RESEARCH ARTICLE

Morphology and burrowing energetics of semi-fossorial skinks (Liopholis spp.)

Nicholas C. Wu1,*, Lesley A. Alton1, Christofer J. Clemente1, Michael R. Kearney2 and Craig R. White1

ABSTRACT

Burrowing is an important form of locomotion in reptiles, but no study has examined the energetic cost of burrowing for reptiles. This is significant because burrowing is the most energetically expensive mode of locomotion undertaken by animals and many burrowing species therefore show specialisations for their subterranean lifestyle. We examined the effect of temperature and substrate characteristics (coarse sand or fine sand) on the net energetic cost of burrowing (NCOB) and burrowing rate in two species of the Egernia group of skinks (Liopholis striata and Liopholis inornata) compared with other burrowing animals. We further tested for morphological specialisations among burrowing species by comparing the relationship between body shape and retreat preference in Egernia group skinks. For L. striata and L. inornata, NCOB is 350 times more expensive than the predicted cost of pedestrian terrestrial locomotion. Temperature had a positive effect on burrowing rate for both species, and a negative effect on NCOB for L. striata but not L. inornata. Both NCOB and burrowing rate were independent of substrate type. Burrows constructed by skinks had a smaller cross-sectional area than those constructed by mammals of comparable mass, and NCOB of skinks was lower than that of mammals of similar mass. After accounting for body size, retreat preference was significantly correlated with body shape in Egernia group skinks. Species of Egernia group skinks that use burrows for retreats have narrower bodies and shorter front limbs than other species. We conclude that the morphological specialisations of burrowing skinks allow them to construct relatively narrow burrows, thereby reducing NCOB and the total cost of constructing their burrow retreats.

KEY WORDS: Egernia, Cost of burrowing, Morphometrics, Locomotion energetics, Metabolic rate

INTRODUCTION

Terrestrial locomotion, such as running, is more energetically expensive than flying or swimming for an animal of similar mass (Schmidt-Nielsen, 1972), but considerably less costly than travelling through a dense, cohesive medium such as soil (Vleck, 1979; Seymour et al., 1998). A specialised burrower such as the Namib Desert golden mole (Eremitaalpa grunts namibensis) expends 26 times more energy burrowing through loose sand (80 J m⁻¹) than running (3 J m⁻¹) on the surface of the sand, though the cost of ‘swimming’ through loose sand is less than a tenth of that expended by mammals that tunnel through compact soil (Seymour et al., 1998). A non-specialised burrower such as the spinifex hopping mouse (Notomys alexis), can expend 5000 times more energy burrowing than running (7.1 kJ m⁻¹ compared with 1.2 J m⁻¹; White et al., 2006b). Despite the considerable energetic cost, burrowing has many benefits. These include food storage, access to underground food, a secure micro-environment free from predators and extreme environmental gradients (Robinson and Seely, 1980), nesting (Seymour and Ackerman, 1980), hibernation (Moberly, 1963) and enhanced acoustics to facilitate communication (Bennet-Clark, 1987).

Animals utilise a range of methods to burrow through soil, depending on soil characteristics (density, particle size and moisture content) and body morphology (limbed or limbless). Regardless of the method of burrowing, for animals that live and forage underground (fossorial species), the energy taken up must exceed the energy invested in burrowing. Fossorial mammals have therefore evolved ways to maximise burrowing capacities and efficiency and show convergent features that include reduction of hind-limbs, tail, eyes and external ears (Hefnner and Hefnner, 1990). Compared with mammals, burrowing reptiles tend to show different morphological features, such as limb reduction, body elongation and general size reduction (Wiens and Slingluff, 2001; Navas et al., 2004), strengthening of the cranium (Lee, 1998), shortening of the head and lower rostral angulation (Andrews et al., 1987; Barros et al., 2011), shortening of the tail (Shine and Wall, 2008) and streamlining of scales (Jackson and Reno, 1975). More specialised fossorial reptiles display reduction of both eyes and ears, fixation of the lower eyelid, a greater number of vertebrae and ribs associated with elongation of the body and atrophy of one lung (Bellairs, 1969).

The work required to excavate soil increases exponentially with body diameter, and fossorial animals therefore tend to be smaller than surface dwellers. Burrow size depends on animal morphology; bipedal animals such as birds construct significantly larger burrows than mammals to accommodate space for their beaks and feet to dig (White et al., 1978). Reptiles, however, tend to be more elongate than birds and mammals of a similar mass, so their burrows have a smaller cross-sectional area (White, 2005). Iguanas are more elongated than fossorial mammals of similar mass and construct smaller burrows (Rand and Dugan, 1983). The small cross-sectional area of burrows constructed by reptiles may therefore reduce their cost of burrowing, but this hypothesis remains untested. The hypothesis would be supported if reptiles are found to have lower burrowing costs than birds and mammals of similar mass.

