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Thermogenic capacity in subterranean *Ctenomys*: Species-specific role of thermogenic mechanisms

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ABSTRACT

One way to understand ecological patterns of species is to determine their physiological diversity on a large geographic and/or temporal scales, in a context of hierarchical biodiversity framework. In particular, macrophysiological studies analyze how environmental factors affect the physiology and therefore the distribution of species. Subterranean species are an excellent model for evaluating the large-scale effects of ambient temperature (T_a) conditions on thermal physiology and distribution, due to their extensive use of burrows that provide a relatively thermal stable environment. Species belonging to the genus *Ctenomys* are all subterranean and endemic of South America. Cold induced maximum metabolic rate (MMR), basal metabolic rate (BMR) and non shivering thermogenesis (NST) were analyzed, as well as the expression of uncoupled proteins (UCP) in brown adipose tissue (BAT). Biogeographical variables appear to have no effect MMR experimentally induced by cold condition within *Ctenomys*. Also, mechanisms of heat production are species-specific, varying from a combination of ST and NST to a complete use of shivering mechanisms. This pattern is correlated at tissue level, since species that use only ST show a smaller interscapular BAT patch, not detectable presence of UCP1 and low COX activity. Thus, other factors, including body mass, that constrain cold induced MMR could affect thermogenic variability among *Ctenomys*. In the evolutionary timescale, if low O_2 levels of burrows impose a ceiling in cold induced MMR, and ST is enhanced due to species-specific life history traits, such as digging effort, then the observed differences among *Ctenomys* species might be explained.

1. Introduction

Macrophysiology has received vigorous attention in recent years from empirical and theoretical approaches (Chown and Gaston, 2008). Currently, macrophysiology links the exploration and understanding of ecosystems and biodiversity with classical physiological and ecological studies (Chown et al., 2004). Thus, the discipline focuses on physiological diversity over large geographical and/or temporal scales, in the context of a hierarchical framework of biodiversity, and the physiological mechanisms underlying those patterns. In particular, physiological responses to ambient temperature provide an excellent opportunity to learn about how local changes in temperature can affect individual performance, species distribution, trophic interactions within a community, and biodiversity. So, to understand those mechanisms that operate to determine ecological features, we must know the underlying physiological mechanisms (Bernardo et al., 2007; Bernardo and Spotila, 2006; Luna et al., 2009).

It has been assumed that the breadth of thermal tolerance might reflect the energy requirements in a given environment (Scholander et al., 1950). So, thermal physiology might influence ecological and evolutionary success of animals, and represents a valuable tool to understand the effect of these changes on animal´s ecology (*e.g.* species distribution). In the case of endotherms, body temperature (T_b) is maintained constant, and in some extent, decoupled from the direct effect of ambient temperature (T_a) through mechanisms that modify heat production and dissipation (Withers et al., 2016). Such mechanisms are part of a coordinated system that balances heat transfer, typically related to energy metabolism and thermal conductance (Naya et al., 2013).

As T_a decreases, energy expended to ensure constant T_b should increase. Thus, changes in thermogenic capacity and thermal insulation are also important to maintain thermal homeostasis. Particularly,

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thermogenic capacity is determined by shivering (ST) and non-shivering thermogenesis (NST). It has been suggested that ST requires more energy than NST because it involves muscular activity (Janský, 1973), being NST a more plastic element in the machinery of heat production (Cannon and Nedergaard, 2004). Although, NST is associated to brown adipose tissue, which is destined almost exclusively to metabolic heat production (Foster and Frydman, 1979). The activation of brown adipose tissue (BAT) is directly related to the presence of norepinefrine (NE) secreted by sympathetic system. Norepinefrine effect is mediated by the presence of uncoupling proteins in BAT, especially UCP1, which causes a fall in mitochondrial membrane potential (Golozoubova et al., 2006).

In this general framework, inhabiting a relatively thermal stable environment can buffer the effect of T_as on physiological variables. Some mammalian species live in underground burrows that can diminish both daily and seasonal ambient temperature fluctuations. Species belonging to the genus *Ctenomys* are all subterranean and endemic of the southern half of South America. These species are morphologically similar as show adaptations to life underground, but differ noticeably in body mass (from 0.1 to 1 kg, approximately; Medina et al., 2007). Moreover, as species are widely distributed (*i.e.* from 10° S to 55° S and from 0 to 5000 masl), they are exposed to different climates. Unlike other subterranean species, *Ctenomys* species make an extensive use of surface, experiencing T_a variations when they exit underground daily to forage, and during a particular period of the year for reproduction and/or dispersal (Begall et al., 2007). Luna et al. (2009) found that climate does not directly affect basal metabolic rate (BMR) within *Ctenomys* genus, but suggested that BMR might be indirectly determined by climatic variability through a direct effect on maximum metabolism. Furthermore, as thermogenic capacity can be estimated by cold-induced maximal metabolic rate (MMR), any climatic effect on whatever component of thermogenic capacity (ST or NST) can be reflected on MMR or vice versa (Wunder and Gettinger, 1996). Albeit, the specific contribution of ST and NST in one species of *Ctenomys* has already been evaluated (Luna et al., 2012) and the magnitude of contribution of each one may vary in species-specific basis (Almeida and Cruz-Neto, 2011; Van Sant and Hammond, 2008). Therefore, the aim of this study is to compare total thermogenic capacity and its components in several species of the subterranean genus *Ctenomys* that live in a relative thermally stable burrows but are exposed to contrasting T_as on surface. In this context, we are able to evaluate the physiological diversity of energetic and thermal variables within *Ctenomys*. We hypothesized that cold induced MMR and the specific contribution of ST and NST, in relation to body mass, are similar among *Ctenomys* species, as consequence of a similar thermal stability within burrows. As an alternative hypothesis, we proposed that *Ctenomys* species that live in colder areas or at higher altitudes show relatively higher cold induced MMR due to wider variations between above and underground T_as.

2. Materials and methods

2.1. Animals and housing

Animals of *Ctenomys talarum* (from 2 populations; Mar de Cobo and Necochea, Buenos Aires Province), *C. australis* (Necochea, Buenos Aires Province), *C. porteusi* (Daireaux, Buenos Aires Province), *C. tuconax* (Tafi del Valle, Tucumán Province) and *C. roigi* (San Lorenzo, Corrientes Province) were live-trapped at different localities of Argentina (Fig. 1). In Necochea, *C. talarum* and *C. australis* inhabits the same area but microspatially occurs in different soil types. For comparative purposes, all individuals were captured in the Austral spring and summer. By capturing animals in these seasons, a basal level of thermogenic capacity variables were ensured, avoiding any effect of winter acclimatization. After capture, individuals were taken to the laboratory and housed in individual cages (0.30 × 0.40 × 0.25 m) with wood shavings as nesting material in a room controlled for light and temperature (LD 12:12; 25 ± 1 °C). Animals were fed with mixed native grasses, carrots, lettuce, corn, alfalfa and sunflower seeds *ad lib*. Water was not provided, as these species do not drink free water. In all cases, individuals were acclimated for 7–10 days before experimental trials. Based on cortisol levels during the first days of captivity, Vera et al. (2011) showed that by day 10 animals completely acclimate to laboratory conditions.

