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ORIGINAL ARTICLE

Kidney form and function vary along an aridity gradient in the African striped mouse, genus *Rhabdomys*

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Abstract

Rodents display various adaptations to maintain water balance, involving morphological, physiological and behavioural mechanisms adapted for their habitats. We investigated variation in kidney form and function in four sister species of African striped mice (genus *Rhabdomys*), occurring along an aridity gradient in South Africa. We collected urine from mice housed in metabolic chambers and measured urine osmolarity using a vapor pressure osmometer. Thereafter, mice were euthanised, and their kidneys were removed and stored in buffered formalin. We dissected both kidneys sagittally and calculated size-corrected average renal medullary thickness (RMT). The arid-occurring *R. pumilio* had larger RMT and more concentrated urine than individuals of species from mesic environments, although no association occurred between RMT and urine concentration within all species except for *R. bechuanae,* which had larger RMT but not higher urine osmolarity than mice from the other mesic locations. *R. d. chakae* from Karkloof Nature Reserve (most mesic environment) had the smallest RMT. While kidney RMT and function are under strong selection pressures in arid African striped mice, mesic populations vary in form but not function. Phylogeny appears to modulate relative RMT in the genus *Rhabdomys*, but RMT and urine osmolarity are likely driven by local environmental conditions along the west–east rainfall gradient in South Africa.

Keywords Cryptic rodents · Environmental adaptation · Renal medullary thickness · Urine osmolarity

Introduction

Arid environments constitute a challenge to water conservation for constituent species (Walsberg 2000). High temperatures and limited environmental water availability require animals to reduce the amount of water lost to the environment through metabolic processes (Al-kahtani et al. 2004; Finotti et al. 2018). Water conservation in these water-stressed environments occurs through behavioural and physiological mechanisms such as limiting activity periods to the cooler parts of the day (Tracy and Walsberg 2000), burrowing to avoid the heat (Oufara et al. 1987), lower metabolic rates (Oufara et al. 1987; Tirado et al. 2007) and producing more concentrated urine (Schmidt-Nielsen and O'Dell 1961; Al-kahtani et al. 2004). Other examples of such adaptations include longer large intestines that increase surface area for water reabsorption (Jackson and Spinks 1998), longer descending thin limbs of the loops of Henle (Urity et al. 2012) and increased medullary thickness (Beuchat 1990). Kidney hypertrophy and concomitant urine hyperosmolality frequently occur in desert-adapted mammals, and smaller mammals such as rodents are particularly efficient in their urine concentration ability (Beuchat 1990; Diaz and Ojeda 1999; Al-kahtani et al. 2004; Diaz et al. 2006; Finotti et al. 2018). Conversely, mammals from mesic environments such as grasslands or forests tend to produce comparatively more dilute urine and smaller relative kidney renal medullary thickness (RMT) than xeric species (Beuchat 1996).

The inner and outer medulla of the kidney function as a counter-current multiplier system, allowing for the accumulation of interstitial salt and urea and preserving fluid in the kidneys, thus aiding in the limitation of water lost to the environment (Schmidt-Nielsen and O'Dell 1961). Solutes are actively transported through the loops of Henle (which extend through the cortex and medulla) and reabsorbed by the ascending limb in the outer medulla but

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passively delivered to the inner medulla (Pannabecker 2015). In contrast to mesic-adapted mammals, arid-adapted mammals often have a greater number of long loops of Henle than short loops of Henle, which increases the surface area of the distal limbs and connecting tubules allowing for increased water reabsorption and urine concentrating ability (Beuchat 1996). Thus, the medulla plays a significant role in urine concentrating ability, with a positive correlation between urine concentration and medullary thickness (Schmidt-Nielsen and O'Dell 1961).

As predicted by the relationship between environmental aridity and kidney form and function, mammals distributed across environments varying in aridity differ in kidney form and urine concentrating capacity. For instance, insectivorous bats that occur more strictly in arid environments produced more concentrated urine and had larger kidneys than mesic-occurring species in a water-restriction experiment (Geluso 1978). Similarly, Otomyinae rodents from arid environments (bush Karoo rats *Otomys unisulcatus*, Brants's whistling rat *Parotomys brantsii* and Littledale's whistling rat *P. littledaleii*) had larger kidneys than closely related species (Angoni vlei rat *O. angoniensis* and southern African vlei rat *O. irroratus*) from mesic environments (Pillay et al. 1994). However, this is not always true; species-level comparisons of caviomorph rodents *Thrichomys laurentius* (semi-arid), *T. aff. laurentius* (dry sub-humid) and *T. fosteri* (humid) from regions in Brazil differing in aridity did not differ in urine concentration under laboratory conditions (Carvalhaes et al. 2015). Similarly, intra-specific comparisons of southern mountain cavies *Microcavia australis* did not show variation in renal medullary thickness along an aridity gradient (Tejo Riquelme et al. 2014). Assessments of renal plasticity with water depravation conditions between populations of a species are common, although few studies have considered urine concentrating capabilities in cryptic species complexes occurring in different environments.