Compared with fully fossorial species, semi-fossorial animals (facultative burrowers) use burrows as retreats and forage at the surface, or construct burrows only for nesting purposes (Seymour and Ackerman, 1980). Semi-fossorial animals construct less complex burrows than fully fossorial species, despite similar cross-sectional area (White, 2005), but their lack of structural adaptations for burrowing may require them to expend more energy per unit of cross-sectional area (White et al., 2006b).
Early work that considered the energetics of burrowing comprised either speculative accounts that featured no direct measurements (Grinnell, 1923; Tryon and Cunningham, 1968; Vaughan, 1974), or considered only basal metabolism and thermoregulatory costs (McNab, 1966). The first studies of burrowing energetics were conducted by Seymour (1973), who measured the metabolic rates of burrowing spadefoot toads (Spea multiplicata; prev. Scaphiopus multiplicatus) and Vleck (1979), who calculated the net energetic cost of burrowing (NCOB: the net energy cost of constructing a unit length of burrow, excluding maintenance costs associated with resting metabolism) of pocket gophers (Thomomys botae). Since then, many studies of burrowing energetics have been conducted on a variety of burrowing mammals (e.g. Seymour et al., 1998; Ebensperger and Bozinovic, 2000a; Withers et al., 2000; White et al., 2006b), and have shown that the ambient air temperature (Luna and Antinucci, 2007) and different substrate characteristics (Luna and Antinucci, 2006; Zelová et al., 2010) can greatly affect the energetic cost of burrowing. For example, the cost of burrowing for mole rats (Bathyergidae) is up to 3.7 times higher when burrowing in dry sand relative to damp sand (Lovegrove, 1989) and burrowing metabolic rate is lowest for tuco-tucos Ctenomys talarum burrowing at ambient temperatures within their thermoneutral zone (Luna and Antinucci, 2007).

To date, there have been no experimental studies of the cost of burrowing in reptiles. Burrowing is an important form of locomotion in reptiles (Lee, 1998) and one-third of Australian skinks are burrowing species that spend most of their lives underground (Wilson and Swan, 2013). The night skink (Liohposis striata) and the desert skink (Liohposis inornata) are nocturnal, semi-fossorial skinks from the genus Liohposis (previously Egerinia; Gardner et al., 2008). In their natural habitat they are important bioturbators that modify the desert environment and produce burrows that provide shelter for other fauna including prickly geckos (Nephrurus levis) and knob-tailed geckos (Acacia binoei) (Wilson and Swan, 2013). The night skink (L. inornata) is more fossorial, and the desert skink (L. striata) is more terrestrial. Some species, because morphology contributes to burrowing efficiency. Hypothesis 1 was tested by comparing the dimensions of burrows constructed by skinks with those constructed by other animals. Hypotheses 1–3 were tested by measuring the effects of soil characteristics and temperature on the burrowing energetics and NCOB for L. striata and L. inornata. Hypothesis 4 was tested by comparing morphometric measurements of L. striata and L. inornata with related skinks of the Egernia group (Gardner et al., 2008), which consists of seven genera (Bellatorias, Corucia, Cyclodromorphus, Egerinia, Liopholis, Lissopleis and Tiliqua), to determine the relationship between size-independent body shape and retreat preferences.

### RESULTS

#### Temperature and sand treatments

The mean ambient air temperatures were 22.6±0.7°C (mean±s.d.) in the 23 and 35°C treatment, respectively. The mean sand temperature at 10 cm depth was lower than the ambient air temperature by 1.0°C (21.6±0.8°C) in the 23°C treatment while in the 35°C treatment, the sand temperature was lower by 3.0°C (32.2±1.3°C). Both temperature treatments showed significant differences between sand and air temperatures (paired *t*-test, *t*6=8.64, *P*<0.001). The mean density of the coarse sand treatment was 1.57±0.02 g cm−3 and mean density of the fine sand treatment was 1.60±0.02 g cm−3 (Student’s *t*-test, *t*6=−3.68, *P*<0.001). Moisture content differed between sand treatments, where the coarse sand treatment contained 3.6±0.4% water and the fine sand treatment contained 5.4±1.4% water (Student’s *t*-test, *t*6=−6.52, *P*<0.001).