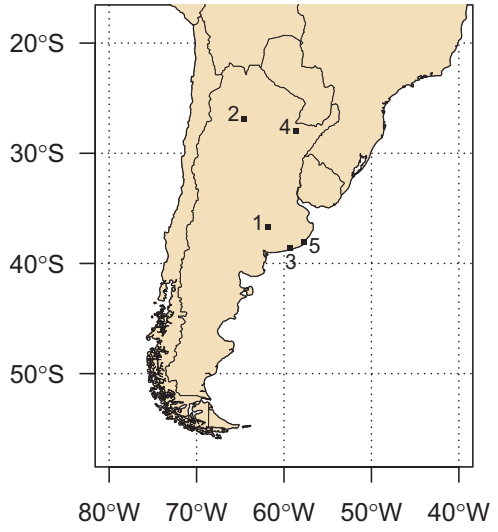

Fig. 1. Geographic distribution of *Ctenomys* species capture sites. 1. Daireaux [*C. porteusi* (n = 5), altitude: 119 masl, $T_a$max: 21.9 °C, $T_a$min: 8.6 °C, anual precipitation: 820 mm]. 2. Tafi del Valle [*C. tuconax* (n = 4), altitude: 3042 masl, $T_a$max: 16.2 °C, $T_a$min: 2.1 °C, anual precipitation: 122 mm]. 3. Necochea [*C. talarum* (n = 5), *C. australis* (n = 4), altitude: 23 masl, $T_a$max: 18.8 °C, $T_a$min: 8.5 °C, anual precipitation: 871 mm]. 4. San Lorenzo [*C. roigi* (n = 2), altitude: 54 masl, $T_a$max: 26.8 °C, $T_a$min: 15.4 °C, anual precipitation: 1141 mm]. 5. Mar de Cobo [*C. talarum* (n = 4), altitude: 3 masl, $T_a$max: 19.6 °C, $T_a$min: 8.0 °C, anual precipitation: 833 mm].

2.2. Physiological and molecular parameters

Respirometric technique (Lighton, 2008) was used to estimate physiological parameters (BMR, NST_{max}, cold-induced MMR). Oxygen consumption was measured using a computerized positive pressure open-flow respirometry system (Sable Systems, Las Vegas, USA). Animals were individually placed in a chamber (1.8 L) that received air at 1.4 L min^{-1} from a flowmeter (Side-Trak Sierra model 830/840, Sierra Instruments, Monterey, USA). Air passed through a CO_2-absorbent (self-indicating IQB®, IQB Laboratories, Quilmes, Argentina) and water scrubber (Drierite®, Hammond Drierite Co. Ltd., Xenia, USA) before going through the chamber. Excurrent air from the chamber was subsampled at 110 ± 10 ml min^{-1}, and was passed through IQB® and Drierite®, before being analyzed by an O_2 analyzer (model FC-1B, Sable Systems, Las Vegas, USA) every 1 s by Expedata - PC program (Sable Systems, Las Vegas, USA). Rates of oxygen consumption were calculated using the equation 4a of Withers (1977), $\dot{V}o_2 = \dot{V}\ (FiO_2 - FeO_2 / 1 - FiO_2)$, where $\dot{V}$ is the flow rate through the system, FiO_2 and FeO_2 are the fractional O_2 concentration in the incurrent and the excurrent air, respectively (FiO_2 was 0.2095). Body mass (M) was measured before each experimental set using an electronic balance (model FX-3000, ± 0.01 g, A&D Company Limited, San Jose, USA), whereas T_b was measured as rectal temperature with a YSI probe (model 93k73545-402) connected to a Cole-Parmer thermistor meter (model 8402-10, ± 0.1_C, Cole-Parmer Instrument Company, Vernon Hills, USA) after each respirometric measurement. Meroi et al. (2014) found in *C. talarum* an arrhythmic daily pattern of O_2 consumption, thus experimental trials were conducted between 08 and 17 h.