The African striped mouse is a small (weighing 30–70 g), diurnal, cryptic species complex belonging to the murid genus *Rhabdomys*, with a widespread distribution in eastern and southern Africa (Monadjem et al. 2015). Within South Africa, *Rhabdomys* taxa are phylogeographically distributed along a west–east aridity gradient, although species are syntopic in parts of the distribution (Ganem et al. 2012; Meynard et al. 2012). Broadly, *R. bechuanae* is presumed to have diverged earliest in the north-central region and occurs in the widest range of climatic conditions (Du Toit et al. 2012; Table 1). *R. pumilio* and *R. dilectus* are sister-clades, having diverged more recently (~2.9 MA), but with vastly different habitat preferences (Du Toit et al. 2012; Table 1). *Rhabdomys dilectus* is comprised of two subspecies, which occur in the mesic north-eastern and south-eastern regions of South Africa, respectively (Meynard et al. 2012).

Rhabdomys is comprised of generalist omnivore species, with specific diets determined by the environments in which they occur (Schradin and Pillay 2005a, b). For instance, *R. pumilio* occurs in environments where rainfall occurs primarily in winter and varies greatly by region, with lower rainfall in the northern range of its distribution (60 mm per annum in the arid areas; Schradin and Pillay 2005b). Its diet consists of insects, seeds, newly emerged plants and wildflowers in the spring, and shrubs and succulents (which serves as the primary source of their water intake) throughout the year (Schradin and Pillay 2005a, b). In contrast, the grassland-occurring *R. dilectus* consume grass seeds and insects in spring and summer, and berries and herbs year round (Schradin 2006). Rainfall is higher (~500–1800 mm per annum) in the east, depending on the region, with the highest rainfall along the eastern coast of South Africa (Reilly and Haskins 1999). Therefore, *R. dilectus* has regular access to free-standing water and better hydrated plants. Yet, little is known about the physiological adaptations of the African striped mouse to the large variation in environmental aridity across its distributional range.

Table 1 Locality information for four taxa of the African striped mouse genus *Rhabdomys*, including the grid position, biome and annual rainfall (mm per annum) for each location. The sample size (n) for each locality is indicated beneath the species name with the number of sexes included for each locality (f = female, m = male)

Species	Locality	Grid position	Biome	Rainfall (per annum)
R. pumilio (n: f = 6; m = 6)	Goegap Nature Reserve, Northern Cape Province	29°40′53″ S, 17°58′9″ E)	Succulent Karoo	160 mm (Schradin and Pillay 2005a, b)
R. bechuanae (n: f = 6; m = 6)	Sandveld Nature Reserve, Free State Province	27°43′ S, 25°5′ E	Kalahari thornveld savanna; Namib desert	500 mm (Fick and Hijmans 2017)
R. d. dilectus (n: f = 6; m = 6)	Sandveld Nature Reserve, Free State Province	27°43′ S, 25°5′ E	Highveld grassland/savanna	500 mm (Fick and Hijmans 2017)
R. d. chakae (n: f = 6; m = 6)	Carletonville, Gauteng Province	26°21′29″ S, 27°23′53″ E	Highveld grassland	470 mm (Fick and Hijmans 2017)
R. d. chakae (n: f = 6; m = 6)	Karkloof Nature Reserve, KwaZulu-Natal Province	29°17′54″ S, 30°13′56″ E	Grassland/mistbelt forest	1023 mm (Gumede et al. 2022)

Our study involves variation in kidney form and function in the genus *Rhabdomys*. The genus is phylogeographically distributed across South Africa's west–east aridity gradient (Du Toit et al. 2012), providing an interesting model for assessing aridity-related variation among its constituent species. We compared mass-corrected renal medullary thickness (RMT) and urine osmolarity in four *Rhabdomys* species, *R. pumilio*, *R. bechuanae, R. dilectus dilectus* and *R. d. chakae*. If the environment in which species occur influences the morphology and function of the kidney, we expected RMT would decrease along the aridity gradient from which subjects originated, with the arid-occurring *R. pumilio* having larger RMT and *R. d. chakae* having smaller RMT, given its presence in a more mesic environment. We also predicted that urine concentration would be greatest in *R. pumilio*, and lowest in *R. d.* chakae.

