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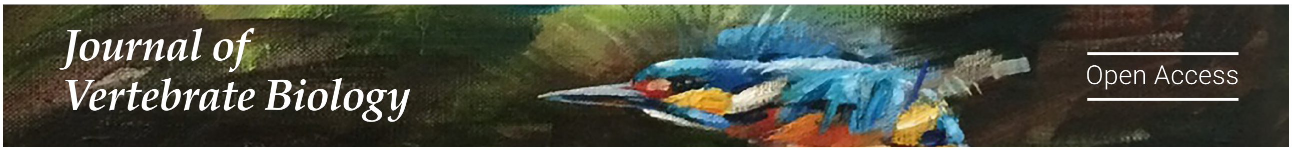

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The effect of elevation on haematocrit in Ethiopian rodents

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Abstract. Key adaptations enabling mammals to cope with oxygen deficiency at high elevations relate to oxygen transfer into the blood. Among others, the efficiency of this mechanism depends on haematocrit (Hct, the volumetric fraction of red blood cells in blood). Although blood of high-elevation mammals is usually characterised by normal or slightly increased Hct, there are contradictory findings from studies along different elevational gradients. The aim of this study was to explore variability of Hct at both inter- and intraspecific levels in six rodent species from lower and higher elevations of Choke Mountain in Ethiopia. We found that *Stenocephalemys* sp. A from higher elevation had higher Hct than its congener *Stenocephalemys albipes* from lower elevation and a similar but weaker tendency was observed intraspecifically in *Lophuromys simensis*. Furthermore, Hct among four species occupying the high-elevation Afroalpine zone was comparable, and higher than in animals from lower elevations. Higher Hct in the three Afroalpine specialists probably contributes to local adaptations for life in high elevation environments under hypobaric hypoxia.

Key words: *Stenocephalemys*, Rodentia, elevational gradient, Ethiopian highlands, blood parameters

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Introduction

Steep elevational gradients have been attracting scientific attention mainly because climatic conditions and habitat characteristics change rapidly as a function of elevation on a relatively small geographic scale. These factors mutually influence survival of any organism living on a gradient. Although relatively consistent and predictable changes in conditions characterise elevational gradients, ecological transitions between successive habitats can be very rapid (Storz et al. 2010). Importantly, such transitions affect less mobile taxa occurring along a gradient, such as rodents, since they have restricted dispersal capabilities. Therefore, rodents are suitable candidates for studying the impact of elevation on various biological adaptations.

In the context of evolutionary physiology, two main selective pressures related to increasing elevation are cold stress and oxygen deficiency. The latter is caused by decreasing barometric pressure, the so-called hypobaric hypoxia (e.g. Barry 2008).

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Therefore, vertebrates living at high elevations have to physiologically compensate for relatively low ambient temperature and its substantial diurnal and seasonal variation, as well as limited access to oxygen (e.g. Tufts et al. 2013). As a result, various adaptations maximising gas exchange and thermogenesis have been commonly found in high-elevation mammals (Withers et al. 2016).

Briefly, key adaptations enabling mammals to cope with hypobaric hypoxia relate to oxygen transfer into the blood. The efficiency of this mechanism depends mainly on the quality and amount of haemoglobin (Hb), Hb-O_2 affinity, the number and size of red blood cells (RBC), and haematocrit (Hct) – the volumetric fraction of RBC in the blood (e.g. Yousef et al. 1972, Lenfant 1973, Monge & León-Velarde 1991, Withers et al. 2016). These parameters mutually influence each other, thus forming relevant trade-offs affecting the efficiency of the whole mechanism. For instance, an increase in Hct results in a linear increase in the O_2 carrying capacity of the blood and also an exponential increase in blood viscosity (Birchard 1997). As a result, while maximal oxygen transport occurs at certain levels of Hct, it decreases at higher and lower Hct due to either reduced oxygen carrying capacity or increased blood viscosity (Birchard 1997). Therefore, the blood of high-elevation species is usually characterised by high Hb-O_2 affinity and normal or slightly increased Hct compared to species/populations living at lower elevations (Monge & León-Velarde 1991, Tufts et al. 2013).

