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Phylogenetic analysis of the allometry of metabolic rate and mitochondrial basal proton leak

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Abstract

The mitochondrial basal proton leak (MBPL) significantly contributes to high body temperatures (T_b) and basal metabolic rates (BMR) in endotherms. In endotherms at a given body mass (M), liver MBPL is higher than in ectotherms, supporting the notion that MBPL may partly explain the evolutionary increase in metabolic rate (MR), fostering endothermy. Here, we re-addressed this assumption by performing a phylogenetic analysis comparing all available liver MBPL data for ecto- and endotherms. While MBPL within endotherms negatively scales with M and BMR as shown previously, MBPL of ectotherms does not scale allometrically with M. Phylogenetic analysis reveals that this result is confounded by a positive scaling coefficient for MBPL with M for reptiles. Strikingly, the reptilian MBPL reaches endothermic levels above a body mass of 6.6 kg. Thus, phylogenetic scaling of MBPL supports previous claims of endotherm-like physiological characteristics in large reptiles. It appears that diversification of ancestral ectothermic tetrapods to a body mass of at least 6 kg may have been required to reach a MBPL that is beneficial for sustained high body temperatures. Novel MBPL data for the lesser hedgehog tenrec, a protoendothermic eutherian that displays reptile-like thermoregulatory patterns, fall within the endo- and ectothermic allometric regressions. Finally, we add additional evidence that within endotherms, phylogenetic differences in MR do not correlate with MBPL. Collectively, these data suggest that MBPL does not universally scale with metabolic rate in ecto- or endotherms and that an increasing MBPL with M may have played an important physiological role in the evolutionary history of reptilian thermoregulation.

Keywords: Mitochondrial proton leak, metabolic rate, allometry, phylogeny, scaling

1 Introduction

The mitochondrial basal proton leak (MBPL, nmol H^+ min^{-1} mg $protein^{-1}$) is a result of the inherent leakiness of the mitochondrial inner membrane for protons (Brand et al., 1994a). Consequently, the proton gradient across the mitochondrial inner membrane, storing the energy to form ATP, is partly dissipated as heat. The study of the relationship between MBPL, body mass (M, g) and metabolic rate, defined as the rate of oxygen consumption (MR, ml O_2 h^{-1}), has evidenced that MBPL in liver and skeletal muscle mitochondria of endotherms significantly contributes to high MRs in endotherms (Brand et al., 1994b; Rolfe and Brown, 1997). The positive correlation of whole body MR and M is accompanied by a decrease in the rate of MBPL in the liver of endotherms (Porter and Brand, 1993).

In contrast, body temperature (T_b) and MR of ectotherms is governed by ambient temperature (T_a) and the relationship between MBPL, MR, T_b and M is less conclusive. Previous comparisons of MBPL between ecto- and endotherms suggested a higher MBPL in endotherms at similar M, proposing a contribution of MBPL to the evolutionary increase in MR leading to endothermy (Brand et al., 1994b). However, a clear phylogenetic pattern could not be verified (Brookes et al., 1998) and MBPL in ectotherms did not show an allometric relationship with M (Hulbert et al. 2002).

Coinciding with this suggestion, MBPL rates in liver mitochondria of crocodilians were surprisingly reported to match those of endothermic rats (Hulbert et al., 2002). Despite not being classified as endotherms, large crocodilians are indeed capable of maintaining higher average daily T_bs when compared to T_a (Seebacher et al., 1999). In recent years, there has been a lively debate surrounding the evolution of thermoregulation in reptiles with some researchers proposing evidence for endotherm-like physiological characteristics in extant crocodiles and extinct dinosaurs (Grady et al., 2014; Seymour et al., 2004).