#### The effect of temperature and sand type on resting and burrowing metabolic rate

For L. striata, resting metabolic rate (RMR) and digging metabolic rate (DMR) was significantly positively affected by temperature (*t*4=2.23, *P*=0.04; *t*4=2.61, *P*=0.02, respectively). Sand treatments did not have a significant effect on RMR (*t*4=0.33, *P*=0.74, Fig. 1A, filled symbols) or DMR (*t*4=0.50, *P*=0.62, Fig. 1A, open symbols). For L. inornata, RMR was significantly positively affected by temperature (*t*4=4.53, *P*=0.0007), but not significantly affected by sand treatments (*t*4=0.30, *P*=0.77, Fig. 1B, filled symbols). DMR was not affected by temperature (*t*4=1.83, *P*=0.09) or sand treatment (*t*4=0.11, *P*=0.91, Fig. 1B, open symbols).
symbols). There were no significant interactions between temperature and sand type \((P>0.05\) in all cases). Data for both \(L.\ striata\) and \(L.\ inornata\) are compared with resting metabolic rates of other reptiles (White et al., 2006a) and digging metabolic rates of giant burrowing cockroaches \(Macropanesthia\ rhinoceros\) (Xu et al., 2014) in Fig. 2.

**The effect of temperature and sand type on burrowing rate and NCOB**

The ambient air temperature had a positive effect on burrowing rate for both \(L.\ striata\) \((t_{14}=6.45, P<0.0001,\ \text{Fig. 3A})\) and \(L.\ inornata\) \((t_{12}=3.25, P=0.007,\ \text{Fig. 3C})\). Air temperature had a negative effect on the NCOB for \(L.\ striata\) \((t_{14}=-3.97, P=0.001,\ \text{Fig. 3B})\) but not for \(L.\ inornata\) \((t_{12}=-1.25, P=0.24,\ \text{Fig. 3D})\). There was no significant effect of sand treatments on burrowing rate for both species \((L.\ striata, t_{14}=1.88, P=0.08;\ L.\ inornata, t_{12}=0.97, P=0.35)\). NCOB was not significantly affected by sand treatments \((L.\ striata, t_{14}=-1.62, P=0.13;\ L.\ inornata, t_{14}=-0.72, P=0.48)\). Interactions between temperature and sand type were always non-significant \((P>0.05\) in all cases). The NCOB was negatively associated with burrowing rate for \(L.\ striata\) \((t_{16}=-5.13, P<0.0001)\) but not \(L.\ inornata\) \((t_{14}=-1.4, P=0.18)\).

**Allometry of NCOB**

Interspecific comparisons of the NCOB with body mass revealed, on average, that both skink species have lower mean NCOB \((L.\ striata, 316\pm160\ \text{J m}^{-1};\ L.\ inornata, 188\pm58\ \text{J m}^{-1})\) than burrow-constructing mammals of similar mass \((\text{Fig. 4})\), but higher than sand-swimming mammals that burrow through loose dry sand. The NCOB for both skink species was higher than the predicted net cost of pedestrian transport \((\text{NCOT})\) for runners and walkers by over two orders of magnitude \((\text{predicted NCOT of } L.\ striata, 0.91\ \text{J m}^{-1} \text{and } L.\ inornata, 0.54\ \text{J m}^{-1})\).

**Burrow dimensions and allometry**

The mean burrow cross-sectional area constructed by \(L.\ striata\) was \(5.3\pm1.4\ \text{cm}^{2}\) and \(2.6\pm0.7\ \text{cm}^{2}\) for \(L.\ inornata\). The body width of \(L.\ striata\), on average took up 51% of their constructed burrow space. The body width of \(L.\ inornata\) took up 56% of the space of their constructed burrow. Video recordings showed skinks would often turn around halfway along a completed burrow and rest in a C shape, with their head and tail end towards the entrance, taking up all the space in the burrow.

Interspecific comparisons of burrow cross-sectional area \(A_{b}\) with body mass \(M_{b}\) revealed allometric scaling for all animals \((\text{Fig. 5})\). Three specific groups were distinguished from the grouped data; birds \(A_{b}=5.46M_{b}^{0.62}, r^{2}=0.84\), reptiles \(A_{b}=0.46M_{b}^{0.74}, r^{2}=0.99\) and verniforms \(A_{b}=0.39M_{b}^{0.63}, r^{2}=0.99\). Reptiles (excluding tortoises) constructed relatively narrower burrows but still within the variation of the grouped data (Tukey’s HSD, \(P=0.02\)). Verniforms constructed significantly smaller burrows (Tukey’s HSD, \(P<0.0001\) and birds
constructed significantly larger burrows than the overall grouped animals of similar mass (Tukey’s HSD, \( P<0.0001 \)).

**Morphometrics**

Linear discriminant analysis (LDA) revealed distinct separations for all retreat groups [hollow logs/rock crevices, hollow logs/burrows, burrows, vegetation (under scrub, grass or bush) and trees (arboreal), Fig. 6] and Wilk’s lambda for size-corrected residual values revealed nine body morphology variables were significantly different among retreat preferences (\( F_{4,19}=5.26, \ P<0.0001 \): hindfoot length, head length, pelvis height, tail width, body width, head width, upper hind leg length, upper foreleg length, and lower foreleg length (supplementary material Table S2).