BMR and NST were measured using the protocol described by

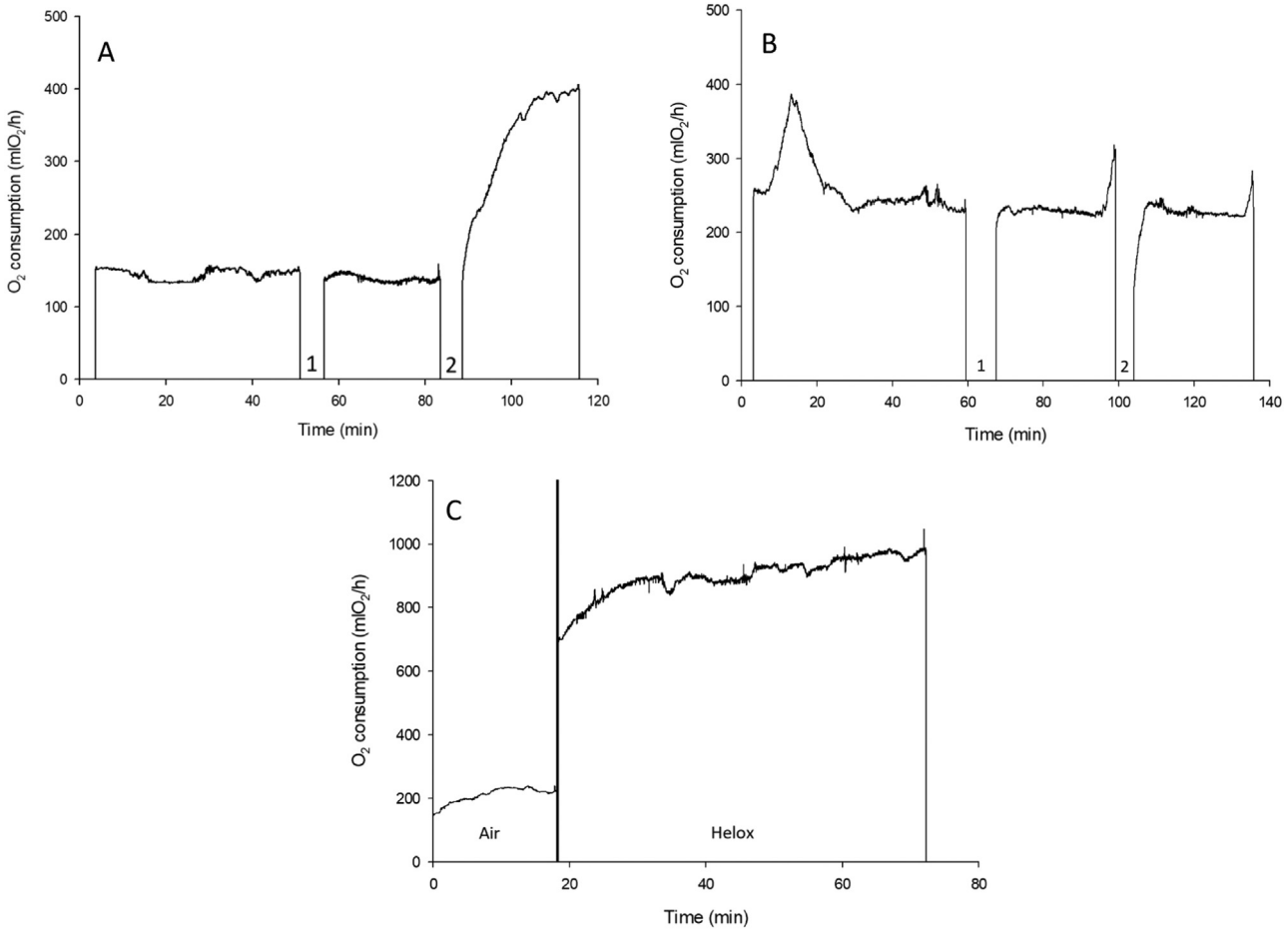

Fig. 2. Records of O_2 consumption for *Ctenomys* species in cold induced maximum metabolic rate (MMR), and basal metabolic rate (BMR) / non shivering thermogenesis (NST) trials. A) O_2 consumption of a 145.42 g *C. talarum* in BMR/NST trial [1: Saline solution injection. 2: norepinephrine (NE) injection]. B) O_2 consumption of a 371.24 g *C. tuconax* in BMR/NST trial [Note that O_2 consumption after NE injection is similar to the basal and saline injection periods]. C) O_2 consumption of a 278.99 g *C. australis* in cold induced MMR trial [Helox: Helium rich atmosphere (79% He – 21% O_2)].

Nespolo et al. (2001) for a subterranean species (*Spalacupus cyanus*), and confirmed by Luna et al. (2012) for *Ctenomys talarum*. Briefly, after a period of habituation inside the chamber (~30 min), O_2 consumption was recorded for 1 h at rest. BMR experimental trial was chosen because it is possible to obtain a 5 − 10 min period during which the lowest steady-state of O_2 consumption can be attained (Luna et al., 2012). After BMR estimation, 30 min of O_2 consumption was recorded after an intramuscular injection of saline solution; and finally, a 30–50 min record after an intramuscular injection of norephinefrine (NE, the same volume as saline solution). In eutherians, O_2 consumption in response to NE occurs 10 min after the injection and lasts at least 5–10 min (Feist and Rosenmann, 1976). Doses of NE were estimated according to Wunder and Gettinger (1996), described as NE ($mg\,kg^{-1}$) doses $= 2.53\,M^{-0.4}$. During this period, a maximum steady-state O_2 consumption of 10-min after the injection of NE was considered to be NST_{max}, which includes both BMR and thermoregulatory NST.

In the case of cold-induced maximum metabolic rate (MMR), it was estimated in a Helium rich atmosphere (Helox), according to the procedure described by (Rosenmann and Morrison, 1974). A mixture of He (79%) and O_2 (21%) was passed through the same chamber used for BMR trials. Before MMR estimation, flow rate was corrected for the He-O_2 gas mix (K factor relative to N_2 = 1.454). As in the case of BMR, the mixture was passed through a CO_2-absorbent (IQB®) and water scrubber (Drierite®) before and after passing through the chamber. After a period of habituation in the chamber (~30 min), O_2 consumption was recorded for 1 h at a T_a of 10 ± 0.1 °C. Experimental T_a for MMR measurement was chosen based on previous studies on *Ctenomys talarum* (Luna et al., 2012). Shivering thermogenesis (ST) was calculated by subtracting estimated NST_{max} to cold induced MMR, following the equation proposed for eutherian mammals (MMR = BMR + NST + ST; Wunder and Gettinger, 1996).

To estimate molecular correlates of NST (content of UCP1 and COXII, and enzymatic activity of COX), interscapular brown adipose tissue (IBAT) was surgically removed following the procedure used in (Luna et al., 2012). For this, each individual was anesthetized by an intramuscular injection of Ketamine hydrochloride ($40\,mg\,kg^{-1}$) and Xylazine ($2\,mg\,kg^{-1}$). After shaving the back of the animal, a 2 cm longitudinal incision was made between the scapulae through the epidermal layer; the skin was carefully opened out and IBAT was completely removed. Hypodermic injection of antibiotic (Dipenisol®) was given to the animals after suturing the incision. Once IBAT was extracted, the tissue was homogenized with a manual homogenizer in STE buffer (250 mM sucrose, 5 mM Tris–HCl, 2 mM EDTA, pH 7.4), with protease and phosphatase inhibitors (10 µM Leupeptin, 10 µM Pepstatin, 0.2 mM PMSF and 0.2 mM Orthovanadate), in a proportion of 10 ml of buffer per g of tissue, and filtered through a layer of inert gauze. Total protein content was determined by the Bradford method (Bradford, 1976). Samples of IBAT were denatured and 40 µg of proteins per line were loaded and run in a SDS-PAGE (3% stacking gel and 12% running gel) according to Laemmli (1970), and electrotransferred onto a nitrocellulose filter, as described by Puigserver et al. (1991), using a Trans-Blot® Turbo™ Transfer System (Bio-Rad, USA). After that, membranes were blocked in 5% nonfat powdered milk in TBS-Tween, and incubated with the corresponding antibodies (UCP1, COXII, Alpha

Diagnostics, USA). Protein bands on the nitrocellulose filters were visualized by Immun-Star® Western C® Kit reagent (Bio-Rad, USA) western blotting detection systems. The chemiluminiscence signal was captured with a Chemidoc XRS densitometer (Bio-Rad, USA), and analyzed with Quantity One Software (Bio-Rad, USA). COX activity was measured using a spectrophotometric method (Chrzanowska-Lightowlers et al., 1993; Wharton and Tzagoloff, 1967). Briefly, aliquots of the IBAT homogenates were incubated in 0.1 M $NaPO_4H_2$, pH 7.0, in the presence of 2 µg/ml Catalase and 5 mM substrate DAB (3, 3′ diaminebenzidine-tetrachloride). After 30 s, 100 µM reduced cytochrome c was added to start the reaction, and the absorbance variation was recorded for 20 min at 450 nm.

2.3. Data analysis and statistics

All data are presented as mean ± SE. ANOVA test was used to compute for differences in BMR, NST_{max}, MMR, content of UCP1 and COXII, and COX enzymatic activity among *Ctenomys* species. Since BMR and NST_{max} were estimated in a single experimental trial for each individual, a repeated measured ANCOVA was used to evaluate differences between BMR and NST_{max} among species (i.e. the independent factor). Body mass was used as a covariate when ANCOVA was performed. Normality and homoscedasticity were tested before analysis. Data was analyzed by generalized mixed-effect models with the R package "nlme" (Pinheiro et al., 2018) in R (R Core Team, 2018) inside RStudio.