Protoendothermic mammals or “basoendotherms”, display significantly lower T_bs than the majority of eutherian mammals. The T_b of the lesser hedgehog tenrec (*Echinops telfairi*) in thermoneutral conditions for example is ≈32°C (Oelkrug et al., 2013). Additionally, *E. telfairi* remains in an “un-heated” mode most of the time, with body temperatures tracking close to ambient temperatures (Lovegrove and Génin, 2008; Oelkrug et al., 2013). Given our previous understanding of the relationship between MBPL and BMR, we would expect that the MBPL of the tenrec is lower than that of eutherian mammals of the same M that display higher T_bs and BMRs.

Recently, we demonstrated that phylogenetic differences in mass-specific BMR within endotherms are not explained by differences in liver MBPL (Polymeropoulos et al., 2012), where marsupials have higher rates of liver MBPL than eutherian mammals, despite having lower T_bs and mass-specific BMRs. This discrepancy challenges the universality of the causative relationship between high MBPLs and MRs and requires careful re-investigation across all taxa, including ectotherms and basoendotherms.

In order to re-assess the relationship between liver MBPL and MR across more taxa and a wide M range and various levels of thermoregulatory ability, we compiled and analysed all available data for MBPL in liver of endo- and ectotherms from the literature. We also add the MBPL from an unpublished study of a protoendothermic mammal as well as two species of fish. Finally, we discuss the significance of the allometry of MBPL and MR in respect to the transition from ecto- to endothermy and potential implications for a function of high MBPL in reptilian thermoregulation.

2 Material and Methods

2.1 Data compilation

Published data for liver MBPL, M and MR of 46 species (19 ectotherms, 27 endotherms) widely ranging in body mass (5.5-46500g in ectotherms, 12.7-150000g in endotherms) were compiled from peer-reviewed literature and standardised to common units (supplement S1 & S2). Here, MR refers to basal metabolic rate (BMR) in endotherms and standard metabolic rate (SMR) in ectotherms (Frappell and Butler, 2004). Any data that were not explicitly specified in the text of the publication were extracted from figures and graphs using the datathief software (v. 1.7).

MR data were only compiled for species for which MBPL data were available in the literature to provide the most accurate representation of the relational data. In the case of the blue shark (*Prionace glauca*) the SMR was estimated based on published SMR data for 21 elasmobranch species greatly ranging in M by fitting ordinary least squares regression (OLS) to Q_{10} standardised (20ºC) data (Bernal et al., 2012).

The MBPL and MR data were normalised to taxon-specific “physiological” temperatures and to the common T_a of 37ºC using taxon-specific Q_{10} coefficients for MR (White et al., 2006) and a Q_{10} of 1.4 for MBPL (Polymeropoulos et al., 2012). Initial relationships of MR and MBPL with changes in M provided in figure 1&2 were calculated using OLS, dependent on i) thermoregulatory ability (ectotherms vs endotherms) and ii) phylogeny.

2.2 Phylogenetic analysis of MR, MBPL and M

MBPL and MR of different species may not be statistically independent and failing to account for this non-independence can result in increased type I error rates if phylogeny is not accounted for (Harvey and Pagel, 1991). To incorporate phylogenetic information into our analysis, we first constructed a phylogenetic tree (supplement S3) by matching all species to a digital supertree (Hinchliff et al., 2015), encompassing all organisms using the ‘rotl’ (open tree of life) package (Francois et al., 2016) within R (R Development Core Team 2012). Depending on which taxa were compared, this tree was then pruned to include only the species/taxa under consideration. Branch length information for this tree was set to the algorithm used by Grafen (1989), where the depth of each node in the tree is related to the number of daughter species derived from that node.

We used a phylogenetic generalized least squares (PGLS) approach to analyse the relationship between log(MR), log(MBPL) and log(M) (Garland, Jr., and Ives, 2000; Grafen, 1989; Martins and Hansen, 1996) with the ‘caper’ (comparative analysis of phylogenetics and evolution in R) package (Orme, 2013) similar to previously established procedures (White et al., 2009).