In general, Hct usually ranges between 30 and 50% of RBC in whole blood in mammals (Windberger & Baskurt 2007). Whereas the highest average Hct of 63% was reported for the Weddell seal *Leptonychotes weddellii* (Guard & Murrish 1975), the alpaca *Vicugna pacos* adapted to high-elevation Andean environments has Hct around 27% (Sillau at al. 1976), the lowest mean Hct recorded for any healthy mammal. Although the effect of elevation on the change in Hct is a relatively well-studied phenomenon in some mammals including humans (e.g. Mortola & Wilfong 2017), rodents (apart from laboratory rats and mice) are rather overlooked in this context. Thus, Hct has been sufficiently studied only in a few rodents from different elevations in the South-American Andes (various species – Morrison et al. 1963a, b), the Rocky Mountains in North America (the North American deer mouse *Peromyscus maniculatus* – e.g. Tufts et al. 2013), and the Caucasus Mountains in Eurasia (the wood mouse *Apodemus sylvaticus* – Kalabuchov 1937). However, the findings from different studies are not consistent; for example, increased Hct levels were found in high-elevation deer mice but not in Andean rodents. Surprisingly, there are very limited data on Hct in rodents living along elevational gradients in Africa. Only Broekman et al. (2006) explored respiratory properties in the subterranean Mahali mole-rat *Cryptomys hottentotus mahali* from low and high elevations and found no significant variation in Hct.

The Ethiopian Highlands, reaching up to 4550 m a.s.l. and subdivided into the eastern and the western part by the Great Rift Valley, is the largest mountainous area in Africa. Due to its topography, some of the steepest elevational gradients on the planet can be found there. These gradients are known for extremely high habitat diversity, which in turn harbours diverse (and elevation-specific) assemblages of endemic rodents (e.g. Bryja et al. 2019a). Some rodent genera likely diverged parapatrically along elevational gradients, and different species now live in various habitats from low-elevation forests to high-elevation Afroalpine grasslands, with the genus *Stenocephalemys* being a prime example (Bryja et al. 2018). Due to their dynamic evolutionary history and parapatric distribution of sister species at different elevations of the same mountains (see Bryja et al. 2018), they represent an excellent model for studying the processes of adaptation and speciation along elevational gradients.

The objective of this study is to explore inter- and intraspecific variability of Hct in rodents from the Ethiopian Highlands by measuring this parameter in six rodent species from different elevations around Choke Mountain and surrounding areas of Ethiopia. At both inter- and intraspecific levels, we tested whether species or populations occupying the high-elevated Afroalpine zone differ in Hct compared to those from lower elevations. At the interspecific level, we focused on the genus *Stenocephalemys* where one species is primarily adapted to high and the other to lower elevation. At the intraspecific level we compared populations of *Lophuromys simensis* occupying either lower or higher elevations.

Material and Methods

Animals and collection

For this study, a total of 75 individuals belonging to six different species (the Ethiopian white-footed mouse *Stenocephalemys albipes*, the narrow-headed