Materials and methods

Subjects

Wild-caught animals used in the experiments were trapped in 1997 during the wet season in their respective habitats. They were genotyped, representing four taxa from four locations: *R. pumilio* from Goegap Nature Reserve (n = 12: six females and six males) in the Northern Cape Province, *R. bechuanae* (n = 12: six females and six males) and *R. d. dilectus* (n = 12: six females and six males) from Sandveld Nature Reserve in the Free State Province, and *R. d. chakae* from Carletonville (n = 12: six females and six males) in Gauteng Province and Karkloof Nature Reserve (n = 11: five females and six males) in the KwaZulu-Natal Province of South Africa (Table 1; Fig. 1). The wild-caught mice were kept at the Milner Park Animal Unit, located at the University of the Witwatersrand. Experiments were approved by the University of the Witwatersrand Animal Ethics Research Committee (AESC 2012/13/2A).

Husbandry

Striped mice were housed individually in clear lab-o-tec cages (15 cm × 42 cm × 15 cm) for a week prior to experiments taking place, under partially controlled environmental conditions. This included a 14:10 light–dark cycle, with lights on at 05h00, the temperature was kept between 22–24 °C and relative humidity between 30–50%. Cages contained nesting boxes (10 cm × 10 cm × 10 cm) filled with paper towelling, wood shavings on the ground, and dry grass and a wooden chew block. Epol™ mouse cubes and water were available ad libitum throughout the experiment. Additionally, mice were given 5 g of fresh vegetables and one teaspoon of millet daily.

Urine collection

Mice were weighed to ± 0.1 g and then transferred to individual metabolic chambers with mesh floors, which separated

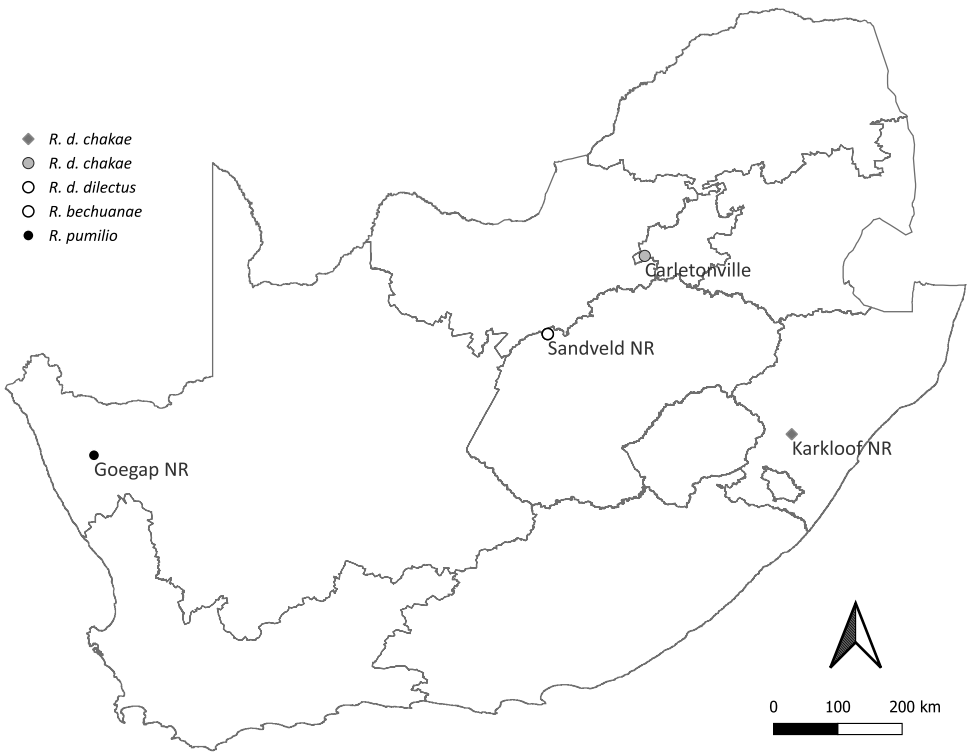

Fig. 1 Map of the origin sites of the populations sampled for four *Rhabdomys* species in South Africa. Populations sampled in this study include *R. pumilio* from Goegap Nature Reserve (n = 6 females, 6 males) in the Northern province, *R. bechuanae* (n = 6 females, 6 males) and *R. dilectus dilectus* (n = 6 females, 6 males) from Sandveld Nature Reserve in the Free State province, and *R. d. chakae* from Carletonville (n = 6 females, 6 males) in the Gauteng province and Karkloof Nature Reserve (n = 5 females, 6 males) in the Kwa-Zulu Natal province

urine and faeces, and housed in these chambers for the duration of the experiment (i.e., two weeks). Urine was collected in 50 ml beakers placed in crushed ice beneath the metabolic chambers for three days. Each day the urine was collected from a beaker with a pipette and analysed using a vapour pressure osmometer (Wescor Vapro 5520) to measure urine osmolarity (mOsm/L). Urine samples were analysed undiluted, and osmolarity of the samples were averaged for each subject.