The first discriminant function (LD1) reduced total variance by 67% and was positively loaded for body width and head length (loading value >1) while negatively loaded for pelvis height, upper hindleg length and hindfoot length. Tree species were loaded negatively, revealing longer hind leg (upper hindleg length and hindfoot length) and pelvic height, whereas vegetation species were loaded positively, with larger heads and bodies, and smaller limbs (Fig. 6).

The second discriminant function (LD2) accounted for 20% of the total variation (supplementary material Table S2) and loaded positively for lower foreleg length, upper hindleg length and head width, but was negatively loaded for upper foreleg length, tail width and body width (Fig. 6). The second function loaded burrowing species positively, revealing longer upper hindleg, shorter upper foreleg, and narrower body width and tail width. Species living in hollow logs and rock crevices showed the opposite traits, with longer upper foreleg length, wider body width and tail width and shorter upper hindleg and head width.

The value of \( \lambda>1 \) indicates that species traits are more similar than expected under Brownian motion (Cooper et al., 2010; Münkemüller et al., 2012). Our value of \( \lambda \) was high (1.42), and significantly different from zero (likelihood ratio test \( P<0.0001 \)), suggesting that related species are more similar to one another than they are to unrelated species.

**DISCUSSION**

**Metabolic rate**

RMRs of both *L. striata* and *L. inornata* are toward the lower range of measurements of other reptiles at similar temperatures (Fig. 2). *L. striata* and *L. inornata* are both desert-adapted species, and arid...
species are often shown to have lower metabolic rates than related non-arid species (e.g. McNab and Morrison, 1963; Lovegrove, 1986; Lighton et al., 2001; Tieleman et al., 2003). There is, however, no association between field metabolic rate and aridity for free-living reptiles (Nagy et al., 1999) and we are not aware of any broad-scale tests for an association between RMR and aridity in reptiles. Thus, although the low RMRs of *L. striata* and *L. inornata* are consistent with the hypothesis that species from arid environments have low RMR, this hypothesis remains to be verified for reptiles more generally.

DMRs of *L. striata* and *L. inornata* at 23°C are around 24–40% lower than the DMR of similarly sized giant burrowing cockroaches *Macropanesthia rhinoceros* measured at 25°C (Fig. 2). DMR increased with temperature in both *L. striata* (*Q*₁₀=1.28) and *L. inornata* (*Q*₁₀=1.29), revealing weak temperature dependence relative to those observed for physiological traits in other species (values of *Q*₁₀ typically fall between 2 and 3; Withers, 1992). For example, the temperature dependence of DMR of the *Liopholis* species considered in the present study is lower than that of a related habitat generalist, *Liopholis whitii* (Bellamy, 2006), which has a *Q*₁₀ of 1.6 for metabolic rate during activity (Huey and Bennett, 1987).

**Energetic cost of burrowing**

Digging is an energetically demanding process, resulting in skinks expending 350 times more energy moving the same distance than the predicted cost of terrestrial locomotion (Fig. 4). Both *Liopholis* skinks have a similar NCOB to spadefoot toads (*Spea multiplicata*) (Seymour, 1973) and expend less energy per unit distance than a mammal of similar mass (Fig. 4). Although among-species variation in NCOB is positively correlated with body mass, there is considerable variation in the NCOB that is not explained by mass (Fig. 4). Differences in air temperature have been shown to affect the NCOB of ectotherms; as temperature increases, the NCOB of scorpions (*Urodacus yaschenkoi*) decreases (White, 2001), as is also generally the case for *Liopholis* skinks in the present study, although the effect of temperature is not significant for *L. inornata* (Fig. 3). This trend was also not observed in *S. multiplicata* (Seymour, 1973).

NCOB is also dependent on the physical and chemical properties of the substrate through which animals burrow (Collis-George, 1959; Vleck, 1979; Zelová et al., 2010). In the present study, the NCOB was not affected by substrate for either species (Fig. 3B,D). Similarities in the NCOB between coarse and fine sand may be due

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**Fig. 5. Interspecific relationship between burrow cross-sectional area (Aᵦ, cm²) and body mass (Mᵦ, g).** Grouped data (filled circles) include mammals, fish, amphibians and invertebrates, while birds (open circles), reptiles (red circles) and vermiform species (×) were separated. The regression line represents an allometric slope of 0.64 (Aᵦ=1.37Mᵦ₀.⁶₄, *r*²=0.96) for grouped data. Data are from published studies presented in supplementary material Table S4.

