al., 2015; Robert & Thompson, 2002; Thompson & Speake, 2006). No study has looked specifically at erythrocyte size in Lepidosauria, and the only similar study showed that erythrocyte size was largest in mid-gestation gravid fish (Ingermann & Terwilliger, 1982), representing a plastic response. Our analysis shows that live birth has led to the selection for larger erythrocytes, perhaps as a result of higher oxygen demands of the female during gestation (Schultz et al., 2008; see also Munns & Daniels, 2007) but it could also be due to greater oxygen requirements of the developing offspring (Foucart et al., 2014). Previously, low oxygen was thought to be a constraint on the evolution of viviparity (Andrews, 2002), but this is unlikely to be the case (see Pincheria-Donoso et al., 2017). Indeed, the number of viviparous snakes and lizards found at higher altitudes suggests the reverse (Feldman et al., 2015; Pincheria-Donoso et al., 2017) and there is a need to consider the interaction between oxygen demand, altitude and the evolution of viviparity (Watson & Cox, 2021). It should be pointed out that our analysis did not take into account whether viviparous species had placental maternal transfer. This could be important because ovo-viviparity involves the retention of embryos *in*
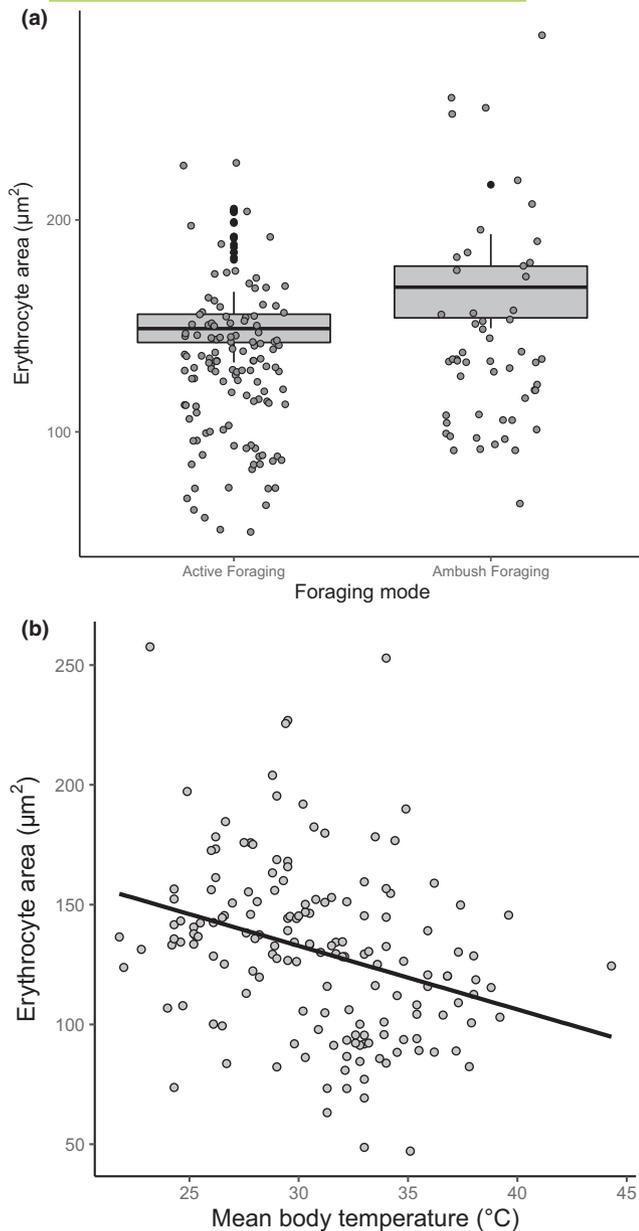
FIGURE 5 The relationship between erythrocyte area (μm²) and (a) foraging mode (active or ambush foraging) and (b) mean recorded body temperature (degrees Celsius) across Lepidosauria

utero but there is no development of a placental-like structure that facilitates transfer of nutrition; this may also affect the transfer of respiratory gases (Blackburn & Stewart, 2011). Although not directly measured, it is inferred that viviparous species with placentation exchange respiratory gases and this is facilitated by changes in maternal blood chemistry that enhances oxygen exchange (Ingerman et al., 1991). To fully understand the link between oxygen demand, oxygen transport and reproductive mode, examples of within-species (Rechnagel et al., 2021) and within-individual variation (Laird et al., 2019), that is, showing both oviparous and viviparous reproductive modes, as well as cross-species comparisons (Foster et al., 2020) all provide ideal opportunities to link evolutionary form to function. Our

analysis suggests that changing blood oxygen-carrying capacity by altering size and/or shape of erythrocytes is one mechanism to avoid constraints of low oxygen and may allow viviparous Lepidosauria to exploit higher altitude areas. In addition, future work should include the diversity of placenta types, and hence the degree of maternal–foetal contact, as an important modifier of oxygen transport and delivery (Stewart & Blackburn, 1988; Thompson & Speake, 2006).

Finally, we found that active foraging species of Lepidosaurs and those with higher mean body temperatures had smaller erythrocytes. In line with this, a handful of studies have found that erythrocyte size negatively correlates with activity, for example, aerobic swimming ability in teleosts (Lay & Baldwin, 1999) and migration distance in birds (Soulsbury et al., 2022). Differences in activity also alter erythrocyte size in some anurans (Atatür et al., 1999). Our work supports the idea that rapid oxygenation and deoxygenation of haemoglobin (Holland & Forster, 1966; Jones, 1979), facilitated by having smaller erythrocytes, is a key component of an active lifestyle. In line with this, species with higher mean body temperatures had smaller erythrocytes. High temperatures lead to higher metabolic rates and oxygen consumption (e.g. Clark et al., 2006) and in turn, across and within species, higher metabolic rates are associated with smaller erythrocyte size (Bury et al., 2019; Czarnoleski et al., 2017; Goodman & Heath, 2010; Gregory, 2002; Starostová et al., 2009). This further emphasizes the importance of mode of life and environmental conditions as important drivers of erythrocyte size.

5 | CONCLUSIONS

This study has demonstrated that across Lepidosauria erythrocyte size and shape can be affected by a range of biotic and abiotic factors and are linked to overall metabolism. Although limited to cell dimensions, the analysis demonstrates that this could be one of the key factors affecting changes in blood oxygenation required by different life histories in different habitats. It would be interesting to extend this analysis to determine whether factors, such as altitude, diving or sustained activity, also affect other key aspects of Lepidosaurian haematology, for example, haematocrit and haemoglobin concentration.

CONFLICT OF INTEREST
The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS
CDS conceived the ideas and analysed the data; ZP and CDS collected the data; CDS and DCD led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

PEER REVIEW

OPEN RESEARCH BADGES

DATA AVAILABILITY STATEMENT

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