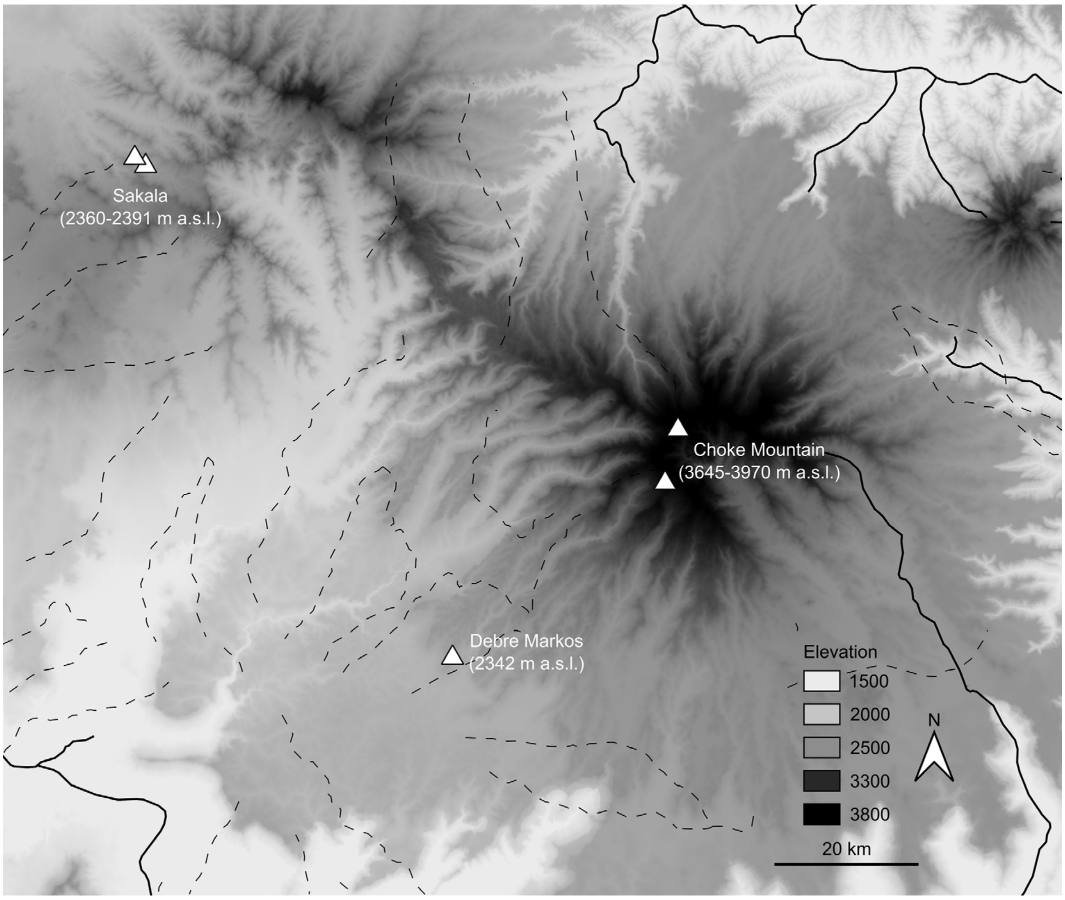

Fig. 1. Map showing higher-elevation sampling sites at Choke Mountain, Ethiopia and lower-elevation sites around Debre Markos and Sakala (black lines denote perennial waters, dashed lines denote non-perennial waters).

rat *Stenocephalemys* sp. A (sensu Lavrenchenko & Verheyen 2006), the Simien brush-furred rat *Lophuromys simensis*, the Abyssinian grass rat *Arvicanthis abyssinicus*, the African root-rat *Tachyoryctes splendens* (clade 2 sensu Šumbera et al. 2018), and the Natal multimammate mouse *Mastomys natalensis*) were captured at three different localities in Ethiopia (Fig. 1, for details of the number of females and males captured for each species or population see Table 1). Murids were captured using Sherman live traps and root-rats with a range of hand-made traps. The higher-elevation locality was close to the peak of Choke Mountain (3645-3970 m a.s.l.; 10.7058° N, 37.8432° E). For comparison, we sampled two lower-elevation localities, near Debre Markos (2342 m a.s.l.; 10.4206° N, 37.5634° E) and at the Sakala area (2360-2391 m a.s.l.; 11.0330° N, 37.1807° E)

Table 1. Haematocrit (Hct) and its coefficient of variation (CV) in six rodent species from higher-elevation sites at Choke Mountain and lower-elevation sites in the Debre Markos and Sakala areas (n_F and n_M denotes to the number of females and males measured, respectively; for *Stenocephalemys* sp. A one individual was not sexed, numbers in parentheses denote range).

Species	Locality (n_F, n_M)	Elevation (m a.s.l.)	Hct (%)	Hct CV
Arvicanthis abyssinicus	Choke Mountain (5, 11)	3733-3965	57.8 ± 5.4 (46.0-68.0)	9.4
Lophuromys simensis	Choke Mountain (9, 9)	3733-3965	59.6 ± 5.0 (51.0-71.0)	8.4
	Debre Markos (3, 4)	2342	57.0 ± 1.9 (55.0-60.0)	3.4
Mastomys natalensis	Debre Markos (n_M = 1)	2342	63.0	-
Stenocephalemys albipes	Choke Mountain (n_M = 1)	3965	70.0	-
	Sakala (4), Debre Markos (4)	2342-2391	54.0 ± 3.0 (50.0-58.0)	5.5
Stenocephalemys sp. A	Choke Mountain (7, 5; 1 NA)	3645-3965	60.2 ± 4.7 (53.0-71.0)	7.8
Tachyoryctes splendens 2	Choke Mountain (7, 3)	3733-3965	57.1 ± 5.0 (50.0-65.0)	8.7