Unlike the method of independent contrasts, in PGLS, the phylogenetic covariance matrix can be modified to account for the deviation of evolution from Brownian motion for the specific trait, achieved by the measure of phylogenetic correlation, λ (Freckleton et al., 2002; Pagel, 1999). For each of the models described here, λ was estimated by fitting PGLS models with values of λ finding the value that maximizes the log likelihood. λ is a multiplier of the off-diagonal elements of the covariance matrix, with λ normally ranging between 0 and 1. If the covariance matrix is based on a Brownian motion model of evolution, then $\lambda = 1$ retains that model, while $\lambda = 0$ represents phylogenetic independence. Intermediate values of λ specify models in which trait evolution is phylogenetically correlated but to a lesser extent than expected under the Brownian motion model.

We tested allometric relationships of log(MR) and log(M), log(MBPL) and log(M) as well as log(MBPL) and log(MR) and distinguished i) groups by thermoregulatory ability (endotherms vs ectotherms) and ii) by taxonomy using PGLS. For MBPL data, ranged major axis (RMA) analysis regressions were performed additionally. In instances where the regression coefficients were similar in different groups, we calculated the common regression coefficients and report their differences in fold-change of untransformed physiological data. Where regression coefficients were statistically different between groups, the Johnson–Neyman technique was used to identify regions within which the two regressions were significantly different (White, 2003). Here, we report the untransformed mass and metabolic rate range of significant difference (kg and ml h^{-1}). Even though in theory three areas can be distinguished using this technique where group x>y, x=y, and x<y, we only report values where x>y or x<y as these comparisons are biologically most relevant for the purpose of this analysis. This was achieved with the R package 'jnt'. Lastly, we evaluated if the measured values of MR and MBPL of the protoendothermic lesser hedgehog tenrec (*E. telfairi*) conformed to the endotherm or ectotherm allometric relationship for each variable. We tested if *E. telfairi* values at T_b (physiological T_b) fall within the 95% prediction confidence limits for the allometry of MR and MBPL of each group (Cooper and Withers, 2006). There does not appear to be a method to fit 95% prediction intervals to a PGLS regression with ML estimated branch length transformations (Lovegrove and Mowoe, 2014), hence the data for the tenrec were only compared to the non-phylogenetically corrected data. All statistics were conducted using R version 3.3.1.

2.3 Proton leak kinetics

The kinetics of mitochondrial proton leak for *Cyprinus carpio* and unpublished species, *Pomatomus saltatrix* and *Echinops telfairi*, were measured by determining the respiration rate required to drive the proton leak similarly to previously described methods (Jastroch et al., 2007; Oelkrug et al., 2013). In brief, measurements were performed with 1.0 mg ml^{-1} liver mitochondria (fish) and 1.5 mg ml^{-1} (tenrec) in buffer containing 4 mg ml^{-1} (fish) and 1 mg ml^{-1} (tenrec) oligomycin (to inhibit phosphorylation of ADP), 110 ng ml^{-1} (fish) and 100 ng ml^{-1} (tenrec) nigericin (dissipating the pH gradient), and 8 mM (fish) and 4.8 mM (tenrec) rotenone (inhibiting complex I) respectively. The mitochondrial membrane potential was measured simultaneously with respiration rate by using an electrode sensitive to the potential-sensitive probe, triphenylmethylphosphonium ($TPMP^+$). The $TPMP^+$-sensitive electrode was calibrated with sequential additions of $TPMP^+$ up to 2.5mmol l^{-1}, and succinate (6 mmol l^{-1} in fish and 4 mmol l^{-1} in tenrec) was added to initiate mitochondrial oxidation. Membrane potential and respiration were progressively inhibited through successive steady states with malonate up to 4 mmol l^{-1} (fish) and 8 mmol l^{-1} (tenrec). Finally FCCP (0.3 uM) was added to dissipate the membrane potential and release $TPMP^+$ from the mitochondria, allowing for correction of baseline drift. Respiration at each steady state was plotted against the corresponding membrane potential to determine the dependence of proton leak rate on the membrane potential. The $TPMP^+$ correction factor was assumed to be 0.4 (Jastroch et al., 2007; Oelkrug et al., 2013).

