Thermal physiology of three sympatric small mammals from southern Africa

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Abstract

Small mammals, and particularly shrews and mice, have relatively high mass-specific metabolic rates and may be constrained to habitats where they can avoid extreme temperatures. Although their phylogeny differs, shrews and rodents often inhabit the same environments and compete for resources due to their similar body masses and dietary overlap. Our aim was to elucidate the variation in thermal parameters of sympatric species. We examined *Myosorex varius, Crocidura flavescens,* and *Mus minutoides*, by measuring metabolic rate, evaporative water loss (EWL), body temperature (T_b), and thermal conductance over a range of ambient temperatures (T_a). Body temperatures of all three species remained above 32°C across the range of T_a. For all species, there was no relation between T_a <35°C and EWL, although EWL in *C. flavescens* was considerably lower compared to the other two species. Dry thermal conductance was much higher in *Mu. minutoides* than in either of the shrews. Resting metabolic rate of all three species declined with T_a < 35°C. The thermoneutral zone of *My. varius* was between T_a = 35°C and T_a = 40°C, whereas that of *C. flavescens* was between T_a = 30°C and T_a = 33°C. No discernible thermoneutral zone was evident for *Mu minutoides*. Our data reveal considerable variation in thermal physiology among three sympatric species of small mammal at a single site and generally conform to the plesiomorphic-apomorphic endothermy model. Differences in body size, life history traits, and evolutionary history may all play a role in the thermoregulatory patterns of these sympatric species.

Key words: Thermal physiology, Myosoricinae, Crocidurinae, Murinae evaporative water loss, metabolic rate, dry thermal conductance

Introduction

Endothermic animals use internal heat production to regulate body temperature (T_b), and fluctuating thermal environments trigger physiological responses (Huey & Bennett, 1990) and/or behavioural modifications (e.g. Cohen, Smale, & Kronfeld-Schor, 2009) to defend a set point T_b. The high surface-area to volume ratio of small endotherms results in high mass-specific rates of heat gain at high ambient temperature (T_a; Kendeigh, 1972). For species that routinely experience T_a approaching or exceeding normothermic body temperature (T_b), evaporative heat dissipation becomes an important factor in thermoregulation (Schmidt-Nielsen & Schmidt-Nielsen, 1950; Van Sant, Oufiero, Muñoz-Garcia, & Hammond, 2012).The rapid heat fluxes between very small endotherms and their environments have driven the evolution of physiological and behavioural avenues for reducing rates of heat gain or loss (Ruf & Geiser, 2015).

Variation in mammalian thermal physiology arises from both phylogenetic history and broad-scale variation in climatic stability of different regions, and this has resulted in distinct taxonomic patterns of normothermic T_b among extant mammals (Lovegrove, 2000, 2012). Indeed, mammalian normothermic T_b varies interspecifically by >10°C, and this variation has important consequences for understanding the evolution of endothermy (Lovegrove, 2012). Lovegrove (2012) proposed three categories of endothermy distinguishable on the basis of resting T_b, basoendothermy (normothermic T_b<35°C), mesoendothermy (normothermic T_b = 35°C - 37.9°C) and supraendothermy (normothermic T_b>37.9°C).

Other mammalian thermoregulatory traits (e.g., basal metabolic rate; BMR) vary predictably among the six biogeographical regions (Lovegrove, 2000), and can vary among even closely-related species with body size, life-history

traits, behaviour, and ambient conditions (Lovegrove & Heldmaier, 1994; Aujard & Vasseur, 2001; Benstaali *et al.*, 2001; Clarke & Rothery, 2008). For instance, two syntopic species of elephant shrews in an arid habitat exhibit divergent patterns of thermoregulation (Boyles *et al.*, 2012). Although such data are scant, observations like those reported by Boyles *et al*. (2012) reiterate the importance of documenting thermoregulatory patterns in the context of understanding how species coexist in time and space.

Due to their similar body mass (M_b) and dietary overlap, shrews and rodents often inhabit the same environments and compete for resources (Gliwicz & Taylor, 2002). Most rodent taxa, including the Murinae*,* are mesoendothermic (Lovegrove, 2012), whereas members of the shrew subfamiles Myosoricinae and Crocidurinae appear to be mesoendothermic bordering on basoendothermic (Dubey *et al.*, 2007; Lovegrove, 2012). However, shrews from the subfamily Crocidurinae typically have lower BMR compared to other subfamilies (Vogel, 1976; Sparti, 1990).

The Eastern Cape province of South Africa is home to the three smallest sympatric mammals found in the country: the pygmy mouse, *Mus minutoides*, (~7 g, the smallest terrestrial mammal in Africa; Skinner & Chimimba, 2005), the forest shrew (~10 g, *Myosorex varius*), and the greater red musk shrew (~45 g, *Crocidura flavescens*). Although all three use nests, *Mu. minutoides* and *My. varius* spend a great deal of time underground in burrows (Bronner, 1992; Burda, Šumbera & Begall, 2007), whereas *C. flavescens* does not burrow extensively (Goulden & Meester, 1978). Burrows are typically buffered from microclimatic fluctuations and the subterranean mammals inhabiting them show more rapid increases in evaporative water loss (EWL), resting metabolic rate and dry thermal conductance (C_{dry}) when exposed to high T_a compared to surface-

dwelling species of similar M_b (McNab, 1966, 1979; Boggs, Kilgore & Birchard, 1984; Reichman & Smith, 1990; Buffenstein & Yahav, 1991; Baldo, Antenucci & Luna, 2015).