We need to address the issue of urine measurement units that are either not reported in the literature or reported inconsistently as either osmolality or osmolarity. While the differences between osmolality (mOsm/kg) and osmolarity (mOsm/L) are well known in chemical analysis, it is important to ensure consistency in how urine concentration in rodents is reported. Nonetheless, despite the different units, both methods should generate similar concentrations when measuring osmotically active particles, especially in the broad ranges that show higher and lower urine osmotic concentrations.

Kidney dissection and measurement

Subjects were euthanized using isoflurane inhalation. We dissected out both the right and left kidneys except in mice where one kidney was damaged (one *R. pumilio* female, one male *R. d. dilectus* from Sandveld Nature Reserve, and three males *R. d. chakae* from Carletonville) during dissection. For these subjects, only the intact kidney was dissected (see below). The kidneys were weighed to ± 0.01 g and fixed in 10% buffered formalin and stored for up to 10 days. The kidneys were removed from the formalin in a fume cupboard and blotted dry on tissue paper. Thereafter, the greatest length, breadth, and height of both kidneys were measured to 0.01 mm using dial callipers (NP). Thereafter, the kidneys were sliced using a surgical blade along the midline sagittal plane under a stereoscopic microscope (10x). We then measured the height of the inner and outer medulla and cortex along the midline under a stereoscopic microscope. RMT was calculated as medullary thickness (the sum of inner and outer medulla) multiplied by 10 and divided by the cube root of the product of the length, breadth, and height of the kidney.

Data analysis

All statistical analyses were conducted in RStudio, R version 4.4.1, (R Core Team 2024). Size-corrected residuals were obtained by regressing the average RMT by the mouse's body mass, and size-corrected RMT and urine osmolality were used as response variables. Response variables were tested for normality and heteroscedasticity. While the size-corrected RMT residuals met the requirements for ANOVA/ANCOVA, urine osmolality was non-normal and so was analysed using Kruskal Wallis tests. An ANOVA indicated no significant difference between mass-corrected RMT and right and left kidneys. Therefore, subsequent analyses used averaged left and right kidney data for all individuals, except in the case of individuals where only one kidney was intact.

A linear model was used to analyse size-corrected RMT (response variable) with species/location (i.e., four taxa from five populations: Goegap NR *R. pumilio* = six females, six males; Sandveld NR *R. bechuanae* = six females, six males; Sandveld NR *R. d. dilectus* = six females, six males; Carletonville *R. d. chakae* = six females, six males; Karkloof NR *R. d. chakae* = five females and six males) and sex included as fixed factors using a type 3 two-way ANOVA with the *car* package. A covariate model was created that included urine osmolarity as a covariate in addition to the two fixed factors. A linear regression of size-corrected RMT and urine osmolarity was performed to assess the relationship between kidney RMT and function for each species. Lastly, two Kruskal Wallis tests with urine osmolarity as the response variable were performed and species/populations and sex as the fixed factors in each test. Because urine could not be collected for the *R. d. chakae* from Karkloof Nature Reserve for logistical reasons, only the first model (simple ANOVA of RMT) included data for species from five populations; all other models considered Goegap Nature Reserve (*R. pumilio*), Sandveld Nature Reserve (*R. bechuanae* and *R. d. dilectus*) and Carletonville (*R. d. chakae*) populations only. The same individuals from each locality were used in all models. We analysed the medullary-cortex ratio by population and sex with two Kruskal Wallis tests, but because the model showed similar differences to the full RMT and covariate models and to avoid unnecessary repetition of results, we include the statistical outputs in Table A in Suppl. Materials.

Significant statistical differences were assessed using pairwise comparisons with Tukey adjustments for multiple comparisons in the 'emmeans' package (Lenth 2024) for ANOVAs and Dunn's test for significant results from the Kruskal Wallis tests with Holm-Šidák adjustments for multiple comparisons. All tests were two-tailed and model significance set at 0.05.