3 Results

The compiled dataset for MBPL and corresponding MR from the literature covers a large mass range for all taxa except amphibians and molluscs (see supplement S1 & S2). Differences in mass scaling coefficients and intercepts between taxa were analysed using PGLS and are reported as fold-changes in MR and MBPL with respect to the values in rows or where the slopes of the regressions were significantly different between taxa, the area (range of M or MR) of significant difference is reported (Table 2).

MR (ml h^{-1}) scaled positively and with a similar mass scaling exponent in endotherms and ectotherms, irrespective of *T* (Fig. 1a, Table 1 & 2). Irrespective of *T*, the intercept of endotherms was significantly higher than that of ectotherms.

MRs of individual taxa universally scaled positively with M (Fig. 1b, Table 1). Here, data for amphibians and the mollusc were omitted from any further comparative analysis due to the small number of species and non-normality of the data.

Despite some intertaxonomic differences between regression coefficients at either *T*, within the mass ranges and species under consideration in this analysis, the MRs of birds were highest, followed by eutherian and methatherian mammals, fish and reptiles. For this data set mass scaling coefficients were similar in birds, marsupials and fish and similar in reptiles and eutherians (Table 1 & 2).

As Q_{10} coefficients to correct data to 37ºC within taxa are the same, allometric scaling coefficients within taxa will not change depending on *T*. However, the differences in MR between groups are generally greatly reduced when data at 37ºC (non-physiological *T*) is considered. When comparing scaling coefficients in fish and marsupials at 37ºC there is no statistical difference between MRs compared to data at T_b where a significant difference was observed (Table 2).

MBPL (nmol H^+ min^{-1} mg $protein^{-1}$) in endotherms generally scaled negatively with M irrespective of *T* (Fig. 2a, Table 1 & 2). Interestingly the allometric relationship for ectotherms did not scale allometrically with changes in M. PGLS analysis revealed that within endotherms, birds and eutherian mammals MBPL scales negatively with M (Fig. 2b). Even though this was not the case for marsupials, RMA analysis supported the significantly negative relationships of MBPL and M for all endothermic taxa. In ectotherms MBPL scales negatively with M except for reptiles, where a significantly positive scaling relationship was observed (Fig. 2b). This discrepancy between reptiles and other ectotherms was also the major cause for the large variability within the data for MBPL in ectotherms and highlights the necessity of the phylogenetically informed analysis. Significant differences in MBPL were observed in endotherms in comparison to ectotherms overall and in reptiles compared to all other taxa, irrespective of *T* (Table 2). Equally an increased MBPL was found in marsupials compared to birds at 37ºC and in marsupials compared to fish at T_b.

MR of *E. telfairi* fell inside the 95% predicition limits of the allometric relationship for ectotherms but not endotherms. The value for MBPL of *E. telfairi* fell within the 95% predicition limit of both the endo- and ectotherm allometric relationship.

MBPL scaled negatively with MR in endotherms but not in ectotherms (Fig. 3a, Table 1). PGLS analysis did not reveal differences in regression coefficients between endothermic taxa. However, the allometric regression in fish is significantly lower compared to endotherms at T_b but not when compared at 37ºC. In reptiles MBPL scaled positively with MR. Here significantly different MR ranges were detected compared to all taxa below a MR of ≈200ml h^{-1}, irrespective of *T* (Figure 3b). Furthermore, the values for MBPL of the tenrec fell within the 95% prediction interval of endoterms as well as ectotherms.