Phylogeny and ecological variables may determine thermoregulatory patterns, and it remains largely unclear how and why the thermal physiology of very small mammals varies among syntopic species. We investigated the physiological and behavioural thermoregulation of *Mu. minutoides, My. varius* and *C. flavescens* occurring at a single site. We hypothesised that differences in evolutionary history, phylogeny, and variation in life history traits between these three sympatric species would be reflected in thermoregulatory traits (e.g., normothermic T_b, BMR, EWL and C_{dry}). We predicted that the T_b of the three species would conform to the endothermy classifications of their subfamilies, and that the BMR of *C. flavescens* would be lower than the other two species. We also predicted that the more fossorial species would exhibit higher EWL and C_{dry} rates than *C. flavescens.*

Materials and Methods

Study site and species

The south-eastern coast of the Eastern Cape province of South Africa is a transitional rainfall region with a seasonally and annually variable climate (Lubke & de Moor, 1988). Eight individuals of each species were caught between the Birha and Gusha river mouths along the southeastern coast of the Eastern Cape province of South Africa (33° 23’ S 27° 20’ E). 100 Sherman live traps (H.B. Sherman Traps, Inc.) baited with oats, peanut butter and minced pilchards (ratio 1:1:0.5) were placed in a 20 x 5 trap grid at approximately 10 m intervals. The traps were baited in the late afternoon and checked for the presence of animals

at dawn each morning. This study was part of a larger project that had a 25% successful capture rate over the course of 2 years.

Captured animals were transported to the University of Pretoria and housed in a controlled-temperature room with T_a set to 25°C ± 1°C, relative humidity of 55 %, and a 12L:12D light cycle (light intensity ~ 400 lux), where they were allowed to habituate for two weeks before experiments.

Animals were housed individually in 30 cm x 40 cm x 60 cm plastic containers with a 5-cm layer of sand under wood shavings with shredded toilet paper provided as nesting material. Shrews were fed roughly 75% of their body mass in tinned cat food daily supplemented with mealworms every other day. Mice were fed on a commercially available cockatiel seed mix supplemented with apple slices and mealworms on alternate days. All animals were provided with water *ad libitum*. At the time of experimentation, *My. varius* weighed 10.4 ± 1.5 g, *C. flavescens* 48.3 ± 5.0 g, and *Mu. minutoides* 6.4 ± 0.6 g.

Body temperature measurements

Temperature-sensitive passive integrated transponder (PIT) tags (Destron Fearing, St. Paul, MN 5507) were injected subcutaneously between the scapulae of each individual. The PIT-tags were calibrated before use over a range of temperatures, using a mercury-in-glass thermometer (NIST-traceable accuracy) in a water bath. Individuals were anaesthetized with isofluorane gas before being injected with PIT-tags. PIT-tag data were recorded using a handheld reader (Pocket Reader *EX*, Destron Fearing, St. Paul, MN) modified to allow the placement of a separate antenna adjacent to each respirometry chamber.

While acclimating in the controlled-climate room, subcutaneous T_b data were collected every 15 minutes for 30 hours using the handheld reader.

Disturbance of the animals during this process was kept to a minimum and data from the first 5 hr following PIT tag injection were excluded, which allowed the animals to become habituated to the movement and beeping of the reader above the cages.

Metabolic rate measurements

All gas exchange experiments took place during the diurnal rest phase. Each individual was weighed before being placed into a 500-mL airtight chamber with an air inlet near the bottom and an outlet near the top to maximise air mixing. Air temperature in the chamber was measured using Cu-Cn thermocouples inserted into the chamber through the lids and sealed. Individuals were placed on a piece of plastic mesh placed above a 5-mm layer of mineral oil at the bottom of the chamber to prevent evaporation from faeces and urine. Air was pushed into the chamber at a rate of 500 mL min^{-1}, which ensured that the O_2 concentration in the chambers remained above 20.4 %. Atmospheric air was drawn from outside the laboratory by a pump (model DOA-P13-BN, Gast Air Pumps, Benton Harbour, Michigan, USA) and either partially dried (i.e., 95% of water vapour removed) through a silica gel column, or dried and scrubbed of CO_2 using an adsorption dryer (model K-MT LAB, Zander Aufbereitungstechnik, Essen, Germany). Bev-A-Line tubing (Thermoplastic Processes Inc., Warren, NJ, USA) fed this air through needle valves (Swagelok, Solon OH, USA) that allowed the flow rate to be set. Flow rates were measured using the mass flow meter of an SS-3 Sub-sampler (Sable Systems, Las Vegas NV, USA), regularly calibrated using a soap-bubble flow meter. Two experimental channels plus a baseline channel allowed for alternating measurements of up to two individuals at a time.

The metabolic chambers were placed in a temperature-controlled cabinet (model KMF 720, Binder, Tuttlingen, Germany) at T_a ranging from 5°C to 35°C in 5°C increments in a randomly assigned sequence, with additional readings taken at 32.5°C and 37.5°C. The animals were allowed to settle and habituate in the chamber for 15 minutes before the measurements commenced. Each set of measurements began at 08:00 and lasted approximately 2 hours at each T_a value, with T_b readings taken at 5-min intervals throughout. Individuals were typically exposed to 2 - 3 T_a values (i.e., 4-6 hours) during a day and were not subsequently measured for 5 days. Due to the death of some animals, we only exposed a few *M. minutoides* to 35°C and did not expose them or *My. varius* to 5°C. In total we obtained 138 measurements from *M. minutoides,* 122 from *My. varius,* and 104 from *C. flavescens.*

Sub-sampled air for baseline and experimental channels were pulled through an RH-300 water vapour analyser (Sable Systems) before passing through a CA-10a CO_2 analyser (Sable Systems) and FC-10a oxygen analyser (Sable Systems) to determine fractional H_2O, CO_2 and O_2 concentrations. These analysers were regularly zeroed and spanned following the methods described by Cory Toussaint & McKechnie (2012).