3. Results

In Fig. 2, time course of O_2 consumption according to different experimental procedures are shown. In some species, O_2 consumption rose abruptly after NE injections (Fig. 2 A), whereas in others did not change (Fig. 2B). In all *Ctenomys* species, O_2 consumption increase after individuals change from air to Helox atmosphere (Fig. 2 C). Mass specific basal metabolic rate was different among species (ANOVA, $F_{5, 18}$ = 10.95, P < 0.001; Fig. 3, Table 1). *Ctenomys talarum* (MC, 174.92 ± 9.90 g; NC, 140.01 ± 11.6 g) showed the highest value (Tukey, all, $P < 0.01$), but BMR did not differ among *C. australis* (247.77 ± 7.14 g), *C. tuconax* (340.39 ± 61.09 g), *C. porteusi* (194.18 ± 20.35 g), *C. roigi* (158.27 ± 41.10 g; all, $P > 0.05$). Mass specific NST differed among *Ctenomys* species (ANOVA, $F_{5, 18}$ = 12.50, $P < 0.001$; Fig. 3, Table 1). In this case, both populations of *C. talarum* showed higher NST than the other species (Tukey, all, P < 0.001). On the other hand, NST values of *C. australis* and *C. tuconax* were similar

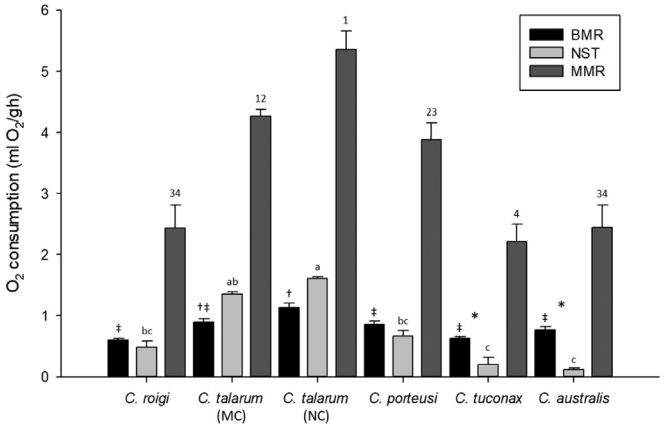

Fig. 3. Basal metabolic rate (BMR), non shivering metabolic rate (NST) and cold induced maximum metabolic rate (MMR) of different *Ctenomys* species. Small letters, numbers or symbols represent statistical differences after ANOVA analysis for each estimated physiological variable. MC: Mar de Cobo Locality. NC: Necochea Locality. *No differences between BMR and NST_{max} (see Results).

($P = 0.99$), as did NST of *C. porteusi* and *C. roigi* ($P = 0.61$). Body temperature after NST_{max} estimation was similar among all the species (ANOVA, $F_{5, 18}$ = 2.62, $P = 0.06$; Table 1). Body mass before MMR estimation was 148.06 ± 35.66 g for *C. roigi*, 176.55 ± 9.14 g for *C. talarum* from Mar de Cobo, 147.10 ± 9.64 g for *C. talarum* from Necochea, 192.63 ± 20.11 g for *C. porteusi*, 327.90 ± 42.05 g for *C. tuconax*, and finally 272.62 ± 11.34 g for *C. australis*. Cold induced MMR was different among species (ANOVA, $F_{5, 18}$ = 18.79, $P < 0.001$; Fig. 3, Table 1). *Ctenomys talarum* showed the highest value for cold induced MMR (Tukey, all, $P < 0.05$). All individuals became hypothermic after cold exposure, showing a great variation of T_b (ANOVA, $F_{5, 18}$ = 6.60, $P < 0.002$; Fig. 3, Table 1). *C. roigi* was found to show the lowest T_b after cold treatment, whereas *C. talarum* slightly decreased its T_b after cold exposure (Tukey, $P < 0.03$). Due to NST_{max} measurement induced by NE injection includes BMR, differences between both variables we analyzed using repeated measured ANCOVA. BMR differed from NST_{max} for all the species ($F_{5, 17}$ = 4.48, $P = 0.01$; all, $P < 0.01$), except for *C. australis* and *C. tuconax* that showed similar values of both parameters ($P = 0.94$; $P = 0.18$, respectively).

Using body mass as a covariate, the log-transformed IBAT mass was different among *Ctenomys* species (ANCOVA, $F_{5, 17}$ = 2.92, $P < 0.04$; Table 1). *Ctenomys australis* had the lowest mass of IBAT while both populations of *C. talarum* showed the highest value. Considering IBAT percentage in relation to body mass, individuals of *C. talarum* from both populations presented similar % IBAT among them (ANOVA, $F_{5, 18}$ = 13.40, $P < 0.001$, Tukey, $P = 0.94$; Table 1), and also in comparison with *C. porteusi* [when contrasting with *C. talarum* individuals from Mar de Cobo ($P = 0.18$) and Necochea ($P = 0.52$)]. However, % IBAT of *C. talarum* differed from *C. australis* and *C. tuconax* (all, $P < 0.02$). Value of % IBAT for *C. roigi* was similar to that found in *C. talarum* (MC, $P = 0.96$; NC, $P = 0.99$), *C. australis* ($P = 0.09$), and *C. porteusi* ($P = 0.94$), but differed from *C. tuconax* ($P = 0.02$). Larger species showed a similar % IBAT (*i.e. C. australis* and *C. tuconax*, $P = 0.83$). Finally, % IBAT for *C. porteusi* was similar to the one for *C. australis* ($P = 0.10$), but not to *C. tuconax* ($P = 0.01$). As it was not possible to detect any signal of UCP1 and COXII antibodies in IBAT for *C. australis* and *C. tuconax*, ANCOVAs were only performed with data for the remaining species. Log-transformed content of UCP1 and COXII in IBAT was similar among species (UCP1, ANCOVA, $F_{3, 11}$ = 0.44, $P = 0.73$; COXII, ANCOVA, $F_{3, 11}$ = 0.31, $P = 0.82$; Table 1). Activity of COX in IBAT was different among species (ANCOVA, $F_{5, 17}$ = 5.23, $P < 0.01$; Table 1). Larger species had the lowest COX activity in IBAT. *Ctenomys tuconax* and *C. australis* showed similar values among them ($P = 0.94$), but differed from *C. porteusi* ($P = 0.002$ and $P < 0.001$, respectively) and *C. roigi* ($P = 0.05$ and $P = 0.02$, respectively). *Ctenomys talarum* from both populations had similar COX activity in IBAT respect *C. tuconax* (MC, $P = 0.15$; NC, $P = 0.26$), but different from *C. australis* (MC, $P = 0.03$; NC, $P = 0.05$). No differences were found for COX activity among *C. porteusi*, *C. roigi*, and both populations of *C. talarum* (all, $P > 0.10$).

4. Discussion

Macrophysiological studies focus on the relationship between the ecological implication of physiological diversity and how it has evolved, including also the underlying explanation of species distribution and physiological characteristics (Osovitz and Hofmann, 2007). In the present study we consistently analyzed total thermogenic capacity (cold induced MMR) and its components (NST or ST) in *Ctenomys* species that differ in body mass and distribution. Maximum cold induced metabolism in *Ctenomys* varies from 2.21 ml O_2/gh to 5.36 ml O_2/gh in *C. tuconax* and *C. talarum*, respectively. As we hypothesized, if thermally stable burrows have had an effect on thermogenic capacity, we would have expected similar cold induced MMR across species. Such pattern was not found among *Ctenomys* species. As MMR experimentally induced by cold has showed a relatively high evolutionary lability

Table 1
Body temperature (*T*b), metabolic variables, interscapular brown adipose tissue (IBAT), UCP1 and COXII content and COX activity in *Ctenomys* species.