**Fig. 6. Linear discriminant function 1 (LD1) and 2 (LD2) for size-corrected residual morphometric dimensions.** Dashed circles represent outer regions of each retreat group and arrows represent loading direction of morphology variables. Retreat preferences and a representative skink for each group represented by the following colours and silhouette image, respectively: burrow retreating (blue, *Liopholis striata*), vegetation retreats (yellow, *Tiliqua scincoides*), hollow logs/rock crevices (green, *Egernia depressa*), hollow logs/burrow retreats (pink, *Bellatorias major*) and trees (red, *Corucia zebrata*). HL, head length; HW, head width; BW, body width; PH, pelvic height; TW, tail width; LFL, lower forelimb; UFL, upper forelimb; UHL, upper hindlimb; FFOOT, forefoot.

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to small differences in density (difference of 0.03 g cm\(^{-3}\)) and moisture content (difference of 1.79%) relative to other studies. The densities of the ‘hard’ and ‘soft’ soil treatments employed by Luna and Antinucci (2006), for example, differed by 0.61 g cm\(^{-3}\), and they found that the NCOB was significantly higher in the ‘hard’ soil treatment compared with the ‘soft’ soil treatment. Similarly, Vleck (1979) used a variety of substrates from fine sand (1.37 g cm\(^{-3}\)) to clay (1.84 g cm\(^{-3}\)) and observed that burrowing energetics were highest in clay substrates and were lowest in gravely sand substrates. The ‘dry’ and ‘damp’ sand treatments utilised by Lovegrove (1989) had moisture contents of 0% and 7.9%, respectively, and resulted in the NCOB being highest when burrowing in dry sand relative to damp sand. Finally, sand-swimming mammals expended less energy to ‘swim’ through loose un-compacted sand than both burrowing mammals and skinks. Since dry loose sand has similar properties to a fluid (Shimada et al., 2009), it is likely that adopting a swimming type of locomotion (e.g. undulatory) in this environment will reduce the energy expenditure.

Burrowing rate

The average burrowing rate of the Liopholis skinks increased at higher air temperature (Fig. 3A,C), as has previously been shown for scorpions (White, 2001). Sand treatment did not significantly affect the burrowing speed in Liopholis skinks; however, other studies have shown that different substrate characteristics affect burrowing speed (Lovegrove, 1989; Luna and Antinucci, 2006; Zelová et al., 2010). The relationship between the energy expenditure during locomotion and locomotory speed has been well documented (Taylor et al., 1970; Full et al., 1990; Seymour et al., 1998). All show a rapid decline in the cost of transport as speed increases. Although total power output increases approximately linearly with speed (Margaria et al., 1963; Heglund et al., 1982), the energy expended to move a given distance decreases non-linearly as speed increases. Liopholis skinks also follow this pattern, where the NCOB decreases with increasing burrowing rate, although the relationship is not significant for L. inornata. Irrespective of the medium travelled through, it seems the cost of transport per unit distance is more efficient at higher speed. However, if an animal travels outside its range of preferred speed without changing gait, then the cost of transport tends to increase (e.g. Hoyt and Taylor, 1981). It is therefore likely that the NCOB will not decrease indefinitely as speed increases, but animals presumably avoid burrowing at speeds that would require the use of anaerobic metabolism (Seymour, 1973) and avoid speeds that are high enough to cause an increase in NCOB. Thus, selecting a burrowing speed that avoids anaerobic metabolism and minimises NCOB allows burrowing animals to tunnel economically.

Adaptations for burrowing in semi-fossorial skinks

The size of a burrow is fundamentally dictated by the body shape and method of burrowing employed by the animal that constructed it. Elongated animals (e.g. marine worms, eels) construct narrower burrows compared with globular animals (e.g. birds) of similar body mass (White, 2005). The advantage of smaller, narrower bodies is that animals can expend less energy excavating burrows, because the energetic cost of burrowing is proportional to the amount of substrate removed (Vleck, 1981). Animals with spherical body shapes are therefore required to undertake more work per unit distance to excavate their wider burrows. Fossorial mammals minimise the cost of burrow construction by developing shorter and stronger limbs (Nevo, 1979) to increase power output and reduce the quantity of soil that must be excavated to burrow a given distance. Fossorial mammals in general are also smaller than semi-fossorial mammals (White, 2003). Fossorial reptiles like lizards and snakes generally have elongated bodies and reduced limbs to reduce energy expenditure during burrowing, and by increasing muscle mass and muscle cross-sectional area longitudinally they are able to do so without sacrificing power (Navas et al., 2004). Both Liopholis skinks examined in the present study constructed smaller burrows than the predicted burrow cross-sectional area, on the basis of their body mass (Fig. 5), but were within the variation observed in the grouped data.