Baseline air was sub-sampled for 3 min at the start of every set of measurements, followed by air from the first animal chamber for 15 min. The baseline air was then sub-sampled for another three minutes before the air from the second animal chamber was sub-sampled for 15 min. This cycle was repeated so that the air from each animal's chamber was sampled three times at each temperature. All data were corrected for lag and drift using the appropriate algorithms in Expedata 1.3.10 software. We used standard equations to

determine the mass-specific metabolic rate for each animal and averages for each species in R (R Core Team, 2014).

Data analysis

We calculated excurrent flow rates using equation 9.3 of Lighton (2008). We then calculated V_{O2} , V_{CO2} , and V_{H2O} using equations 9.4 - 9.6 (Lighton, 2008). Mean resting metabolic rates and EWL rates were calculated from the lowest constant sections of traces of V_{O2}, V_{CO2} and V_{H2O}, and data from periods obviously associated with activity were excluded. Respiratory exchange ratios (RER; V_{CO2} / V_{O2}) were calculated and the thermal equivalence data in Table 4.2 of Withers (1992) used to convert VO_2 exchange measurements to metabolic rates in Watts (W).

Mean dry thermal conductance (C_{dry}; mW °C^{-1} cm^{-2}) was calculated for each species at each temperature using the equation $C_{dry} = \frac{MR-EHL}{(T_b-T_a)SA}$, where MR is the mass specific rate of heat production (mW g^{-1}), EHL is rate of evaporative heat loss (mW g^{-1}), and SA is body surface area (cm^2). We predicted SA for our study species as $SA = 13.2{M_b}^{0.64}$ (Calder, 1984). We also calculated total conductance to facilitate comparisons with values predicted from allometric analyses (Bradley & Deavers, 1980). Since all animals were post-absorptive at the onset of the experiments, the mean minimum RMR of each individual at thermoneutrality (i.e., T_a without regulatory changes in metabolic heat production or evaporative heat loss) was taken to represent BMR.

To test whether T_b, EWL, RMR, and C_{dry} were correlated with T_a while controlling for pseudoreplication caused by multiple recordings from individuals, we used linear mixed-effect models (LME; packages *nlme* 3.1-127, *lme4* 1.1-10 and *MuMIn* 1.15.6 in R version 3.4.2; R Core Team, 2014) with individual as a

random predictor. We also used the regression analysis package *segmented* R (R Core Team, 2014) and a Davies' test to test for a non-constant regression parameters in the linear predictor (i.e., significant change of slope) and determine if the relation between physiological variables and T_a had a distinct break point. If the Davies' test confirmed a breakpoint, we corroborated this by using a general linear model (GLM) to determine if a simple linear model was outperformed by a model with a breakpoint. If the simple linear model was outperformed by a segment linear model, we split the data at the breakpoint and modelled the data above and below the breakpoint separately. In all linear mixed models, P represents the significance of the full model and R^2 represents the total proportion of variance described by explanatory variables and random effects (*sensu* Nakagawa & Schielzeth, 2013). All data are presented as mean ± S.D. and we used $P<0.05$ as our significance threshold.

Results

Body temperature

The three species showed considerable variation in their thermal physiology (Table 1), but conformed approximately to the classic Scholander-Irving model of endothermic homeothermy (Scholander *et al.*, 1950a, 1950b) with T_b regulated around a clear set point T_b (Fig. 1). Between T_a = 10 - 30°C, *My. varius* T_b ranged between 32.8 – 37.0°C and T_b increased gradually with T_a. Between T_a = 30 - 40°C T_b increased sharply to a maximum of 40.5°C. A Davies' test identified a breakpoint at T_a = 31.8°C ($P<0.01$) and an ANOVA suggested that segmented linear regression was better at describing the data than a simple linear regression ($F=8.1$, $df=2$, $P<0.01$). At $T_a>31.8$°C there was a positive correlation

Table 1 Comparisons of body temperature (T_b) at thermoneutrality, evaporative water loss (EWL) at T_a = 25°C, basal metabolic rate (BMR), and average dry thermal conductance (C_{dry}) below thermoneutrality between *Myosorex varius, Crocidura flavescens*, and *Mus minutoides.*

	T_b (°C)	EWL (mg/g/hr)	BMR (mW/g)	C_{dry} (mW/cm^2)
My. varius	35.55 ± 1.0	6.63 ± 1.15	8.03	0.022 ± 0.005
C. flavescens	34.87 ± 0.58	0.83 ± 0.42	6.84	0.006 ± 0.002
Mu. minutoides	36.2 ± 0.63	6.17 ± 2.82	12.33	0.04 ± 0.008