(Fig. 1). The genus *Stenocephalemys* is endemic to Ethiopia and Eritrea where its representatives often comprise the majority of small mammal communities in the highlands (Bryja et al. 2018, Mizerovská et al. 2020). In short, *S. albipes* is the most widespread species of the genus inhabiting a wide elevational range from 800 to 3800 m a.s.l., but its occurrence at high elevations is usually related to human settlements. *Stenocephalemys* sp. A (see Mizerovská et al. 2020, for more details and a formal description of this taxon) is a high-elevation specialist inhabiting only the northern part of the Ethiopian highlands. Similarly, brush-furred rats of the genus *Lophuromys* rank among the most abundant rodents in the Ethiopian highlands. *Lophuromys simensis* is mostly found above 3000 m a.s.l., but records from elevations as low as 1800 m a.s.l. are known (e.g. Lavrenchenko et al. 2004, 2007, Bryja et al. 2019a). *Arvicanthis abyssinicius* is a typical Afroalpine species inhabiting the moorland zone usually above 3500 m a.s.l. (Bryja et al. 2019b). The genus *Tachyoryctes* comprises relatively large highly fossorial rodents. *Mastomys natalensis* is a widespread sub-Saharan rodent living usually in a low-elevation savannah-type habitats. For more details about the genetic diversity and evolutionary relationships among different rodent species of Choke Mountain and surrounding areas see Kostin et al. (2020). Each captured individual was sexed, weighed and body measurements were taken and used for other research purposes.

Blood sampling

From each individual, approximately 130 µL of blood was drawn from the retro-orbital sinus with a heparinised capillary for measurement of Hct (inner diameter 1.5 mm, height 75 mm) immediately after sacrificing by cervical dislocation for other research purposes. Each capillary was sealed and centrifuged using a powered centrifuge at 5000 rpm for 10 min. The blood separated into two distinct phases. The upper transparent phase contained plasma and the dark red lower phase contained packed RBC. The lengths of both phases were measured with a Vernier calliper and served as a measure of their volume; total blood volume was calculated as the sum of the two phases. Haematocrit was calculated as the proportion of the volume of packed RBC to the total blood sample volume. All fieldwork complied with legal regulations in Ethiopia, and sampling was carried out with the permission of the Ethiopian Wildlife Conservation Authority and the Department of Biology of the Debre Markos University and the Environment, Forest and Wildlife Protection and Development Authority of the Amhara National Regional State.

Genotyping

DNA was extracted using commercial kits, and all samples were identified to species by DNA-barcoding using the mitochondrial gene for cytochrome *b* (cyt*b*). The protocol used for PCR and Sanger sequencing is described in Bryja et al. (2014). Obtained sequences were aligned with available datasets from recent cyt*b*-based studies (e.g. Bryja et al. 2018, 2019b, Šumbera et al. 2018). A single specimen of *Mastomys* was identified by the species-specific PCR-typing of cyt*b* using the protocol from Martynov & Lavrenchenko (2018).

Statistical analyses

For each species/population and elevation, we calculated the mean, standard deviation, and coefficient of variation of Hct. Since all Hct values were expressed as proportions, they were arcsine transformed prior to analyses. There was only one individual *M. natalensis* captured at lower elevation (2342 m a.s.l.) and one individual *S. albipes* at higher elevation (3965 m a.s.l.). These two individuals were excluded from all analyses unless specified otherwise. First, we computed a linear model (LM) to test whether Hct differed between males and females for each of the tested species; in *L. simensis* the populations from lower and higher elevations were treated as separate groups. Second, we used LM to test the effect of species on Hct. Thereafter, we used two-tailed t-tests to test a hypothesis of different Hct in species/populations originating from higher and lower elevation. Specifically, we compared two species from the genus *Stenocephalemys* (*S.* sp. A *vs. S. albipes*) and two populations of *L. simensis*. To assess whether the Hct value of a single *S. albipes* from higher elevation differed from Hct characterising the lower-elevation population of this species, the difference was considered statistically significant if the value fell outside the 0.025 and 0.975 quantiles of a normal distribution approximating arcsine-transformed Hct values of the lower-elevation population. All tests were performed using the R statistical software (R Core Team 2019). Throughout the text, means are given ± SD and with ranges in parentheses.

Results

The highest Hct values of 70 and 71% were found in four individuals, two *L. simensis*, one *S. albipes*