Results

Variation in average RMT

Populations ($F_{4,49} = 5.93$, $p < 0.001$), but not sex ($F_{1,49} = 6.96$, $p = 0.408$) and the population*sex interaction ($F_{1,49} = 0.25$, $p = 0.911$), was a significant predictor of mass-corrected RMT. Post hoc comparisons indicated *R. pumilio* individuals from Goegap Nature Reserve had significantly larger RMT values than *R. d. chakae* individuals from Carletonville

and Karkloof and *R. d. dilectus* individuals from Sandveld Nature Reserve (Fig. 2; Table 2). Additionally, *R. d. chakae* individuals from Karkloof had significantly smaller RMT index values than individuals of *R. bechuanae* from Sandveld Nature Reserve (Fig. 2). No other differences reached statistical significance.

Variation in RMT with osmolarity as a covariate

In the model where urine osmolality was included as a covariate (excludes *R. d. chakae* from Karkloof Nature Reserve), population ($F_{3,37}=6.45$, $p=0.001$), but not sex ($F_{1,37}=2.50$, $p=0.122$), the population*sex interaction ($F_{3,37}=0.78$, $p=0.512$) or urine osmolarity ($F_{1,37}=0.10$, $p=0.751$), was a significant predictor of the mass-corrected RMT. Individuals of *R. pumilio* from Goegap Nature Reserve had significantly larger mass-corrected RMT than individuals of *R. d. dilectus* from Sandveld Nature Reserve and *R. d. chakae* from Carletonville (Fig. 2). Mass-corrected RMT was also significantly larger in individuals of *R. bechuanae* from Sandveld Nature Reserve than in individuals of *R. d. dilectus* from Sandveld Nature Reserve and *R. d. chakae* from Carletonville (Fig. 2). No other pairwise comparisons reached statistical significance. Additionally, although we found statistical differences among the species, Levene's tests (species = 0.57, d.f. = 4, 54, $p=0.686$) showed that the differences in means were not accompanied by differences in variability, indicating that the spread of RMT values between species is similar, even though their means differ.

Table 2 Mean ± SE renal medullary thickness (RMT) and average urine osmolality for five populations of the African striped mouse genus *Rhabdomys*. Urine osmolality was not collected for one population (*R. d. chakae* from Karkloof) and is indicated with a hyphen. NR = Nature Reserve

Population	RMT	Urine osmolarity (mOsm/L)
Goegap NR (*R. pumilio*)	6.74 ± 0.23	3741.82 ± 128.81
Sandveld NR (*R. bechuanae*)	5.99 ± 0.15	3367.41 ± 109.86
Sandveld NR (*R. d. dilectus*)	6.47 ± 0.16	3221.42 ± 118.73
Carletonville (*R. d. chakae*)	5.89 ± 0.26	3261.18 ± 60.48
Karkloof NR (*R. d. chakae*)	5.31 ± 0.26	–

Variation in urine osmolarity

Population was a significant predictor of urine osmolarity (KW-$\chi^2=9.42$; d.f. = 3, 37; $p=0.024$). Dunn's test with holm adjustment for multiple comparisons indicated that *R. pumilio* had significantly higher urine osmolality than *R. bechuanae* and *R. d. dilectus* from the Sandveld Nature Reserve and *R. d. chakae* from Carletonville (Fig. 3). No other pairwise comparisons were significantly different. Sex was not a significant predictor of urine osmolarity ($\chi^2=0.09$; d.f. = 1, 37; $p=0.758$). Levene's test (species = 0.94, d.f. = 3, 42, $p=0.430$) indicated similar variability in osmolarity values between species.