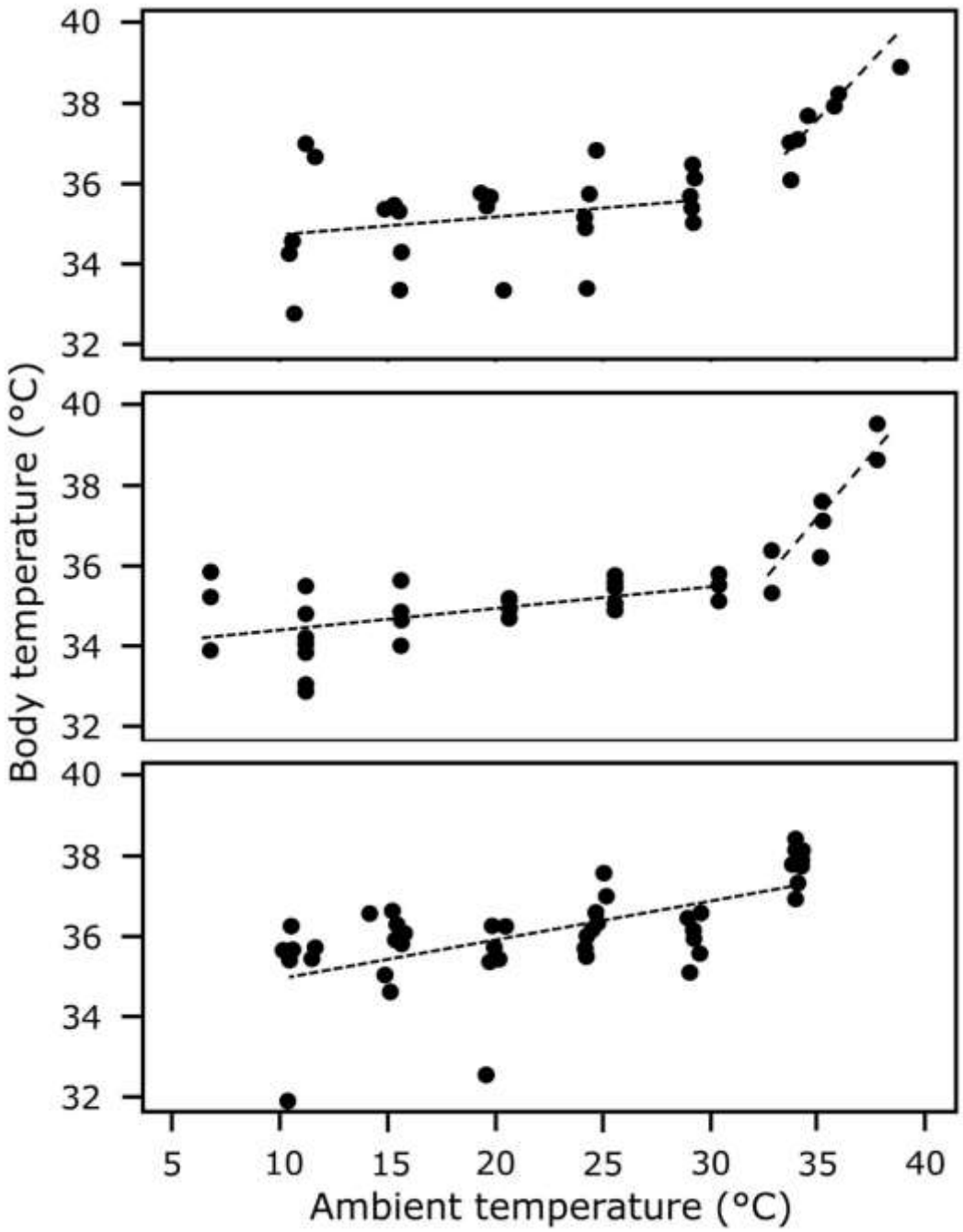

Figure 1 Body temperatures (°C) of *Myosorex varius* (top) *Crocidura flavescens* (middle) and *Mus minutoides* (bottom) when exposed to an ambient temperature range of 5°C to 37.5°C. Break points were determined using a Davies' test. Dotted and dashed lines represent linear regression using linear mixed models. Top: at T_a > 31.8°C there was a positive correlation between T_a and T_b (T=5.38, df=3, P=0.01, R^2=0.79), but not at T_a<31.8°C (T=0.12, df=19, P=0.12, R^2=0.44). Middle: there was a positive correlation between T_a and T_b at T_a>32.4°C (T=4.77, df=3, P=0.02, R^2=0.79), and at T_a <32.4°C (T=3.1, df=19, P<0.01, R^2=0.34). Bottom: the relationship between T_a and T_b was positively correlated (T=5.67, df=34, P<0.01, R^2=0.58).
*The regression line for *Mus minutoides* should be treated with caution as we did not expose individuals to T_a > 35°C and may have missed higher T_b values, which may have changed the shape of the regression.

between T_a and T_b (T=5.38, df=3, P=0.01, R^2=0.79); however, there was no relationship at T_a<31.8°C (T=0.12, df=19, P=0.12, R^2=0.44).

The T_b of *C. flavescens* ranged from 31.2 – 40.5°C. There was a breakpoint at T_a = 32.7°C in the relationship between T_a and T_b for *C. flavescens* (P<0.01) and a GLM revealed that segmented linear regression was better at describing the data than a simple linear regression (F=6.87, df=2, P<0.01). There was a positive correlation between T_a and T_b at T_a>32.7°C (T=4.77, df=3, P=0.02, R^2=0.79), and at T_a <32.4°C (T=3.1, df=19, P<0.01, R^2=0.34).

In *Mu. minutoides*, T_b ranged from 31.8 - 38.4°C and increased linearly with T_a (T=5.67, df=34, P<0.01, R^2=0.58). There was no change in slope (P=0.1), and an ANOVA suggested that segmented linear regression was not better at describing the data than a simple linear regression (F=3.22, df=2, P=0.051). However, it should be noted that, because of the death of some individuals at T_a>35°C we did not expose many of the mice to T_a>35°C. Visual inspections suggests an inflection point between T_a = 30 – 35°C, and it is possible that, had we recorded T_b at T_a>35°C, a break point would have been more apparent.