	Ctenomys species					
	C. roigi	*C. talarum* (MC)	*C. talarum* (NC)	*C. porteusi*	*C. tuconax*	*C. australis*
Cold induced MMR (ml O_2/gh)	2.43 ± 0.38^{34}	4.27 ± 0.11^{12}	5.36 ± 0.30^{1}	3.88 ± 0.28^{23}	2.21 ± 0.29^{4}	2.44 ± 0.37^{34}
T_b after MMR (°C)	26.6 ± 0.5^{a}	32.6 ± 0.7^{ab}	35.2 ± 0.2^{b}	32.4 ± 0.3^{ab}	30.4 ± 1.8 ^{ab}	30.5 ± 2.0 ^{ab}
BMR (ml O_2/gh)	0.60 ± 0.03‡	0.90 ± 0.05†‡	1.13 ± 0.08†	0.86 ± 0.05‡	0.63 ± 0.03‡	0.77 ± 0.05‡
NST (ml O_2/gh)	0.48 ± 0.10^{bc}	1.35 ± 0.04^{ab}	1.61 ± 0.03^{a}	0.67 ± 0.09^{bc}	0.20 ± 0.12^{c}	0.12 ± 0.01^{c}
T_b after NSTmax (°C)	36.0 ± 0.6^{a}	36.9 ± 0.3^{a}	37.2 ± 0.3^{a}	37.2 ± 0.3^{a}	36.2 ± 0.3^{a}	37.0 ± 0.1^{a}
ST (ml O_2/gh)	1.35 ± 0.31	2.02 ± 0.10	2.62 ± 0.23	2.35 ± 0.20	1.38 ± 0.23	1.56 ± 0.41
IBAT mass (g)	0.62 ± 0.22^{ab}	1.03 ± 0.11^{a}	1.33 ± 0.27^{a}	1.01 ± 0.17^{ab}	0.57 ± 0.05^{ab}	0.49 ± 0.03^{b}
% IBAT	0.72 ± 0.17^{a}	0.82 ± 0.08^{a}	0.74 ± 0.02^{a}	0.61 ± 0.06^{ab}	0.30 ± 0.03^{c}	0.40 ± 0.01^{bc}
UCP1 (au/g IBAT)	22.39 ± 13.75^{a}	21.36 ± 7.13^{a}	34.43 ± 6.56^{a}	44.53 ± 22.76^{a}	*	*
COXII (au/g IBAT)	91.46 ± 28.20^{a}	55.8 ± 10.39^{a}	96.52 ± 43.39^{a}	52.36 ± 13.99^{a}	*	*
COX activity (nKat/g IBAT)	71.00 ± 24.09^{c}	37.02 ± 8.26^{bc}	30.52 ± 5.51^{bc}	115.62 ± 37.91^{c}	13.19 ± 2.85^{ab}	9.33 ± 2.45^{a}
UCP1/COXII	0.22 ± 0.08	0.37 ± 0.13	0.53 ± 0.22	0.78 ± 0.30	–	–

Small letters, numbers or symbols represent statistical differences after ANOVA analysis for each estimated physiological or molecular variable. MC: Mar de Cobo Locality. NC: Necochea Locality. Shivering thermogenesis (ST) was computed for each individual as cold induced maximum metabolic rate (MMR) minus basal metabolic rate (BMR) minus non shivering thermogenesis (NST). % IBAT: percentage of IBAT mass related to body mass. *not detectable. au: arbitrary units. nKat (nanokatal): defined as a unit of catalytic activity.

and been negatively correlated to T_a (Rezende et al., 2004), variations in MMR among *Ctenomys* might be related to local environment characteristics. Thus, if surface T_a was the main factor affecting total thermogenesis among species, species that live in colder southern areas or at high altitudes would show higher thermogenic capacity. Again, it appears that there is not a clear trend between surface T_a and MMR. For example, *Ctenomys talarum* and *C. australis* live sympatrically in coastal grasslands, hence they are exposed to the same environmental conditions. However, cold induced MMR in *C. australis* is roughly 45% lower than in *C. talarum*. Opposite, species living at different altitudes, like *C. australis* (at sea level) and *C. tuconax* (~ 3200 masl), have showed a similar cold induced MMR.

Interspecific variation of MMR is mainly associated to body mass. In rodents, body mass alone explains generally around 70% of the variation of either BMR and MMR (Rezende et al., 2004). In *Ctenomys*, body mass is the main factor affecting BMR, explaining more than 65% of its variation (Luna et al., 2009). Interestingly, when comparing to the allometric equation proposed for surface dwelling rodents (Luna et al., 2012; Rezende et al., 2004), cold-induced MMR among *Ctenomys* exhibits a great variation. Maximum cold induced metabolism of *Ctenomys talarum* is about the expected for surface species of similar body mass. However, in *C. tuconax* and *C. australis* MMR is around 50% of the expected according to their body mass (Table 2). Such differences can have a profound impact on T_b. Even all species became hypothermic after cold exposure, *C. talarum* was more efficient defending T_b than *C. roigi*. A high surface/volume ratio could account for the hypothermic response, since heat production can be more advantageous when a low surface-volume ratio is present.

Table 2
Net metabolic scope (MMR-BMR) and percentage of observed value to the allometric equation for MMR and NST in *Ctenomys* species.

	Net Aerobic Scope (ml O_2/gh)	% allometric[a] MMR	% allometric[b] NST
C. roigi	1.84 ± 0.41	46	23
C. talarum (MC)	3.37 ± 0.14	88	71
C. talarum (NC)	4.23 ± 0.30	102	73
C. porteusi	3.02 ± 0.25	80	35
C. tuconax	1.58 ± 0.31	56	–
C. australis	1.57 ± 0.41	59	–

[a] Allometric equation for cold induced MMR proposed for rodents by Luna et al. (2012): MMR (ml O_2/gh) = 29.58 $M^{0.65}$.

[b] Allometric equation for NST proposed for rodents by Rodríguez-Serrano and Bozinovic (2009): NST (ml O_2/gh) = 24.66 $M^{0.50}$. MC: Mar de Cobo Locality. NC: Necochea Locality.

In this context, the interplay between T_as and total thermogenic capacity appears to be more complex than expected. One of the approaches that could be used to examine this relationship is to analyze the net aerobic scope (NAS), because it represents the capacity for simultaneous aerobic processes (*i.e.* MMR) above minimal metabolism (*i.e.* BMR; see Nespolo et al., 2017 for a discussion of the use of NAS). Despite the small and limited data, *Ctenomys* species show similar NAS to those found in the small related subterranean *Spalacopus cyanus* (body mass ~ 100 g, NAS = 3.03 ml O_2/gh; Nespolo et al., 2001), but lower than surface dwelling rodents of comparable size (Luna et al., 2015). Opposite to the finding in subterranean rodents of different lineages (Luna et al., 2017), surface T_a is not a good predictor for residual BMR variations within *Ctenomys* (Luna et al., 2009). Therefore, if BMR and MMR were correlated in *Ctenomys* species on a large scale, maximum metabolism would be unlikely related to geographic differences in modal T_a. In fact, species with similar body weight but from noticeably different climates evidence similar NAS (see Table 2). Such is the case of *C. australis* and *C. tuconax*.