4 Discussion

MR in endotherms is historically very well investigated and experimentally a highly controllable and defined measure. Measurements of MBPL in mammals and birds also display a high degree of reproducibility, presumably due to the physiological relevance of MBPL for MR at high T_b. The measurement of MBPL in ectotherms is experimentally more challenging. It is evident that attaining standard experimental and temperature acclimation conditions such as a post-absorptive state and constant body condition in ectotherms is more difficult and therefore MBPL displays greater intra- and interspecific variability in ectotherms (data in this analysis and Jastroch pers. obs.). Furthermore, the measurement of MBPL is highly prone to measurement error in ectotherms and marginal differences in isolation techniques and measurement protocols between laboratories may lead to significant discrepancies of the results. As expected, the variability of M, and especially of MBPL was much larger in endotherms than in endotherms, which made the formulation of clear conclusions problematic. $TPMP^+$ correction coefficients vary when comparing MBPL from different animals and tissues and are a potential additional source of error.

We are also aware that the use of constant Q_{10} values to extrapolate the data for MR and MBPL to 37°C is problematic. While our assumption of a constant Q_{10} may influence our findings, this possibility remains speculative in the absence of studies that have investigated the effects of temperature on MBPL and MR in the different taxa using e.g. a resampling-based analysis.

We would further like to acknowledge the relatively small number of available species taken into consideration for each taxon here. Nonetheless, although reptiles are represented by only five species, all measurements were performed in the same laboratory, diminishing the likelihood of discrepancies resulting from different measurement techniques. Data for a large number of mammals and all birds stem from the same laboratory, suggesting that if experimental error had occurred, it would be comparable for all taxa.

The data compiled for this analysis accurately reflect previous allometries of MR for endotherms as well as ectotherms (White et al., 2006). For any given mass, MRs of endotherms were one order of magnitude higher than ectotherms. The allometry of MBPL in endo- and ectotherms was similar to previous suggestions where endotherm MBPL scales negatively with M but ectotherm MBPL does not change with M (Hulbert et al., 2002; Porter and Brand, 1993).

While the initial mammalian MBPL allometry only contained eutherian mammals (Porter and Brand, 1993), a follow-up study with additional data and separation of marsupials and eutherian mammals strongly suggested the need for phylogenetically informed analysis to elucidate inter-taxonomic differences in MBPL allometry (Polymeropoulos et al., 2012). In the present study, increased availability of MBPL data enabled the adoption of a phylogenetically informed approach to more taxa and species of endo- and ectotherms for which data on liver MBPL were available. We demonstrate that MBPL heterogeneously scales within ectothermic taxa due to a positive mass scaling coefficient in reptiles. We also add further support to previous findings that an increase in MR between endothermic taxa is not generally accompanied by an increase in MBPL. However, an increase in MBPL from ecto- to endotherms was found with the exception of large crocodiles.

With the data available, the comparison of the MBPL allometry between fish and reptiles shows that MBPL scales heterogeneously with M and MR among ectotherms. Despite previous observations that MBPL in crocodiles is as high as in rodents (Hulbert et al., 2002), the positive correlation among reptiles has not been explicitly discussed. We propose that an increase in MBPL with increasing M may provide a thermodynamic advantage in reptiles. Assuming a reptilian body temperature of 31ºC, a T_b that large crocodiles may indeed naturally experience (Seebacher et al., 1999), the reptilian and eutherian regressions for MBPL and M intersect at ≈10.5 kg and statistically there is no difference in MBPL beyond 6.6 kg between reptiles and endotherms at a T_b of 20ºC. Hence, this M might represent a body size where high T_bs, attained behaviourally (e.g. basking), are facilitated by cellular futile heat dissipation through the MBPL. Considering that important mitochondrial mechanisms such as brown fat non-shivering-thermogenesis, which enables small mammals to survive in cold climates, is not relevant in endotherms larger than 10 kg (Oelkrug et al., 2015), the contribution of other tissues to T_b maintenance should be larger. It has been shown that liver MBPL is an important contributor to BMR and thus, maintenance of thermogenesis (Brand et al., 1994b; Rolfe and Brown, 1997). The total MBPL with liver as a selected contributor, does increase with M. As the data suggest that specific MBPL increase with M in reptiles, the contribution to thermogenesis is larger in big reptiles. The regression further suggests that at a critical M and MR, MBPL becomes indistinguishable between large mammalian and reptilian species. It is thus possible that at large sizes the energy required to maintain a high T_b is comparatively small. This could then be further facilitated by insulation such as fur or feathers. Despite potentially having the mitochondrial prerequisites allowing high rates of MBPL at large M, reptiles may not have developed the high oxidative capacity characteristic of endotherms and thus cannot maintain the higher MRs. The high MBPL in crocodiles could have evolved convergently but the overall positively scaling relationship is suggestive of an autapomorph trait. If high reptilian MBPL supported higher T_bs attained by large animals, we wonder what significance this has for the archosaurian ancestors of crocodiles and birds. If a crocodile sized archosaurian ancestor displayed endotherm or mesotherm like characteristics as suggested by some researchers (Grady et al., 2014; Seymour et al., 2004), a high reptilian MBPL may be an evolutionary vestige.