Resting metabolic rate

Resting metabolic rate for all species declined with increasing T_a until T_a = 35°C (Fig. 2). However, at T_a = 35 - 40°C *My. varius'* RMR remained low (5.17 – 8.64 mW g^{-1}), suggesting this may be its thermoneutral zone. At T_a>35°C, *C. flavescens'* RMR increased suggesting the thermoneutral zone was relatively narrow (T_a = 30 - 33°C). *Mus minutoides'* were not exposed to T_a > 35°C and so we were not able to establish their thermoneutral zone.

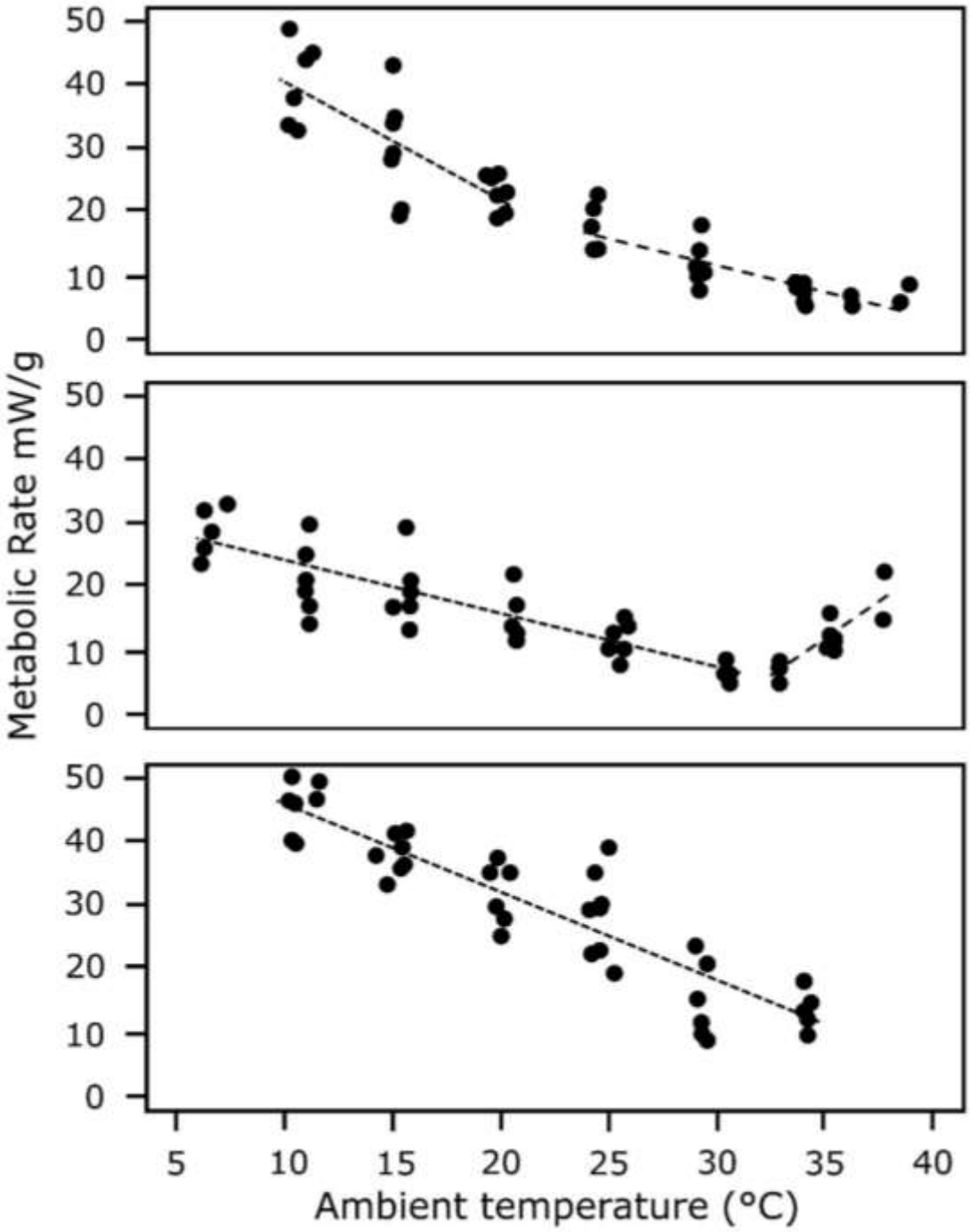

Figure 2 Mass-specific resting metabolic rate metabolic rate (mW g^{-1}) of *Myosorex varius* (top) *Crocidura flavescens* (middle) and *Mus minutoides* (bottom) when exposed to an ambient temperature range of 5°C to 37.5°C. Breakpoints were determined using a Davies' test. Dotted and dashed lines represent linear regression using linear mixed models. Top: At T_a > 22.2°C there was a negative correlation between T_a and RMR (T=-10.94, df=57, P<0.01, R^2=0.69), and at T_a < 22.2°C (T=-7.2, df=46, P<0.01, R^2=0.55). Middle: at T_a>31.8°C there was a negative correlation between T_a and RMR (T=5.99, df=5, P<0.01, R^2=0.8), and there was a negative correlation at T_a < 31.8°C (T=-11.77, df=28, P<0.01, R^2=0.82). Bottom: there was a negative correlation between T_a and RMR (T=-15.5, df=37, P<0.01, R^2=0.84).

For *My. varius,* RMR ranged from 5.17 - 48.8 mW g^{-1} and declined with increasing T_a. There was a breakpoint at T_a = 22.2°C (P<0.01), and a GLM suggested that segmented linear regression fitted the data better than a simple linear regression (F=6.33, df=2, P<0.01). At T_a>22.2°C there was a negative correlation between T_a and RMR (T=-10.94, df=57, P<0.01, R^2=0.69), and at T_a<22.2°C (T=-7.2, df=46, P<0.01, R^2=0.55), though the slopes differed.