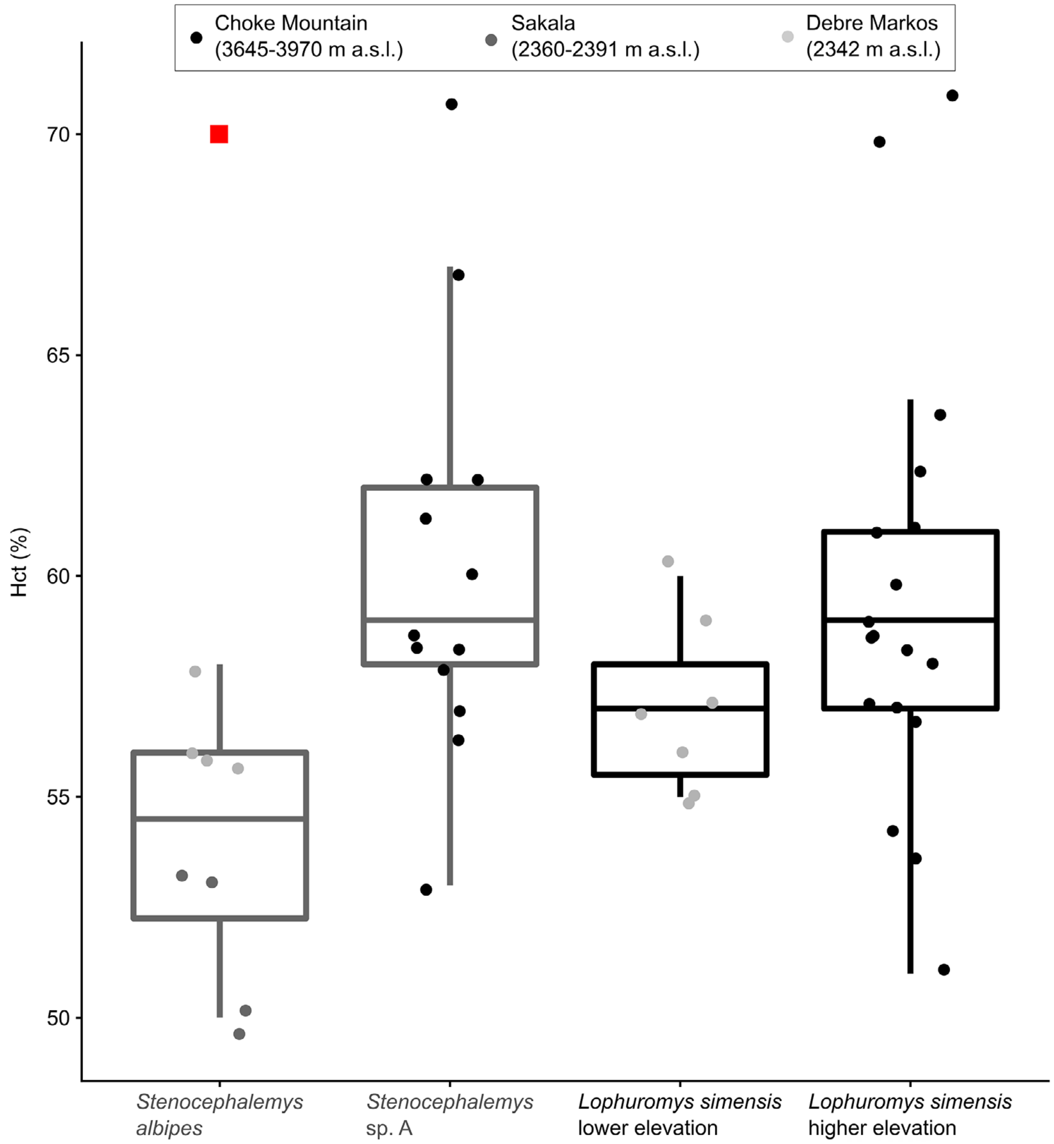

Fig. 2. Haematocrit (Hct) of two *Stenocephalemys* species (*S. albipes* from lower elevation and *S.* sp. A from higher elevation) and populations of *Lophuromys simensis* from both elevations (a red square represents an individual of *S. albipes* captured at the same higher-elevation site as all *S.* sp. A; the midline is the median of the data, with the upper and lower limits of the box being the third and first quartile respectively and the whiskers extend up to 1.5 times the interquartile range from the top/bottom of the box to the furthest data point within that distance; the single specimen of *S. albipes* from higher elevation was not included into the calculation of box-plots).

and one *S.* sp. A, all from higher elevations (3779-3965 m a.s.l.) (Table 1). The lowest Hct of 46% was detected in one individual of *A. abyssinicius* from elevation of 3965 m a.s.l. There was no difference in Hct between males and females in either of the studied species (LMs: *A. abyssinicus*: F = 0.4, *p* = 0.518; *S.* sp. A: F = 0.3, *p* = 0.729; *S. albipes*: F = 0.5, *p* = 0.504; *L. simensis* higher elevation: F = 2.8, *p* = 0.114; *L. simensis* lower elevation: F = 1.6, *p* = 0.265; *T. splendens*: F = 0.5, *p* = 0.491).