Behavioural adaptations such as utilising a sit-and-wait foraging strategy from the safety of a burrow may allow energy conservation and reduce the risk of predation. Active foraging lizards have daily rates of energy expenditure about 1.3–1.5 times greater than sit-and-wait lizards in the same habitats, however the net food gain is about 1.3–2.1 times greater for foraging lizards (Huey and Pianka, 1981). Both Liopholis skink species use a sit-and-wait strategy where they sit inside the entrance of their burrows and wait for prey that pass by (Pianka and Giles, 1982), although some L. striata are occasionally seen foraging at night. Sociality in subterranean animals has been shown to facilitate the partitioning of burrowing workload among multiple individuals, thereby reducing each individual’s total energy cost (Hansell, 1993; Ebensperger and Bozinovic, 2000b). Sociality allows the construction of complex multi-entrance burrows, which provide protection from predators (Rand and Dugan, 1983) and, for endotherms, provides a greater ability to thermoregulate (Yahav and Buffenstein, 1991; Kauffman et al., 2003). Given their higher individual energy costs, solitary species tend to be more efficient diggers than social species, as compared in two African mole rats (Bathyergidae, Rodentia). Solitary Heliophobius argenteocinereus expend less energy digging over a given distance than communal-living Fukomys mechowii, but F. mechowii shares the workload of burrow construction in a cooperative group (Zelová et al., 2010). Most Egernia group skinks, including the semi-fossorial skinks, show some degree of social communal living among closely related kin (Pianka and Giles, 1982; McAlpin et al., 2011; Fenner et al., 2012), which, in combination with the relatively low NCOB afforded by an elongate body form, may contribute to minimising the individual cost of burrow construction.

Morphological specialisations for burrowing

The relationship between body shape and habitat specialisation has been well studied (Vitt et al., 1997; Thompson and Withers, 2005; Grizante et al., 2012). Some studies have shown no relationship between morphology and habitat preference in lizards (Jakić et al., 1980). Others found anti-predator escape (distance a lizard moved away from potential predator) was correlated with body shape [particularly body width and pelvic dimensions (Schulte et al., 2004)]. Egernia skinks occupy a wide range of habitats (Chapple, 2003) and show diverse body shapes, from very elongated, short-limbed Cyclodomorphus species to the large, heavy built Tiliqua (Wilson and Swan, 2013). Divergence of body shapes in the Egernia skinks relating to retreat preferences seems to be conserved and dependent on phylogeny (λ=1.42) as related species have similar retreat preferences (supplementary material Fig. S1). However, morphological differences between
retreat preferences have been observed at a sub-species level (e.g. cranial differences in *Egernia depressa*; Doughty et al., 2011; Hollenshead, 2011).

LDA revealed distinct separation between rock crevices, hollow logs and burrow retreat groups. Burrowing species have narrower bodies, smaller heads, hindfoot and upper forelimb, and longer upper hindlimbs (Fig. 6). Similar specialisations for burrowing as those observed in the present study for *Egernia* group skinks have also been identified in Australian *Ctenophorus* (Agamidae) and *Varanus* (Varanidae), where burrowing representatives of all three groups have wider heads and longer, narrower bodies than their non-burrowing relatives (Thompson and Withers, 2005; Thompson et al., 2008). There is no consistent association between burrowing and limb dimensions; however, burrowing skinks and varanids have relatively long upper hindlimbs, while burrowing skinks and agamids have relatively short upper forelimbs. Shorter forelimbs for both skinks and agamids may allow them to increase their power output during excavation as they tend to burrow head first with their front limbs. However, the benefits of burrowing with shorter limbs can only be properly examined through biomechanical and kinematic studies.

In conclusion, digging requires a high level of specialisation to ensure that the benefits of burrowing outweigh the cost of a burrowing lifestyle. Locomotion via burrowing has been shown to greatly affect energy expenditure in various animals. The present study has provided the first estimates of the energy expenditure of burrowing in skinks and reveals that the semi-fossorial skinks, *L. striata* and *L. inornata* exhibit specialisation towards a subterranean lifestyle. These features include a relatively low NCOB compared with other tunnel-constructing species, low temperature dependence of metabolic rate during burrowing, construction of narrower burrows than mammals, and exhibition of morphological traits such as narrower bodies and smaller limbs compared with other *Egernia* group species. Future research on the energetic cost of burrowing in specialised fossorial reptiles (e.g. *Lerista, Amphibbasenia* and *Serpentes*) should be compared with the measurements of semi-fossorial reptiles obtained in the present study, to verify whether fossorial species expend less energy on burrow excavation than semi-fossorial reptiles, and establish the causality of why fully fossorial reptiles show convergence towards legless, elongated bodies and lower metabolic rate.