Resting metabolic rate for *C. flavescens* ranged from 5.33 - 32.97 mW g^{-1} and declined steadily with increasing T_a until T_a > 35°C when it began to steadily increase. There was a breakpoint at T_a = 31.8°C (P<0.01), and a segmented linear regression provided a better fit than a simple linear regression (F=20.64, df=2, P<0.01). At T_a > 31.8°C there was a positive correlation between T_a and RMR (T=5.99, df=5, P<0.01, R^2=0.8), and there was a negative correlation at T_a < 31.8°C (T=-11.77, df=28, P<0.01, R^2=0.82). The lowest values of RMR occurred at T_a = 30°C - 33°C, suggesting this was the thermoneutral zone

For *Mu. minutoides,* RMR varied between 6.67 and 46.66 mW g^{-1}, RMR declined with increasing T_a, and there was no change in slope (P=0.06) for this relationship (T=-15.5, df=37, P<0.01, R^2=0.84; Fig. 2). The lack of data points at T_a>35°C precluded us from identifying a thermoneutral zone.

Evaporative water loss

For all three species, EWL remained relatively constant at T_a = 10 – 35°C (Fig. 3). However, the ranges of EWL for *My. varius (*4.4 - 11.8 mg g^{-1} hr^{-1}) and *Mu. minutoides* (3.1 - 15.8 mg g^{-1} hr^{-1}) were considerably larger than *C. flavescens* (0.02 – 4.1 mg g^{-1} hr^{-1}).

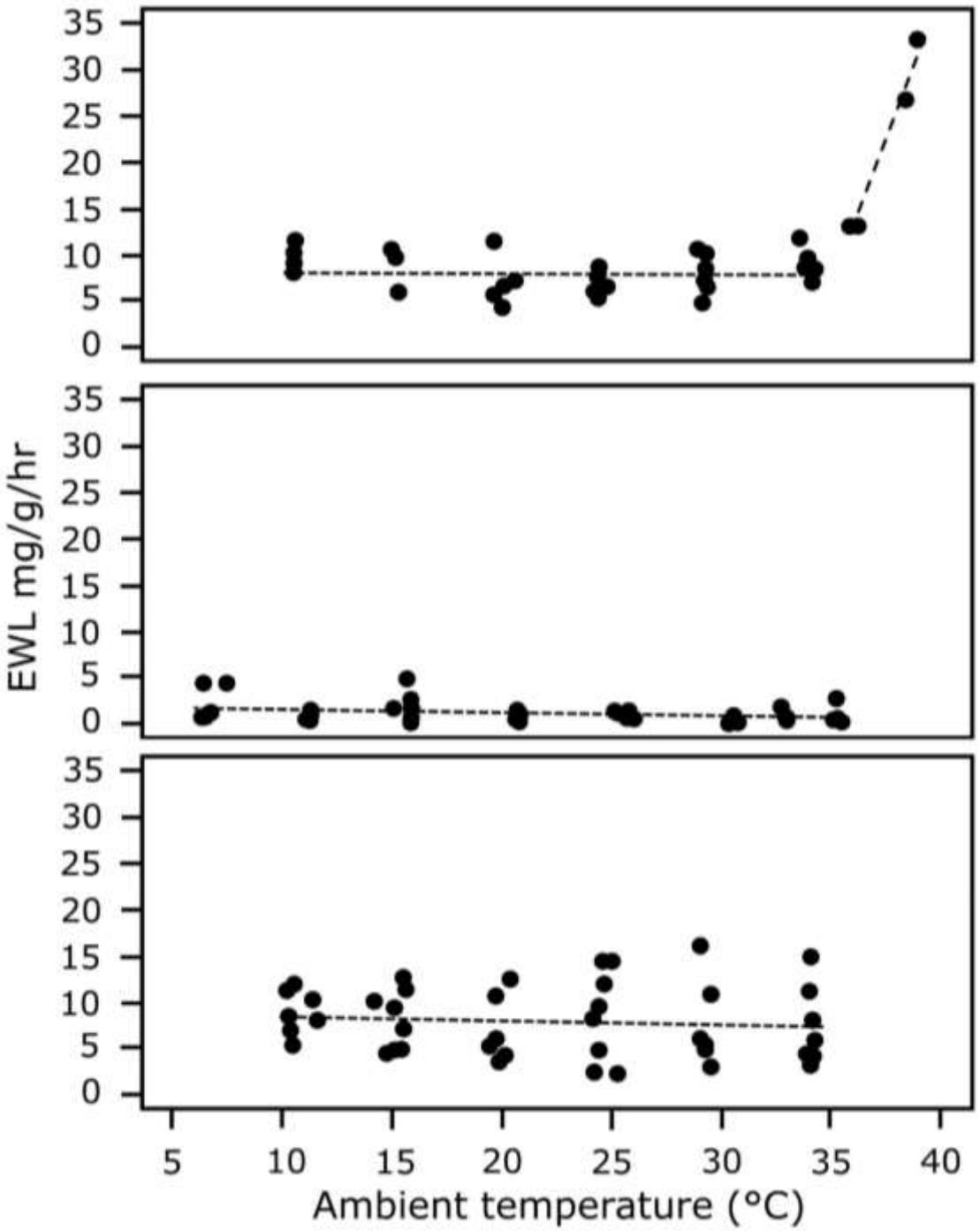

Figure 3 Mass-specific evaporative water loss (mg g^{-1} hr^{-1}) of *Myosorex varius* (top) *Crocidura flavescens* (middle) and *Mus minutoides* (bottom) when exposed to an ambient temperature range of 5°C to 37.5°C. Break points were determined using a Davies' test. Dotted and dashed lines represent linear regression using linear mixed models. Top: at T_a > 35.1°C there was a positive correlation between T_a and EWL (T=10.45, df=2, P=0.01, R^2=0.99), and no correlation at T_a < 35.4°C (T=-0.27, df=23, P=0.79, R^2=0.09). Middle: T_a and EWL were not correlated (T=-1.22, df=97, P=0.22, R^2=0.15). Bottom: T_a and EWL were not correlated (T=-0.79, df=37, P=0.43, R^2=0.44).