The variability of Hct tended to be higher for species (or population in the case of *L. simensis*) from higher elevation (Table 1). The highest Hct variability was found in *A. abyssinicius*, whereas *L. simensis* population from lower elevation showed the lowest variability (Table 1). Although being not significant, Hct tended to differ among the all studied species (LM: $F_{4,\,67}$ = 2.4, *P* = 0.06). The four species occupying the Afroalpine habitats (only the population from the higher elevation was included for *L. simensis*) of Choke Mountain had similar Hct (LM: $F_{3,\,53}$ = 1.0, *P* = 0.395). When we compared Hct in two species of the genus *Stenocephalemys*, *S.* sp. A living at higher elevation had significantly higher Hct than *S. albipes* living at lower elevation (Fig. 2; t-test: t = –3.7, df = 19, *P* = 0.002). The Hct value of the only individual of *S. albipes* from the

Table 2. Haematocrit (Hct) values for rodents occurring at different elevations from different continents. Hct values are given as mean ± SD (Hct of the fossorial African root-rat is presented in Table 3 with other fossorial species).

Continent, country	Species	Elevation (m a.s.l.)	Hct (%)
Africa, Ethiopia[a]	*Arvicanthis abyssinicius*	3733-3965	57.8 ± 5.4
	Lophuromys simensis	3733-3965	59.6 ± 5.0
		2342	57.0 ± 1.9
	Stenocephalemys sp. A	3645-3965	60.2 ± 4.7
	Stenocephalemys albipes	3965	70.0
		2342-2391	54.0 ± 3.0
South America, Chile[b]	*Abrocoma* sp.	450	42.5
	Abrothrix sp.	450	52.2 ± 3.0
	Akodon andinus	4200-4700	51.3 ± 3.5
	Akodon berlepschii	4200-4700	57.1 ± 4.3
	Notiomys	450	42.3
	Octodon sp.	450	48.8 ± 6.0
	Oryzomys sp.	450	54.6
	Phyllotis darwini rupestris	4200-4700	43.0 ± 3.5
	Phyllotis darwini darwini	0	42.9 ± 5.2
South America, Peru[c]	*Akodon amoenus*	3900	54.2 ± 3.0
	Akodon berlepschii	3900	54.4 ± 3.5
	Akodon boliviensis	4540	50.0 ± 2.8
		3900	46.7 ± 1.8
	Akodon jelsikii	4540	48.5
		3900	47.8 ± 2.6
	Hesperomys sorella	4540	42.7 ± 4.8
	Mus musculus	4540	56.5 ± 4.8
		0	46.5 ± 5.0
	Phyllotis darwini posticalis	4540	35.0 ± 3.8
	Phyllotis darwini chilensis	3900	42.3 ± 4.0
	Phyllotis darwini pictus	3900	38.3 ± 2.1
	Phyllotis darwini limatus	0	47.3 ± 6.7
	Rattus rattus	650	41.8 ± 5.7
North America, USA[d]	*Peromyscus maniculatus*	4350	59.7 ± 4.8
		430	48.9 ± 4.8

References: a – this study, b – Morrison et al. 1963a, c – Morrison et al. 1963b, d – Tufts et al. 2013.

higher elevation was above the 97.5% quantile of Hct values characterising the lower-elevation population of *S. albipes*. In *L. simensis*, the animals from higher elevation tended to have higher Hct than the animals from lower elevation (Fig. 2; t-test: $t = 1.9$, $df = 23$, $P = 0.077$).

Discussion

Compared to previously published data, the Hct values recorded in several Ethiopian rodent species in the present study are among the highest recorded for free-living rodents (Table 2) and for mammals generally (Windberger & Baskurt 2007). We found that species occupying Afroalpine habitats close to the peak of Choke Mountain had similar values of Hct (58-60%) which tended to be slightly higher compared to species or populations from lower elevations (*S. albipes* 54% and the lowland population of *L. simensis* 57%). Similar findings were obtained in a study on the North American deer mouse, *Peromyscus maniculatus*, which showed an increase of 11 percentage points in Hct in mice from Evans Mountain (4350 m a.s.l., Hct = 60%)

Table 3. Haematocrit (Hct) for fossorial rodents from various elevations and different continents (Hct values are given as mean ± SD).