At the intersection of the allometry of MBPL at log(M) = 0, representative of approximately the smallest mass of a mammal, in theory, the maximal attainable MBPL in eutherians based on the present data is 340.09 nmol H^+ min^{-1} mg $protein^{-1}$. In order for a reptile to reach this level of MBPL at a T_b of 20ºC, the theoretical M for the reptile would be ≈41.5 tons. This M is very similar to the average M of 40.4 tons of the largest 10 extinct dinosaurs (Benson et al., 2014). If, hypothetically, the MBPL was indeed an indirect indicator of MR, and if dinosaurs displayed similar mitochondrial characteristics as extant crocodiles, then large dinosaurs may have been endothermic.

Our analysis supports previous claims that evolutionary increases in MR are not generally reflected in increases of MBPL in endotherms. Although clear differences in whole body MR are present between endotherm taxa, there is no statistical difference in MBPL. However, our data confirm that the increase in MR between ecto- and endotherms is accompanied by an increase in MBPL. In addition to this, the values of MR for the protoendothermic (but eutherian) tenrec were not different to that of ectotherms overall whereas the values for MBPL were not discernable from either endo- or ectotherms. Despite this possibly being a consequence of the large variability of MBPL data, especially in ectotherms, it appears that the MBPL value is at an intermediate level compared to endo- and ectotherms. This suggests that the lower T_b and BMR of the tenrec is not reflected in lower MBPL. Clearly, more data on the MBPL in ectotherms and other protoendotherms are required to clarify this relationship.

Overall, the liver MBPL does not appear to be an appropriate indicator of the magnitude of MR in endotherms but possibly the evolutionary process leading from ecto- to endothermy.

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Figure captions

Figure 1: Allometry of metabolic rate (MR), classified by endo- and ectotherms (a) and separated by phylogeny (b). Dashed linear regressions and diamonds denote Q_{10} extrapolations of metabolic rate data from each group's and taxon's respective body temperature (T_b) to a common temperature of 37ºC.

Figure 2: Allometry of mitochondrial basal proton leak (MBPL) in liver, classified by endo- and ectotherms (a) and separated by phylogeny (b). Dashed linear regressions and diamonds denote Q_{10} extrapolations of MBPL data from each group's and taxon's respective body temperature (T_b) to a common temperature of 37ºC. For further details, see Table 1&2.

Figure 3. Relationship between liver mitochondrial basal proton leak (MBPL) and metabolic rate (MR) in endo- and ectotherms. Dashed linear regressions and diamonds denote Q_{10} extrapolations of MBPL and MR data from each taxon's respective body temperature (T_b) to 37°C.