As we have stated, total thermogenic capacity can vary according to the modification of its components, either NST and/or ST. Wunder and Gettinger (1996) allometrically analyzed the contribution of thermogenic mechanisms in mammals, finding that at low body mass (*i.e.* 2–3 g) the main thermogenic mechanisms is NST; on the opposite, at high body mass (*i.e.* 8–10 kg) maximal thermogenic capacity is determined by ST alone. However, the specific contribution of each mechanism to cold induced MMR appears to vary within a restricted range of body mass. For example, in surface dwelling rodents, such as *Phyllotis xanthopygus*, ST is the main mechanism for heat production (~ 60% of total thermogenic capacity; Nespolo et al., 1999). On the other hand, the constitution of NST and ST in *Peromyscus maniculatus* is more balanced (~38% and ~47%, respectively; Van Sant and Hammond, 2008). In bats, species uses both NST and ST as heat producing mechanisms, albeit differences in magnitude (Almeida and Cruz-Neto, 2011). To our knowledge, just one study evaluates total thermogenic capacity other genera of subterranean species and it shows that NST in *Spalacopus cyanus* is the exclusive heat-producing mechanism (Nespolo et al., 2001). In the case of *Ctenomys*, thermogenic mechanisms are different within the small range of body mass of the species analyzed (from ~100 g to ~350 g), varying from a combination of NST and ST (*e.g. C. porteusi*; Fig. 4), to an exclusive contribution of ST to total thermogenic capacity (*e.g. C. australis* and *C. tuconax*; Fig. 4). Thus, at least in *Ctenomys*, body mass alone cannot account for differences in the relative importance of the mechanism of heat production during thermoregulation.

Particularly, NST has received more attention than ST, since it can

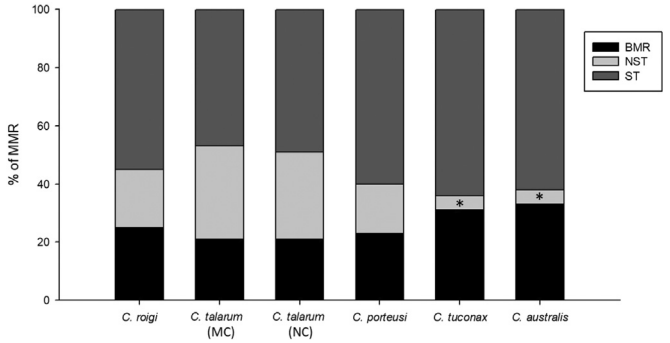

Fig. 4. Percentage of basal metabolic rate (BMR), non shivering thermogenesis (NST) and shivering thermogenesis (ST) related to each species cold induced maximum metabolic rate (MMR). *No differences between BMR and NST_{max} (see Results).

be easily estimated and it is also considered the plastic element and most efficient component of the heat-producing machinery (Cannon and Nedergaard, 2004). In rodents, Rodríguez-Serrano and Bozinovic (2009) found that NST is negatively correlated with body mass and T_a, evidencing that cold climate rodent species exhibit higher NSTs than species from warm climates. Non shivering thermogenesis among *Ctenomys* ranges from a relatively high contribution (*e.g. C. talarum*; Fig. 4) to a complete absence (*e.g. C. tuconax*; Fig. 4). In *Ctenomys opimus* and *C. magellanicus*, it falls within the range of our dataset [2 mlO_2/gh and 1.48 mlO_2/gh, respectively; Rodríguez-Serrano and Bozinovic (2009)], and is also similar to values obtained for other subterranean rodents (Luna et al., 2012). The observed NST pattern at the organismic level is correlated with that observed at tissue level. Indeed, mass of the interscapular brown adipose tissue (IBAT) matches with the magnitude of NST. Brown adipose tissue of *C. australis* and *C. tuconax*, both species with null NST is small and similar to white adipose tissue, with no detectable uncoupling proteins (UCP1). Furthermore, it is generally assumed that active thermogenic tissue such as BAT, must have high oxidative capacity to ensure NST (Klingenspor, 2003). Cytocrome *c* oxidase (COX) is a marker enzyme for mitochondrial membrane, and particularly COXII content is used as a proxy of the respiratory capacity of BAT mitochondrias (Klaus et al., 1988; Klingenspor et al., 1996). Also, in *C. australis* and *C. tuconax*, COXII was not detectable and a low enzymatic activity of COX was found.

Consequently, with the available information about thermogenic mechanisms, we observed no clear thermogenic capacity trend in *Ctenomys*. Neither body mass nor T_a did apparently affect the upper metabolic limit. However, specific contribution of NST and ST appears to be constrained by body mass but not by T_a. As mentioned earlier, for species living in the same locality and hence exposed to similar climatic conditions, the contribution of NST varied from more than 30% of MMR in *C. talarum* to total absence in *C. australis*. So, other factors not evaluated in this study, may account for the observed differences in thermogenic capacity.

Digging activities require different muscles types that allow the maintenance of high effort for long periods. Candidates for this task are fatigue-resistant muscles (Alvarez et al., 2012). This type of muscle fibers (*i.e.* slow twitch fibers) are also recruited for shivering, and its proportion can establish individual´s thermogenic potential (Hohtola, 2004; Rowland et al., 2015). Therefore, if species had differences in the type of muscles they use to excavate due to differences in the kind of soil where they inhabit, they would also present differences in the capacity of these muscles to ensure ST. During the construction of new tunnels, digging strategies of *C. talarum* and *C. australis* differ due variations in soil hardness (Vassallo, 1998). Thus, in larger *Ctenomys* species, muscles differences related to digging effort can promote shivering mechanisms. In this context, it has been hypothesized that atmospheric conditions inside burrows is the driven factor affecting MMR in *Ctenomys talarum* (Luna et al., 2012, 2009). *Ctenomys* species live in burrows characterized by atmospheres with low O_2 and high CO_2 concentrations (Baldo et al., 2015), so a limited O_2 availability might have determined a ceiling in maximum metabolism. Then, if O_2 concentration imposes limitations in the O_2 delivery system (Hayes and Chappell, 1986), maximum metabolism would be invariable independent of any performed activity (*e.g.* maximum digging metabolism) or physiological mechanism (*e.g.* thermogenesis). Digging metabolic rate of *C. australis* is fairly similar to cold induced MMR (~ 2.50 ml O_2/gh), but it is not the case of *C. talarum* whose digging metabolism is ~30% lower than cold induced MMR (~ 3.80 ml O_2/gh vs. ~ 5.30 ml O_2/gh, respectively; Luna and Antinuchi, 2007). Therefore, in larger *Ctenomys* species, a combination of a low MMR and enhanced ST via differential muscle activity during digging, can restrict NST increments. Even though, this explanation appears not to fit with *C. roigi*, this species is extremely hypothermic after cold exposure. Hence, it should be taken with caution because MMR might be lower than expected in this condition (Cannon and Nedergaard, 2011).

Finally, our analysis provides coarse evidence that a commitment between burrow and surface use could affect thermogenic features of *Ctenomys* species. However, thermogenic mechanisms are plastic and can account for the observed specific differences. Although captures were made at the same season, we do not neglect that phenotypic plasticity might mask a different pattern. For example, in surface-dwelling species such as *Peromyscus maniculatus*, NST varies ~ 42% after cold acclimation (Van Sant and Hammond, 2008) similarly to *Phyllotis xanthopygus*, in which NST and ST differ by almost 50% and 200%, respectively (Nespolo et al., 1999). However, acclimation to different ambient temperatures in *C. talarum*, does not modify NST at least in the short term (Luna et al., 2012). So, it is expected that a similar pattern might occur in the other *Ctenomys* species.