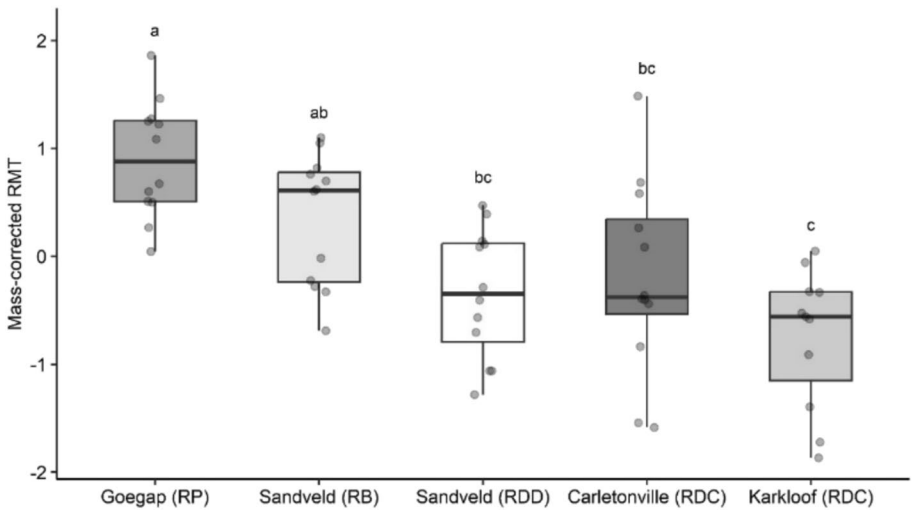

Fig. 2 Mass-corrected renal medullary thickness (RMT) between five populations of four species within the genus *Rhabdomys*. Populations have been plotted following the aridity gradient of their occurrence: from Goegap NR (most arid) to Karkloof (most mesic). Species have been abbreviated as RP = *R. pumilio* (n) = 12 (six females and six males, RB = *R. bechuanae* (n) = 12 (six females and six males, RDD = *R. dilectus dilectus* (n) = 12 (six females and six males) and RDC = *R. dilectus chakae* (Carletonville: n = 12 six females and six males; Karkloof NR: n = 11, five females and six males). Significant differences between groups are indicated with different letters

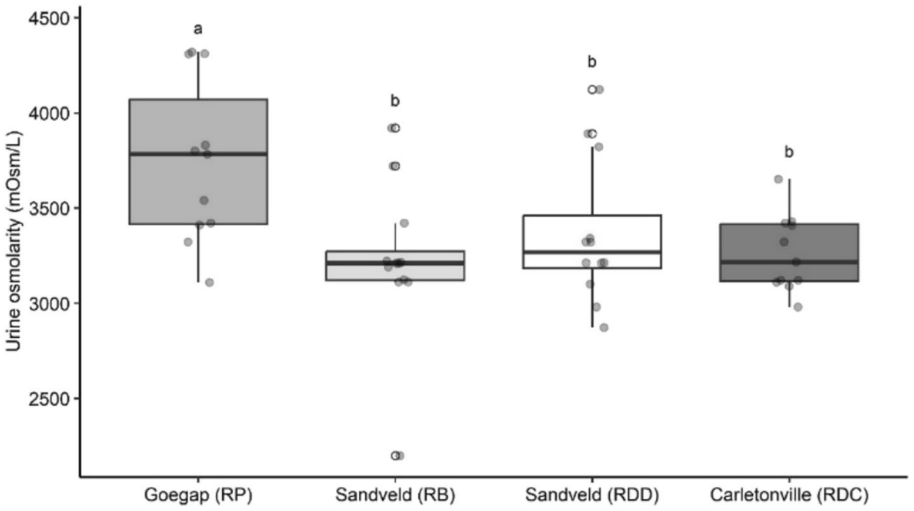

Fig. 3 Urine osmolality of four species within the genus *Rhabdomys*. Populations have been plotted following the aridity gradient of their occurrence: from Goegap NR (most arid) to Karkloof (most mesic). Species have been abbreviated as RP = *R. pumilio*, RB = *R. bechuanae*, RDD = *R. dilectus dilectus* and RDC = *R. dilectus chakae*. For each category plotted, sample size (n) = 12 (six females and six males). Significant differences between groups are indicated with different letters

Relationship between RMT and urine osmolarity

A linear regression between average RMT and urine osmolality indicated a significant but moderate positive association for *R. bechuanae* ($F_{1,10} = 5.38$, $R^2 = 0.350$, $p = 0.043$). There was no significant relationship between mass-corrected RMT and urine osmolality for all other *Rhabdomys* populations (*R. pumilio*: $F_{1,9} = 0.03$, $R^2 = 0.030$, $p = 0.613$; *R. d. dilectus*: $F_{1,10} = 0.99$, $R^2 = 0.090$, $p = 0.342$; *R. d. chakae*: $F_{1,9} = 0.11$, $R^2 = 0.013$, $p = 0.751$).

Discussion

Our results support our predictions of larger average RMTs relative to body weight and more concentrated urine in the arid-dwelling *R. pumilio*. We found that RMT increased with environmental aridity, with *R. pumilio* from Goegap Nature Reserve showing the largest RMT and *R. d. chakae* from Karkloof Nature Reserve (the most mesic environment) showing the smallest RMT. Similarly, urine osmolarity was highest in the arid-dwelling *R. pumilio*, but the more mesic species did not differ from one another. Interestingly, we found no or a weak association between RMT and urine osmolarity in each species.