In *My. varius,* EWL remained relatively constant until T_a > 35°C, above which it increased rapidly to a maximum of 33.2 mg g^{-1} hr^{-1}. A Davies's test identified a break point at T_a = 35.1°C (P<0.01), and a GLM suggested that segmented linear regression was better at describing the data than a simple linear regression (F=83.6, df=2, P<0.01). At T_a > 35.1°C there was a positive correlation between T_a and EWL (T=10.45, df=2, P=0.01, R^2=0.99), and no correlation at T_a < 35.1°C (T=-0.27, df=23, P=0.79, R^2=0.09).

In contrast, EWL of *C. flavescens* remained consistently low across the full T_a range we investigated. There was no change in the slope (P=0.99), and no correlation between T_a and EWL (T=-1.22, df=97, P=0.22, R^2=0.15). The EWL of *Mu. minutoides* also remained relatively constant at all T_a values. There was no change in slope (P=0.92), a segmented linear regression did not provide a better fit than a simple linear regression (F=0.57, df=2, P=0.57), and T_a and EWL were not significantly correlated (T=-0.79, df=37, P=0.43, R^2=0.44).

Dry thermal conductance

Dry thermal conductance varied substantially among the three study species. At T_a = 10°C - 35°C the mean C_{dry} of *My. varius* was 0.019 ± 0.007 mW cm^{-2}, that of *C. flavescens* was 0.007 ± 0.002 mW cm^{-2}, and that of *Mu. minutoides* was 0.041 ± 0.01 mW cm^{-2}. Moreover, each species exhibited a different pattern with increasing T_a. C_{dry} decreased in *My. varius,* whereas it increased sharply at T_a>30°C for *C. flavescens* and increased more gradually for *Mu. minutoides.*

For *My. varius,* C_{dry} gradually declined, especially at T_a > 30°C. A Davies' test suggested there were two distinct slopes with a breakpoint at T_a = 33.4°C in the relationship between T_a and C_{dry} (P<0.01), and a GLM suggested that segmented linear regression fitted the data better than a simple linear

regression (F=7.2, df=2, P<0.01). However, there was no relationship between T_a and C_{dry} at T_a > 33.4°C (T=-2.2, df=4, P=0.09, R^2=0.49) or T_a < 33.4°C (T=-1.8, df=15, P=0.086, R^2=0.39).

For *C. flavescens,* C_{dry} gradually increased over T_a = 10 - 30°C (0.004 – 0.01 mW cm^{-2}) and then sharply increased to a maximum of 0.03 mW cm^{-2} at T_a = 35°C. There was a breakpoint at T_a = 34.6°C (P<0.01), and a GLM suggested that segmented linear regression fitted the data better than a simple linear regression (F=67.8, df=2, P<0.01). A lack of data at T_a > 34.6°C precluded us from fitting a regression model; however, at T_a < 34.6°C there was a positive correlation for C_{dry} (T=3.5, df=12, P<0.01, R^2=0.5). In *Mu. minutoides,* T_a and C_{dry} were positively correlated (T=2.99, df=32, P<0.01, R^2=0.34), and a Davies' test did not detect a change in slope (P=0.06).

Discussion

The study of thermal physiology affords insight into the natural histories of species and interspecific comparisons may reveal how thermal traits have evolved within and among taxa. In sympatric species, physiological similarities suggest similar evolutionary pressures, whereas divergence may be indicative of adaptation. Consistent with our hypothesis, our data reveal considerable variation in thermal physiology among three sympatric small mammals. Generally, *Mu. minutoides* had the highest values for the physiological variable we studied and *C. flavescens* the lowest. Specifically, we found that our results mirrored the evolutionary relationships of these three species and our results conform to Lovegrove's (2012) classification of endothermy. One limitation of our data set concerns the fact that not all individuals were exposed to T_a>35°C,

and the extent which we can draw direct comparisons between species is thus limited.

Body temperature

Body temperatures of all three species remained relatively high (>32°C) across the range of T_a we investigated, consistent with the pattern expected for classic endothermic homeotherms (Scholander *et al.*, 1950a, 1950b; Bligh & Johnson, 1973). Our results conform with the continuum proposed by Lovegrove (2012), with *Mu. minutoides*' T_b associated with mesoendothermy, *My. varius'* T_b on the low threshold of mesoendothermy, and *C. flavescens* with on the high threshold of basoendothermy (Lovegrove, 2012). Size effects could partially explain this difference as *Mu. minutoides* weighs only ~7 g, compared to ~11 g and ~48 g for *My. varius* and *C. flavescens* respectively. Surface area to volume ratios correlate positively with mass-specific rates of heat loss and heat gain (Kendeigh, 1972), and the linear relationship between T_b and T_a we observed could be due to simple thermodynamics. Phylogeny is another important factor to consider as rodents, in general, are mesoendothermic, whereas crocidurine shrews are at the high end of basoendothermy (Lovegrove, 2012). During the late Micocene (11 – 5 MYA) the earth was cooling, especially during the Messinian (Herbert *et al.*, 2016). The families Crocidurinae and Murinae both diverged in Eurasia during the early Miocene; however, shrews of the genus Crocidura are thought to have colonised Africa approximately 9.3 MYA, whereas members of the genus *Mus* 5 - 6 MYA (Steppan *et al.*, 2004; Dubey *et al.*, 2007; Lecompte *et al.*, 2008). Therefore, crocidurine shrews likely colonized Africa during a more tropical climate than mice and this may have, in part, led to their lower T_b evident today as a result of stabilizing selection and the retention of

thermal traits associated with the group's tropical Asian origin (Lovegrove, 2012).