5. Conclusion

Biogeographical factors that influence cold induced MMR in surface dwelling species appear to have no effect on subterranean *Ctenomys* species. Also, that mechanism of heat production is species-specific. In the smallest species, a combination of ST and NST have been used to maintain T_b at low T_as. In larger species, total thermogenic capacity is driven only by shivering mechanisms. This pattern is correlated at tissue level, since larger species show a smaller interscapular BAT patch, not detectable presence of UCP1 and low COX activity. Thus, other factors that constrain cold induced MMR should be further explored to assess their effect on thermogenic variability among *Ctenomys*. Although cold-induced MMR appears to be infrequently reached, it is an ecologically relevant trait (Bozinovic and Rosenmann, 1989; Garland and Carter, 1994) and is plausible to be affected by natural selection (Rezende et al., 2004; Sadowska et al., 2005). Thus, in the evolutionary timescale, if low O_2 levels impose a ceiling in cold induced MMR, and ST is enhanced due to species-specific life history traits (*e.g.* digging effort depending on soil type), then the observed differences among *Ctenomys* species might be explained.

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Author contributions

All authors contributed to the design of the experiment. The experimental trials were conducted by F.L. with help from J. S-S. Data analyses were performed by F.L. and J.S-S. The manuscript was written by F.L with all authors contributing to revisions.

References

Almeida, M.C., Cruz-Neto, A.P., 2011. Thermogenic capacity of three species of fruit-eating phyllostomid bats. J. Therm. Biol. 36, 225–231. https://doi.org/10.1016/j.jtherbio.2011.03.005.

Alvarez, G.I., Díaz, A.O., Longo, M.V., Becerra, F., Vassallo, A.I., 2012. Histochemical and morphometric analyses of the musculature of the forelimb of the subterranean rodent *Ctenomys talarum* (Octodontoidea). Anat. Histol. Embryol. 41, 317–325. https://doi.org/10.1111/j.1439-0264.2012.01137.x.

Baldo, M.B., Antenucci, C.D., Luna, F., 2015. Effect of ambient temperature on evaporative water loss in the subterranean rodent *Ctenomys talarum*. J. Therm. Biol. 53, 113–118. https://doi.org/10.1016/j.jtherbio.2015.09.002.

Begall, S., Burda, H., Schleich, C.E. (Eds.), 2007. Subterranean Rodents: News from Underground. Springer, Berlin, London.

Bernardo, J., Ossola, R.J., Spotila, J., Crandall, K.A., 2007. Interspecies physiological variation as a tool for cross-species assessments of global warming-induced endangerment: validation of an intrinsic determinant of macroecological and phylogeographic structure. Biol. Lett. 3, 695–699. https://doi.org/10.1098/rsbl.2007.0259.

Bernardo, J., Spotila, J.R., 2006. Physiological constraints on organismal response to global warming: mechanistic insights from clinally varying populations and implications for assessing endangerment. Biol. Lett. 2, 135–139. https://doi.org/10.1098/rsbl.2005.0417.

Bozinovic, F., Rosenmann, M., 1989. Maximum metabolic rate of rodents: physiological and ecological consequences on distributional limits. Funct. Ecol. 3, 173. https://doi.org/10.2307/2389298.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Cannon, B., Nedergaard, J., 2011. Nonshivering thermogenesis and its adequate measurement in metabolic studies. J. Exp. Biol. 214, 242–253. https://doi.org/10.1242/jeb.050989.

Cannon, B., Nedergaard, J., 2004. Brown adipose tissue: function and physiological significance. Physiol. Rev. 84, 277–359. https://doi.org/10.1152/physrev.00015.2003.

Chown, S.L., Gaston, K.J., 2008. Macrophysiology for a changing world. Proc. R. Soc. B Biol. Sci. 275, 1469–1478. https://doi.org/10.1098/rspb.2008.0137.

Chown, S.L., Gaston, K.J., Robinson, D., 2004. Macrophysiology: large-scale patterns in physiological traits and their ecological implications. Funct. Ecol. 18, 159–167. https://doi.org/10.1111/j.0269-8463.2004.00825.x.

Chrzanowska-Lightowlers, Z.M.A., Turnbull, D.M., Lightowlers, R.N., 1993. A microtiter plate assay for cytochrome c oxidase in permeabilized whole cells. Anal. Biochem. 214, 45–49. https://doi.org/10.1006/abio.1993.1454.

Feist, D.D., Rosenmann, M., 1976. Norepinephrine thermogenesis in seasonally acclimatized and cold acclimated red-backed voles in Alaska. Can. J. Physiol. Pharmacol. 54, 146–153. https://doi.org/10.1139/y76-023.

Foster, D.O., Frydman, M.L., 1979. Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. Can. J. Physiol. Pharmacol. 57, 257–270. https://doi.org/10.1139/y79-039.

Garland, T., Carter, P.A., 1994. Evolutionary physiology. Annu. Rev. Physiol. 56, 579–621. https://doi.org/10.1146/annurev.ph.56.030194.003051.

Golozoubova, V., Cannon, B., Nedergaard, J., 2006. UCP1 is essential for adaptive adrenergic nonshivering thermogenesis. Am. J. Physiol. -Endocrinol. Metab. 291, E350–E357. https://doi.org/10.1152/ajpendo.00387.2005.

Hayes, J.P., Chappell, M.A., 1986. Effects of cold acclimation on maximum oxygen consumption during cold exposure and treadmill exercise in deer mice, *Peromyscus maniculatus*. Physiol. Zool. 59, 473–481. https://doi.org/10.1086/physzool.59.4.30158600.

Hohtola, E., 2004. Shivering Thermogenesis in Birds and Mammals, In: Life in the Cold: Evolution, Mechanisms, Adaptation, and Application. Twelfth International Hibernation Symposium. Biological Papers of the University of Alaska, Fairbanks, pp. 241–252.

Janský, L., 1973. Non-shivering thermogenesis and its thermoregulatory significance. Biol. Rev. 48, 85–132. https://doi.org/10.1111/j.1469-185X.1973.tb01115.x.

Klaus, S., Heldmaier, G., Ricquier, D., 1988. Seasonal acclimation of bank voles and wood mice: nonshivering thermogenesis and thermogenic properties of brown adipose tissue mitochondria. J. Comp. Physiol. B 158, 157–164. https://doi.org/10.1007/BF01075829.

Klingenspor, M., 2003. Cold-induced recruitment of brown adipose tissue thermogenesis. Exp. Physiol. 88, 141–148. https://doi.org/10.1113/eph8802508.

Klingenspor, M., Ivemeyer, M., Wiesinger, H., Haas, K., Heldmaier, G., Wiesner, R.J., 1996. Biogenesis of thermogenic mitochondria in brown adipose tissue of Djungarian hamsters during cold adaptation. Biochem. J. 316, 607–613.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lighton, J.R.B., 2008. Measuring Metabolic Rates: a Manual for Scientists. Oxford University Press, Oxford; New York.