**MATERIALS AND METHODS**

**Animal collection and maintenance**

Six *Liopholis striata* (formerly *Egernia striata* Sternfeld 1919) (mean±s.d. SVL and mass of 93.6±3.4 mm and 27.8±3.7 g, respectively) were collected from the goldfields region of Western Australia (Government of Western Australia Department of Environment and Conservation Licence SF008358) in October 2011. Six *Liopholis inornata* (formerly *Egernia inornata* Rosén 1905) (mean±s.d. SVL and mass of 76.4±3.5 mm and 12.8±1.3 g, respectively) were collected from Big Desert State Forest, Victoria (Victoria Department of Sustainability and Environment Permit 10005993) in October 2011. Skinks were housed individually in white plastic tubs (60×40×26 cm) which contained sand (25–30 mm deep) (Ki-carma*, Ormeau, Australia) in a temperature-controlled room (20±5°C). Each tub was maintained under two linear fluorescent bulbs with a 12 h:12 h light:dark photoperiod cycle; one bulb emitted only visible radiation (Crompton Lighting, Padstow, NSW, Australia) and the other bulb emitted visible, ultraviolet-A (320–400 nm) and ultraviolet-B (290–320 nm) radiation (Repti Glo 10.0, Exo Terra*, Rolf C. Hagen Inc., Montreal, QC, Canada). Skinks were provided with two black plastic refuges: one refuge was situated at one end of the tub directly under a 50 W halogen lamp (Crompton Lighting, Padstow, NSW, Australia) that heated the top of the refuge to 35±2°C, the inside of the refuge to 30±2°C, and the far end of the tub to 25±2°C for 8 h in the middle of the 12 h light cycle; the second refuge was positioned at the other end of the tube away from the halogen lamp with a wet sponge positioned on top to maintain a mean relative humidity of 60% in the tub.

Skinks were given access to water at all times and were maintained on a diet of finely processed raw food that consisted of 50% vegetables (butternut pumpkin, green beans and rocket), 40% meat (turkey mince) and 10% fruit (strawberries). This food mix was supplemented with a reptile-specific multi-vitamin (Herpetivite™, Rep-Cal, Los Gatos, CA, USA) and calcium powder (phosphorus-free calcium with vitamin D₃ Ultrafine, Rep-Cal) at the recommended dose of 15 ml of each supplement per 1 kg of food. *L. striata* were given 2.5 ml and *L. inornata* were given 1.2 ml of this food and supplement mix once or twice a week. Faeces were removed and water was replaced twice a week; the sand was replaced every six months. All skinks were handled in accordance with the Queensland Department of Environment and Resource Management Scientific Purposes Permit WISP10698712 and the University of Queensland Animal Ethics Approval Certificate SBS/288/11/ARC.

**Experimental treatments**

To determine whether temperature and substrate characteristics affect the NCOB, each skink was individually subjected to an environment with two variables: air temperature (23 and 35°C) and substrate characteristics (coarse sand and fine sand), which were presented in a full factorial combination.

**Sand characteristics**

Two grades of commercial sand were used to produce sand treatments that varied in particle size distribution. Washed fine sand (Joint Fill fine graded sand, Cement Australia Pty Ltd, Darra, QLD, Australia) consisting of ~95% silica dioxide sand and ~5% mineral and organic impurities (particle size: 0.06–0.25 mm) and washed coarse sand (Easy mix Tiler’s coarse sand, River Sands Pty Ltd, Carbrook, Australia) consisting of <1% silt (particle size: 0.5–1.5 mm).

Sand bulk density (compacted) was measured as weight of soil per cubic centimetre (g cm⁻³), and the moisture content (%) of sand during burrowing trials was regulated by adding measured amounts of water to dry sand until a standard consistency was achieved (damp enough to be moulded by hand; White, 2001; White et al., 2006b). The coarse sand treatment consisted of a mixture of 70% coarse sand and 30% fine sand, because coarse sand alone
would not bind at all without the addition of some fine sand. The fine sand treatment consisted of 100% fine sand. Soil temperature during burrowing trials was measured 10 cm under the surface of the sand using a type K thermocouple (QM1538 Digitech®, www.jaycar.com.au).

**Respirometry**

Positive pressure flow-through respirometry (Lighthorn, 2008) was used to measure resting metabolic rate (RMR) and digging metabolic rate (DMR) as the rate of CO2 production ($\dot{V}_{CO2}$, ml h$^{-1}$). A gas analyser sub-sampler pump (SS-3, Sable Systems International, Las Vegas, NV, USA) was used to pump outside air scrubbed of CO2 (using soda lime, Chem-Supply, Adelaide, Australia) and water vapour (using Drierite, W. A. Hammond Drierite Co. Ltd, Xenia, OH, USA) to a mass flow controller (GFC17, Adelaide, Australia) and a NIST-traceable bubble film flow meter (1-10-500 ml, Bubble-O-Meter, Dublin, OH, USA). After passing through the mass flow controller, air was pushed through a respirometry chamber, which was a 2 l airtight cabinet (ERI140, ProSciTech, Thuringowa, Australia) that regulated the air temperature at ±1°C. Average burrow dimensions (length, width and height) were measured to the nearest mm with a digital vernier caliper (for $L. inornata$) or to the nearest cm with a tape measure (for $L. striata$). The respirometry chamber was angled at approximately 10 deg, which generally allowed the skinks to burrow at the top, making it easier to measure burrow parameters.