Family	Species	Elevation (m a.s.l.)	Hct (%)
Bathyergidae	*Cryptomys h. hottentotus* (South Africa)[a]	150	50 ± 2
	Cryptomys h. mahali (Lesotho)[b]	3200	51 ± 6
	Cryptomys h. mahali (South Africa)[b]	1600	49 ± 5
	Fukomys damarensis (Namibia)[a]	1636	43 ± 4
	Georychus capensis (South Africa)[a]	120	40 ± 6
	Heterocephalus glaber (Kenya)[c]	830	46
Spalacidae	*Tachyoryctes splendens* 2 (Ethiopia)[d]	3733-3965	57 ± 5
	Myospalax baileyi (China)[e]	3200	46 ± 3
	Spalax carmeli (Israel)[f]	100-320*	51
	Spalax galili (Israel)[f]	130-850*	52
	Spalax golani (Israel)[f]	370-1100*	51
	Spalax judaei (Israel)[f]	410-760*	48
Ctenomyidae	*Ctenomys australis* (Argentina)[g]	1*	41 ± 2
	Ctenomys talarum (Argentina)[g]	20*	39 ± 2
	Ctenomys peruanus (Peru)[h]	3900	54
Octodontidae	*Spalacopus cyanus* (Chile)[i]	10*	41 ± 3
Geomyidae	*Thomomys bottae* (USA)[j]	250	46 ± 1
	Thomomys u. melanotis (USA)[j]	3150	47 ± 1

References: a – Weber et al. 2017, b – Broekman et al. 2006, c – Johansen et al. 1976, d – this study, e – Wei et al. 2006, f – Arieli et al. 1986, g – Busch 1987, h – Morrison et al. 1963b, i – Tirado et al. 2019, j – Lechner 1977; * approximate elevation, precise locations are not given.

compared to mice from a low-elevation locality at Nine Mile Prairie, Nebraska (430 m a.s.l., Hct = 49%) (Tufts et al. 2013). The near threefold difference in Hct between our study and that of Tufts et al. may be explained by the much greater elevational difference between low- and high-elevation sites in the latter (3965 *vs.* 2342 m a.s.l.). A gradual increase in Hct with increasing elevation was documented in another study on deer mice (Hammond et al. 1999). However, there may also be other factors that influence Hct levels. For example, in studies conducted on highland and lowland rodents from Peru and Chile the considerable variability in their Hct was not explained by elevation (Hct 47% for highland 3900-4700 m a.s.l. and 46% for lowland 0-650 m a.s.l. species), but less agile species from both elevations (43 and 41% for species from lower and higher elevation, respectively) had lower Hct than their more agile counterparts (51 and 52% for species from lower and higher elevation, respectively) (Morrison et al. 1963b). Although there are only limited behavioural data on most of the African species included in our study, it seems that for example *S. albipes* adapted to semi-arboreal life in various forest types is more agile than its congeners adapted to more terrestrial life in the grassy habitats of the Afroalpine zone (our unpublished data based on open field test observations). Nevertheless, the fact that *S.* sp. A and other species from higher elevation tended to have higher Hct than *S. albipes* from lower elevation implies that the differences in Hct are more likely an effect of elevation than agility.

Stenocephalemys congeners from low and high elevation, together with a single individual of *S. albipes* preferring generally lower elevations but captured in the natural Afroalpine habitat at high elevation give us an opportunity to explore the effect of elevation on Hct in more detail. Our findings showed that highland *S.* sp. A has Hct levels six percentage points higher than its sister species *S. albipes* from lower elevations. In this regard, it is important to mention that many high-elevation specialists have normal or only slightly increased Hct levels, which could be a result of the positive relationship between Hct and blood viscosity (e.g. Birchard 1997, Barve et al. 2016). This is a typical example of the cascade effect when high Hct increases blood viscosity that subsequently reduces oxygen delivery to the tissues. Nevertheless, it might still be possible that the six percentage-point increase in Hct in *S.* sp. A has an adaptive value for life in the Afroalpine environment under hypobaric hypoxia. Yet other adaptations may be helping to counter the hypobaric hypoxia. Among these, increased Hb concentration, higher Hb-O_2 affinity and general adaptations related to hypoxia tolerance (see e.g. Barve et al. 2016) should be considered in future studies in rodents occurring along elevational gradients in the Ethiopian Highlands.