Luna, F., Antenucci, C.D., 2007. Energy and distribution in subterranean rodents: Sympatry between two species of the genus *Ctenomys*. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 147, 948–954. https://doi.org/10.1016/j.cbpa.2007.02.032.

Luna, F., Antenucci, C.D., Bozinovic, F., 2009. Comparative energetics of the subterranean *Ctenomys* rodents: breaking patterns. Physiol. Biochem. Zool. 82, 226–235. https://doi.org/10.1086/597526.

Luna, F., Bozinovic, F., Antenucci, C.D., 2015. Macrophysiological patterns in the energetics of Caviomorph rodents: implications in a changing world. In: Biology of Caviomorph Rodents: Diversity and Evolution, SAREM Series A - Mammalogical Research. SAREM, Buenos Aires, Argentina, pp. 245–272.

Luna, F., Naya, H., Naya, D.E., 2017. Understanding evolutionary variation in basal metabolic rate: an analysis in subterranean rodents. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 206, 87–94. https://doi.org/10.1016/j.cbpa.2017.02.002.

Luna, F., Roca, P., Oliver, J., Antenucci, C.D., 2012. Maximal thermogenic capacity and non-shivering thermogenesis in the South American subterranean rodent *Ctenomys talarum*. J. Comp. Physiol. B 182, 971–983. https://doi.org/10.1007/s00360-012-0675-6.

Medina, A.I., Martí, D.A., Bidau, C.J., 2007. Subterranean rodents of the genus Ctenomys (Caviomorpha, Ctenomyidae) follow the converse to Bergmann's rule: converse Bergmann's rule in Ctenomys. J. Biogeogr. 34, 1439–1454. https://doi.org/10.1111/j.1365-2699.2007.01708.x.

Meroi, F., Luna, F., Antenucci, C.D., 2014. Variación estacional de la tasa metabólica de reposo en *Ctenomys talarum* (Rodentia, Ctenomyidae): Ausencia de efectos ambientales. Mastozool. Neotrop. 21, 241–250.

Naya, D.E., Spangenberg, L., Naya, H., Bozinovic, F., 2013. Thermal conductance and basal metabolic rate are part of a coordinated system for heat transfer regulation. Proc. R. Soc. B Biol. Sci. 280https://doi.org/10.1098/rspb.2013.1629. (20131629–20131629).

Nespolo, R.F., Bacigalupe, L.D., Rezende, E.L., Bozinovic, F., 2001. When nonshivering thermogenesis equals maximum metabolic rate: thermal acclimation and phenotypic plasticity of fossorial *Spalacopus cyanus* (Rodentia). Physiol. Biochem. Zool. 74, 325–332.

Nespolo, R.F., Opazo, J.C., Rosenmann, M., Bozinovic, F., 1999. Thermal acclimation, maximum metabolic rate, and nonshivering thermogenesis of *Phyllotis xanthopygus* (Rodentia) in the Andes mountains. J. Mammal. 80, 742–748. https://doi.org/10.2307/1383243.

Nespolo, R.F., Solano-Iguaran, J.J., Bozinovic, F., 2017. Phylogenetic analysis supports the aerobic-capacity model for the evolution of endothermy. Am. Nat. 189, 13–27. https://doi.org/10.1086/689598.

Osovitz, C.J., Hofmann, G.E., 2007. Marine macrophysiology: studying physiological variation across large spatial scales in marine systems. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 147, 821–827. https://doi.org/10.1016/j.cbpa.2007.02.012.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Core Team, R., 2018. nlme: linear and nonlinear mixed effects models. R. Package Version 3, 1–137.

Puigserver, P., Lladó, I., Palou, A., Gianotti, M., 1991. Evidence for masking of brown adipose tissue mitochondrial GDP-binding sites in response to fasting in rats made obese by dietary manipulation. Effects of reversion to standard diet. Biochem. J. 279, 575–579. https://doi.org/10.1042/bj2790575.

Core Team, R., 2018. R: a language and environment for statistical computing. R Found. Stat. Comput., Vienna, Austria.

Rezende, E.L., Bozinovic, F., Garland, T., 2004. Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. Evolution 58, 1361–1374.

Rodríguez-Serrano, E., Bozinovic, F., 2009. Interplay between global patterns of environmental temperature and variation in nonshivering thermogenesis of rodent species across large spatial scales. Glob. Change Biol. 15, 2116–2122. https://doi.org/10.1111/j.1365-2486.2009.01854.x.

Rosenmann, M., Morrison, P., 1974. Maximum oxygen consumption and heat loss facilitation in small homeotherms by He-O2. Am. J. Physiol. -Leg. Content 226, 490–495. https://doi.org/10.1152/ajplegacy.1974.226.3.490.

Rowland, L.A., Bal, N.C., Periasamy, M., 2015. The role of skeletal-muscle-based thermogenic mechanisms in vertebrate endothermy: non-shivering thermogenic mechanisms in evolution. Biol. Rev. 90, 1279–1297. https://doi.org/10.1111/brv.12157.

Sadowska, E.T., Labocha, M.K., Baliga, K., Stanisz, A., Wróblewska, A.K., Jagusiak, W., Koteja, P., 2005. Genetic correlations between basal and maximum metabolic rates in a wild rodent: consequences for evolution of endothermy. Evolution 59, 672–681.

Scholander, P.F., Hock, R., Walters, V., Johnson, F., Irving, L., 1950. Heat regulation in some arctic and tropical mammals and birds. Biol. Bull. 99, 237–258. https://doi.org/10.2307/1538741.

Van Sant, M.J., Hammond, K.A., 2008. Contribution of shivering and nonshivering thermogenesis to thermogenic capacity for the deer mouse (*Peromyscus maniculatus*). Physiol. Biochem. Zool. 81, 605–611. https://doi.org/10.1086/588175.

Vassallo, A.I., 1998. Functional morphology, comparative behaviour, and adaptation in two sympatric subterranean rodents genus *Ctenomys* (Caviomorpha: octodontidae). J. Zool. 244, 415–427. https://doi.org/10.1111/j.1469-7998.1998.tb00046.x.

Vera, F., Antenucci, C.D., Zenuto, R.R., 2011. Cortisol and corticosterone exhibit different seasonal variation and responses to acute stress and captivity in tuco-tucos (*Ctenomys talarum*). Gen. Comp. Endocrinol. 170, 550–557. https://doi.org/10.1016/j.ygcen.2010.11.012.

Wharton, D.C., Tzagoloff, A., 1967. Cytochrome oxidase from beef heart mitochondria. In: Methods in Enzymology. Elsevier, pp. 245–250. https://doi.org/10.1016/0076-6879(67)10048-7.

Withers, P.C., 1977. Measurements of metabolic rate, VCO2, and evaporative water loss with a flow through mask. J. Appl. Physiol. 42, 120–123.

Withers, P.C., Cooper, C.E., Maloney, S.K., Bozinovic, F., Cruz-Neto, A., 2016. Ecological and environmental physiology of mammals. Ecological and Environmental Physiology Series. Oxford University Press, Oxford, United Kingdom.
Wunder, B.A., Gettinger, R.D., 1996. Effects of body mass and temperature acclimation on the nonshivering thermogenic response of small mammals, In: Adaptations to the Cold: Proceedings of the Tenth International Hibernation Symposium. University of New England Press, Armidale, pp. 131–139.

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