The arid-dwelling *R. pumilio* experiences long periods of arid conditions in its locality, and its ability to conserve water is crucial, particularly during prolonged dry seasons lasting up to 8 months per annum (Schradin and Pillay 2005a, b). Renal hypertrophy and urine hyperosmolarity are common adaptations among rodents in xeric environments (Beuchat 1990; Rocha et al. 2021). While *R. pumilio* did produce more concentrated urine and had larger RMTs compared to the other species in our study, the differences were not as large as we expected compared to other xeric-occurring species. For example, kangaroo rats (*Dipodomys merriami*; Urity et al. 2012) and the sand rat (*Psammomys obesus*; KaiBling et al. 1975) have larger kidneys or more complex internal structures (e.g., shorter prebend regions, longer tubule lengths, and complex expression of aquaporin channels in different regions in the loops of Henle of kangaroo rats *Dipodomys merriami*; Urity et al. 2012). Strictly desert-adapted rodents produce hyper-concentrated urine in the range of 6340 mOsm/L (*P. obesus*; KaiBling et al. 1975) to 9374 mOsm/L (*Notomys alexis*; MacMillen and Lee 1967), although some desert-occurring species may concentrate urine at lower osmolalities (Beuchat 1996), utilising other behavioural or physiological mechanisms to maintain water balance (e.g., nocturnal or crepuscular activity periods, Tracy and Walsberg 2000; lower metabolic activity, Tirado et al. 2007; reduced evaporative water loss, Cavieres et al. 2024). In contrast, mesic-occurring species such as the prairie vole (*Microtus ochrogaster*) and the meadow vole (*M. pennsylvanicus*) produce urine with osmolarities of 2544 mOsm/L and 1666 mOsm/L, respectively (Heisinger et al. 1973).

The species from the most mesic environment (*R. d. chakae* from Karkloof Nature Reserve) had the smallest RMT of all species. Although we did not record the urine osmolarity for this population, we expect that individuals from this population will produce less concentrated urine than the arid species. We studied *R. d. chakae* occurring in two different locations, occurring approximately 500 km apart with different rainfall levels: Carletonville, which occurs in the highveld grassland biome and receives an average of 470 mm rainfall per annum (Fick and Hijmans 2017), and Karkloof Nature Reserve, which occurs in the Mistbelt Forest biome and receives almost twice as much rainfall (1013 mm per annum; Gumede et al. 2022). We expected that kidney size in highveld grassland *R. d. chakae* would be more similar to the Sandveld populations given the similarity in annual rainfall between the grassland locations (500 mm rainfall per

annum; Du Toit et al. 2012; Fick and Hijmans 2017) than the Karkloof population. Yet, we found no differences in RMT between the *R. d. chakae* populations or between the *R. dilectus* subspecies. This may suggest phylogenetic constraints on the renal morphology in *R. dilectus* despite differences in their local environments, which may have arisen from the evolution of the species in the highveld grasslands (du Toit et al. 2012). Alternatively, the conditions experienced by the species at a micro-habitat level in the drier highveld grassland might mitigate against changes in renal morphology and clearly warrants further study. Similarly, populations of black-tailed tree rat (*Thallomys nigricauda*) from grassland/savanna regions differing in environmental aridity did not differ in either RMT or urine concentration capacity, which the authors speculated might indicate a genetic basis for RMT or that the species utilised behavioural or physiological mechanisms such as altering their activity period or lower metabolic responses to maintain a water balance (Coleman and Downs 2009).

Species occurring within the same environment and experiencing similar annual rainfall should produce urine at a similar concentration and show similar RMTs. However, we did not detect differences in osmolarity between syntopic *R. bechuanae* and *R. d. dilectus*, and the RMT of *R. bechuanae* was similar to that of the arid-occurring *R. pumilio*, and greater than that of *R. d. dilectus*. The two species originated from the Sandveld Nature Reserve, which is located in the savanna biome (in Kalahari Thornveld vegetation, a more arid vegetation type found in central South African savannas) but occupy different micro-habitats. *R. bechuanae* prefers shrubs and open areas, while *R. d. dilectus* prefers covered grasslands (Dufour et al. 2015). A recent study of physiological markers of liver and kidney function suggests that *R. bechuanae* copes better with drier conditions than syntopic *R. d. dilectus* (Keilani et al. unpublished). Despite the lack of urine osmolarity differences between the species, the differences in RMT between *R. bechuanae* and *R. d. dilectus* supports the ecological and physiological differences between the species in syntopy.

The study species did not show the extremes in RMT and urine osmolality reported in other rodent species (Beuchat 1990, 1996), despite occurring in habitats with vastly different vegetation composition and rainfall. In the only other study to consider urine concentration capacity in this genus, Buffenstein (1984) measured urine osmolality of water-deprived *R. pumilio* from Namibia. The mice produced hyper-concentrated urine of 4450 mOsm (full units not reported). The publication does not mention the specific locality in Namibia where the mice originated; much of Namibia is desert, and some parts are as dry as western South Africa, like the habitat of *R. pumilio* in our study. Additionally, based on updated taxonomic changes within the genus (Du Toit et al. 2012), it is likely that Buffenstein (1984) studied *R. bechuanae*, not *R. pumilio*. Despite the lack of taxonomic clarity and location, the study illustrated the extremes of urine concentrating ability in the genus, particularly in arid-occurring species. Nonetheless, it appears that the urine concentration potential in our study and that of Buffenstein (1984) is lower than the concentrations reported for desert/arid rodents in the literature (see above).