Metabolic rate

The evolution of mammalian endothermy has resulted in distinct taxonomic patterns of normothermic T_b among extant mammals, arising in part from the past climatic stability of different regions (Lovegrove, 2012). The species we investigated here largely conformed to these broad trends. Physiological traits either within mesoendothermy or bordering on basoendothermy in the two shrews are congruent with this taxon's evolution and distribution in tropical regions (Dubey *et al.*, 2007, Lovegrove, 2012). Small mammals from unpredictable, mesic environments at subtropical latitudes may experience large fluctuations in climatic conditions, and lower BMRs similar to those of tropical and desert-dwelling species likely confer adaptive benefits in terms of energy balance when conditions are extreme. The higher BMR of *Mu. minutoides* reflects the inheritance of mesoendothermy and possibly of being highly apoendothermic as is the case for many murid rodents (Lovegrove, 2012).

Evaporative water loss

In the present study, *Mu. minutoides* and *My. varius* had EWL equivalent to 127% and 186%, respectively, of the predicted values for species in mesic habitats (Cortes *et al.*, 2000). By burrowing, many fossorial and semi-fossorial mammals avoid high T_a and use a microclimate with higher humidity and lower temperatures, which in turn, lowers the gradient for water loss (Reichman & Smith, 1990). *Mus minutoides* and *My. varius* prefer burrows that are likely to confer similar benefits (Bronner, 1992; Burda *et al.*, 2007). This suggests that

many have a limited capacity for evaporative cooling when T_a approaches or exceeds T_b, or that they cannot meet the water requirements for evaporative cooling in their daily water budget (Kellner & Swihart, 2014). The drastic increase in EWL for *My. varius* above 35°C is similar to that of subterranean naked mole rats (*Heteocephalus glaber*) that increase their EWL to nearly 25 x minimal rates at T_a = 30 – 35°C (Buffenstein & Yahav, 1991). Indeed, one *My. varius* increased its EWL nearly 7-fold between T_a = 29°C and 39°C. It is likely that, for *Mu. minutoides* and *My. varius,* the gradient driving EWL is much steeper in the dry air of our experimental set up compared to their natural environments.

Conversely, *C. flavescens* had the lowest EWL, equivalent to just 25% of the predicted value (Cortes *et al.*, 2000). This variation could reflect differences in microhabitat preferences as, unlike *Mu. minutoides* and *My. varius, C. flavescens* prefers rocky shelters and makes use of abandoned nests or hollows (Goulden and Meester, 1978). These rocky nests are likely to be less insulated from environmental conditions than those of *Mu. minutoides* and *My. varius*, which may necessitate greater physiological control over EWL resulting in the low and constant rates of EWL we observed. Indeed, many fossorial mammals show greater temperature-induced increases in EWL compared to surface dwelling mammals (Buffenstein & Yahav, 1991; Baldo *et al.*, 2015). We suggest that future studies should elucidate the differences in the microclimate conditions each species.

Thermal conductance

In mammals and birds, thermal conductance scales negatively with M_b (Aschoff, 1981). Between 10°C and 35°C, *Mu. minutoides* had a mean C_{dry} more than

double that of *My. varius* and nearly 6 x that of *C. flavescens*. The total thermal conductance of *Mu. minutoides* and *My. varius* were equivalent to 115% and 105% of the predicted values (Bradley & Deavers, 1980), while the mean value for *C. flavescens* was equivalent to just 49% of the predicted value. Many fossorial and semi-fossorial species have higher than expected conductance to enhance heat dissipation via non-evaporative avenues (Edwards & Haines, 1978; Reichman & Smith, 1990). It may thus be that the high thermal conductances we recorded for *Mu. minutoides* and *My. varius* arise from a combination of a semi-fossorial lifestyle and small M_b.

Conclusion

Our study shows that aspects of thermal physiology vary among sympatric small mammals. The larger shrew species, *C. flavescens,* exhibited lower T_b, RMR EWL, C_{dry}, than *My. varius,* while the smallest species *Mu. minutoides* showed the highest values except for EWL compared to the two shrews. Because not all individuals were exposed to T_a > 35°C, the slope of the regressions, especially for *Mu. minutoides,* may change and values from T_a > 35°C should not be compared. Our results support Lovegrove (2012)'s endothermy distinctions, with *My. varius* on the lower threshold of mesoendothermy, *C. flavescens* on the border between mesoendothermic and basoendothermic traits, and *Mu. minutoides* displaying traits associated with mesoendothermy.

Acknowledgements

CH is a recipient of a DST-NRF SARChI bursary awarded by NCB. NCB acknowledges support from the DST-NRF SARChI chair for Behavioural Ecology and Physiology to fund the research. We acknowledge permits from the Eastern

Cape Department of Nature Conservation to undertake the research and export the animals as well as the Gauteng Department of Nature Conservation to import animals to the University of Pretoria. We also thank two anonymous reviewers whose comments on an earlier version of this manuscript greatly improved its quality. This work is based on research supported in part by the National Research Foundation of South Africa. The opinions, findings and conclusions are those of the authors alone, and the National Research Foundation accepts no liability whatsoever in this regard.

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