Mean $\dot{V}_{CO2}$ (converted to ml h$^{-1}$) was calculated following Withers (2001): 

$$\dot{V}_{CO2} = \frac{\left(\dot{V}_i \times F_{ECo2}\right) \times F_{CO2}}{1 + ((1/RER) - 1) \times F_{CO2}}$$

where $\dot{V}_{CO2}$ is rate of CO2 production (ml CO2 min$^{-1}$), $\dot{V}_i$ is rate of incident airflow (ml min$^{-1}$), $F_{ECo2}$ is ex-current fraction of CO2 and RER is respiratory exchange ratio, which was assumed to be 0.8. Rate of burrowing energy expenditure (J h$^{-1}$) was calculated by subtracting resting $\dot{V}_{CO2}$ from burrowing $\dot{V}_{CO2}$ and multiplying by the energy equivalent of 1 ml CO2 production (25.6 J; Withers, 1992). The NCOB (J m$^{-3}$) was determined by dividing rate of energy expenditure by burrowing rate (m h$^{-1}$).

Comparative data for NCOB and body mass were compiled from the literature for a variety of vertebrate and invertebrate studies (supplementary material for this study).
material Table S3). Species were grouped taxonomically (mammals, reptiles, amphibians and invertebrates), and the effects of temperature and substrate were examined. Data for NCOB were compared with the predicted scaling relationship for the net cost of pedestrian transport (NCOT) of runners and walkers from Full et al. (1990).

**Burrow dimensions**
The average burrow cross-sectional area ($A_b$, cm$^2$) for *L. striata* and *L. inornata* was calculated based on an ellipse shape: $A_b = \pi \alpha \beta$, where $\alpha$ is half of the burrow width and $\beta$ is half of the burrow height. Additionally, the proportion ($\%$) of body width space taken up within the burrow (burrow width) was compared with the total burrow width. For comparisons between burrow dimensions with other animals, published measurements of burrow cross-sectional area were obtained from White (2005), supplemented with additional studies (supplementary material Table S4). If multiple variables were available, the average burrow dimension was calculated and when body mass was not stated, an appropriate mass was obtained from multiple published sources. Data were log$_{10}$ transformed and classified into the body mass was not stated, an appropriate mass was obtained from multiple published sources. Data were log$_{10}$ transformed and classified into the following groups: grouped data (mammals, fish, amphibians and invertebrates), birds, reptiles, and vermiforms (worm-like shape). Scaling exponents of burrow area with mass ($g$) from each group were calculated by linear regression.

**Morphometrics**
Body proportions of skink species from the *Egernia* group were compared with *L. striata* and *L. inornata*. A digital vernier caliper (Part no: 2351, Kincombe® Australia Pty Ltd., Victoria, Australia) or one metre ruler (for larger specimens) was used to measure 17 body variables to 0.01 mm or length in millimetres (L). In addition, two individual species were analysed using linear mixed effects models in R (2013). An independent student’s t-test was used to test the null hypothesis that sand density and moisture do not differ between treatments and a paired t-test was used to determine if sand temperature was significantly different from the ambient air temperature. The effect of air temperature (23 or 35°C) and substrate characteristics (coarse or fine) on the NCOB and burrowing rate for *L. striata* and *L. inornata* were analysed using linear mixed effects models in the R ‘nlme’ package (Pinheiro et al., 2013) with sand and temperature as fixed effects and individual identity as a random effect to account for repeated measurements of each individual. Two individual *L. striata* were repeated twice in the experiment. Means+s.d. are presented, $\alpha$ was set at 0.05 for all statistical tests.

**Burrow allometry**
Analysis of covariance (ANCOVA) was used to test for differences in burrow cross-sectional area among species [grouped data (mammals, fish, amphibians and invertebrates), birds, reptiles, and vermiforms] with mass as a fixed continuous predictor.

**Morphometrics: linear discriminant analysis**
Linear discriminant analysis (LDA) was used to calculate a set of weightings from body length for each morphology variable using linear models prior to LDA. A forwards stepwise model using greedy.wilks function from the ‘klcr’ package (Weihs et al., 2005) was performed to extract significant variables depending on the Wilk’s lambda criterion. The results were used for the following discriminant function analysis by the lda function from ‘MASS’ package (Venable and Ripley, 2002). Phylogenetic signal was also calculated using LDA scores to determine if related species resemble one another using (Pagel, 1999) lambda ($\lambda$).

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**Competing interests**
The authors declare no competing or financial interests.

**Author contributions**

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**Supplementary material**
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**References**