Overall, our results indicate that the genus occupies an intermediate position compared to several global rodent species (Beuchat 1990, 1996). Species divergence within the genus is associated with the establishment of the current biomes. *R. bechuanae* is thought to have diverged from a common ancestor earlier than its sister species under moderate rainfall conditions characterized by rainfall of approximately 500 mm per annum (Fick and Hijmans 2017) in central and east South Africa (Du Toit et al. 2012). Therefore, it is possible that the renal morphology and urine concentration ability of the ancestral form have been retained in the genus following range expansion and speciation approximately 4 million years ago. Additionally, *R. bechuanae* is exposed to the most diverse rainfall and temperature conditions across its distribution, occurring in the Namib desert, central savanna and highveld grasslands of South Africa (Du Toit et al. 2012; Ganem et al. 2012). The extremes in habitat types within which species occur likely selected for phenotypic changes.

In comparison to its sister species, our data indicate that *R. pumilio* would face challenges in its arid environment because of its renal structure and function. In support, extreme conditions lead to renal damage and death in *R. pumilio* (Schoepf et al. 2017), particularly in individuals that are in poor condition. The species possibly compensates by obtaining water from succulent plants that sustain it through the driest times (Schradin and Pillay 2005a, b). Additionally, it reduces energy expenditure during the dry season by reducing metabolism and physical activity (Rimbach et al. 2018), which might also decrease water need. Another contributor to osmoregulation is the nonapeptide arginine vasopressin (AVP), which increases water reabsorption induced by dehydration (Yoshimura et al. 2021). A field study of *R. pumilio* found increased plasma AVP levels in the dry season than the wet season (Schoepf et al. 2015). In contrast, the reasons for the renal structure in *R. d. chakae* from Karkloof, the wettest locality in our study, remain unclear, because the species does not experience prolonged dry periods and the vegetation in its habitat should be sufficient to sustain it during dry periods in winter.

Captive rodents can quickly adjust to ad libitum water availability in captivity. For example, species of *Thrichomys* from phylogeographically distinct regions of Brazil did not differ in urine osmolality when water was available ad libitum under laboratory conditions (Carvalhaes et al. 2015). Additionally, white-footed mice (*Peromyscus leucopus*)

from drier South Dakota habitats produced more concentrated urine than mesic conspecifics when water-restricted but similar concentrations when water was available ad libitum (Oswald 1998). The mice in our study were provided with ad libitum water for the few weeks they were housed in captivity. While we found species-level differences that supported our predictions of variations along an aridity gradient, it is also possible that the constant availability of water could have diluted the osmolarity scores, masking the extremes. Water availability in captivity could also explain the lack of association between kidney RMT and osmolarity within species/populations. The arid-occurring species may display plasticity in renal function, producing more concentrated urine under depravation conditions. However, we cannot exclude the possibility that the mesic-occurring species might similarly show some plasticity under water restrictions under natural conditions.

In conclusion, African striped mice species at the extreme ends of an aridity gradient in South Africa showed differences in renal morphology. The arid-dwelling *R. pumilio* had the highest urine osmolarity compared to the other species. In central South Africa, where rainfall levels fall between the extremes found elsewhere in the country, renal morphology differed between the syntopic species, *R. bechuanae* and *R. d. dilectus*. Additionally, a population of *R. d. chakae* occurring in this drier region had renal morphology similar to a population occurring in the most mesic location in the study. These outcomes reveal a complex interplay between environmental and phylogenetic factors in shaping renal structure in the genus. Future studies of the physiology of free-living *Rhabdomys* species under wet and dry weather conditions are needed to assess the adaptive capacity of renal systems in modulating osmoregulation of the different species.

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Author contributions CNN and NP contributed to investigation, formal analysis equally. CNN served as lead for visualisation, and writing – original draft. NP served as lead for conceptualisation, resources, methodology, data curation, funding acquisition, project administration, supervision, and writing – review and editing.

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Data availability The datasets generated during the study are available in the 'figshare' repository, (https://doi.org/https://doi.org/10.6084/m9.figshare.28380158).

Declarations

Conflicts of interest The authors have no relevant financial or non-financial interests to declare.

Compliance with ethical standards This study complies with the ethics standards of the Animal Ethics Research Committee of the University of the Witwatersrand (AESC 2012/13/2A).

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