It is a well-known phenomenon that changes in Hct can be environmentally induced. For instance, Tufts et al. (2013) showed that Hct in deer mice native to high elevation decreased from 60 to 50% after six weeks acclimation in normoxia (i.e. 300 m a.s.l.), which was similar to the values of low-elevation natives (50% Hct). This indicates that Hct differences in deer mice are largely attributable to physiological plasticity rather than being genetically fixed (Tufts et al. 2013). A tendency for slightly lower Hct in *L. simensis* from lower elevation might represent such a decrease caused by physiological plasticity in this primarily highland species. An extremely high Hct value of one *S. albipes* (70%; 10 percentage points higher than the mean Hct of high-elevation adapted *S.* sp. A and 16 percentage points higher than its conspecifics from lower elevation) captured at almost 4000 m a.s.l. may represent another case of environmentally induced change in Hct, so-called acclimatization. During this process, individuals of species living at lower elevations usually respond to hypobaric hypoxia by increased Hct (e.g. Ou et al. 1985, Lui et al. 2015). Available data show that Hct increase during acclimatization varied greatly among species. For instance, whereas it was up to 11 percentage points in humans (5600 m a.s.l., Richalet et al. 1994), a 58% increase (from 47.9 to 75.6%) was documented in laboratory rats (acclimated for 28 days to elevation of 6580 m a.s.l., Shertzer & Cascarano 1972), 10 percentage points in deer mice (acclimated for one month to elevation of up to 3801 m a.s.l., Hammond et al. 1999), and 16 percentage points in *S. albipes* (this study). Because of its nature, a typical acclimatization response is temporary and Hct drops back after descending to lower elevation (e.g. Zubieta-Calleja et al. 2007).

The difference in Hct found between the two *Stenocephalemys* species might also indicate existence of genetically based differences in this haematological trait. If the differences in Hct have a genetic background, this might be one of the traits contributing to the ecological speciation suggested in *Stenocephalemys* (Bryja et al. 2018) and the ability of the genus to colonize new and extreme habitats, such as Afroalpine grasslands (see also possible adaptive mutations in one of the oxidative phosphorylation genes (cyt*b*) in this genus in Kostin & Lavrenchenko (2018). Whether the differences in Hct between *S.* sp. A and *S. albipes* are genetically or environmentally based could be clarified by reciprocal-transplant experiments, i.e. those conducted at both low- and high-elevation study sites.

A specific case among rodents is fossorial species as conditions in their burrows are usually hypoxic (Buffenstein 2000). Thus, fossorial rodents from lower elevations might be adapted to equivalent oxygen conditions to those experienced by their non-fossorial counterparts from higher elevations. However, the fossorial rodents studied here do not seem to show any predictable differences in Hct when compared with other rodents (Tables 2 and 3), which corroborates the recent findings of Weber et al. (2017) who did not find any consistent patterns in Hct or intrinsic Hb-O_2 affinity among African mole-rats. Further, when the Hct of lowland and highland populations of the same species are compared, neither pocket gophers *Thomomys bottae* nor common mole-rats *C. h. mahali* show any significant differences (Table 3). It should however be mentioned that the fossorial rodent from the highest elevation analysed, *T. splendens* from Choke Mountain, had the highest Hct of any fossorial rodent measured (Table 3). The effect of elevation on Hct in fossorial rodents is thus unclear and future studies should aim to clarify this issue. Certainly, other mechanisms than Hct may be involved. For instance, highland common mole-rats showed increased Hb concentration compared with lowland animals (Broekman et al. 2006) suggesting that high-elevation hypoxic adaptations might apply to other haematological traits. Likewise, there was a convergence in mechanisms regulating Hb-O_2 affinity between African mole-rats and epigeic (active on the surface) rodents adapted for high elevations (Weber et al. 2017).

Conclusion and future perspectives

The results of the present study provide evidence that the species occupying the high-elevation Afroalpine zone have comparable Hct that is slightly higher than the Hct of animals from lower elevations. The same applies to two congeners of *Stenocephalemys*, as *S.* sp. A living at higher elevation had higher Hct than *S. albipes* from lower elevation. Future research should look at other of the diverse rodent communities along elevational gradients in Africa to establish whether slightly higher Hct might be a general phenomenon promoting the survival of Afroalpine specialists under environmental conditions of lower oxygen availability. It would also be interesting to study the genetic background of the evolution of Hct and Hb-O_2 affinity, the latter trait being universally considered as a key characteristic of hypoxia-tolerant mammals (Withers et al. 2016), using reciprocal transplant experiments. Narrow-headed rats of the genus *Stenocephalemys* would be ideal candidates for this purpose, because parapatric ecological speciation along the elevational gradient has been proposed for them (Bryja et al. 2018).

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