Table 1. Phylogenetic generalised least squares regression for log(MR, ml h^{-1}) and log(M, g), log(MBPL, nmol H^{+} min^{-1} mg protein^{-1}) and log(M) as well as log(MBPL) and log(MR) for all taxa and groups, corresponding to log transformed data in table S1 & S2. For MBPL, ranged major axis (RMA) analysis regressions were performed additionally. MR = aM^{b}; a) allometric coefficient, b) allometric exponent, λ = evolutionary model, T = temperature, T_b = body temperature. Asterisks denote the level of significance where: *; p = 0.05, **; p = 0.01, ***; p = 0.001***.

		MR/M				MBPL/ M							MBPL/MR						
		PGLS				PGLS				RMA			PGLS				RMA		
Group/ Taxon	T	a	b	R^2	λ	a	b	R^2	λ	a	b	R^2	a	b	R^2	λ	a	b	R^2
Endotherms	T_b	0.60	0.72***	0.93	0.62	2.44	-0.22***	0.61	0	2.59	-0.28**	0.61	5.04	-1.36**	0.33	0.88	7.70	-2.81**	0.57
	37 °C	0.61	0.72***	0.96	0.30	2.46	-0.23***	0.61	0	2.62	-0.29**	0.61	5.17	-1.42***	0.37	0.90	7.27	-2.57**	0.61
Eutheria	T_b	0.46	0.77***	0.99	0	2.52	-0.29*	0.60	0.45	2.75	-0.33**	0.6	5.61	-1.61**	0.51	0.95	7.02	-2.41**	0.60
Aves	37 °C	0.96	0.63***	0.98	0	2.27	-0.17**	0.70	0	2.34	-0.21**	0.70	5.93	-1.74*	0.39	1	8.20	-3.00*	0.67
	T_b	1.07	-	-	-	2.23	-	-	-	2.39	-	-	6.11	-	-	-	8.00	-	-
Metatheria	37 °C	0.70	0.65**	0.98	0	2.46	-0.16	0.54	0	2.58	-0.23*	0.54	5.91	-1.87	0.40	1	7.49	-2.77*	0.40
	T_b	0.52	-	-	-	2.39	-	-	-	2.51	-	-	5.60	-	-	-	7.86	-	-
Ectoth	37	0.0	0.73	0.8	0	1.5	0.04	0.0	1	-0.	0.5	0.0	0.1	0.90	0.	0.65	-1.	2.1	0.1

erms	°C	1	**	5		5		2		25	7	2	4		13	5	26	2*	3
	T_{b}	-0.58	0.75***	0.81	-	1.30	-	-	-	-0.002	-	-	0.48	0.86	0.122	0.60	-1.07	1.99**	0.13
Reptilia	37 °C	-0.26	0.73***	0.99	1	0.33	0.26	0.72	0	0.43	0.31*	0.71	-1.12	2.26	0.76	0	-1.53	2.57*-	0.76
	T_{b}	-0.91	-	-	-	0.58	-	-	-	0.18	-	-	-1.21	-	-	-	-1.52	-	-
Pisces	37 °C	-0.03	0.67**	0.86	0	2.17	-0.15	0.5	0.88	2.74	-0.31*	0.77	2.79	-0.23	0.012	0.35	4.54	1.72	0.08
	T_{b}	0.33	-	-	-	1.93	-	-	-	2.49	-	-	2.36	-	-	-	5.33	-	-

Table 2. Comparison of the allometric relationships of MR and M, MBPL and M as well as MBPL and MR in endo- and ectotherms and their representative taxa. Where slopes of regressions of log-transformed data between groups were equal (PGLS), common regression coefficients of raw data (physiological) were computed and fold-change between group regressions are reported. Here, we present the fold-change in respect to the groups & taxa in rows. Where significantly different slopes of regressions between taxa were found, we report regions of significance as determined by the Johnson-Neyman technique. Here < & > indicate from which boundary value up- or downwards, significant differences were detected for the respective denominator variable. Regions of equality have not been explicitly displayed. These values are to be read in conjunction with the figures. Asterisks denote the level of significance where: *; p = 0.05, **; p = 0.01, ***; p = 0.001***. *T* = temperature, T_{b} = body temperature.

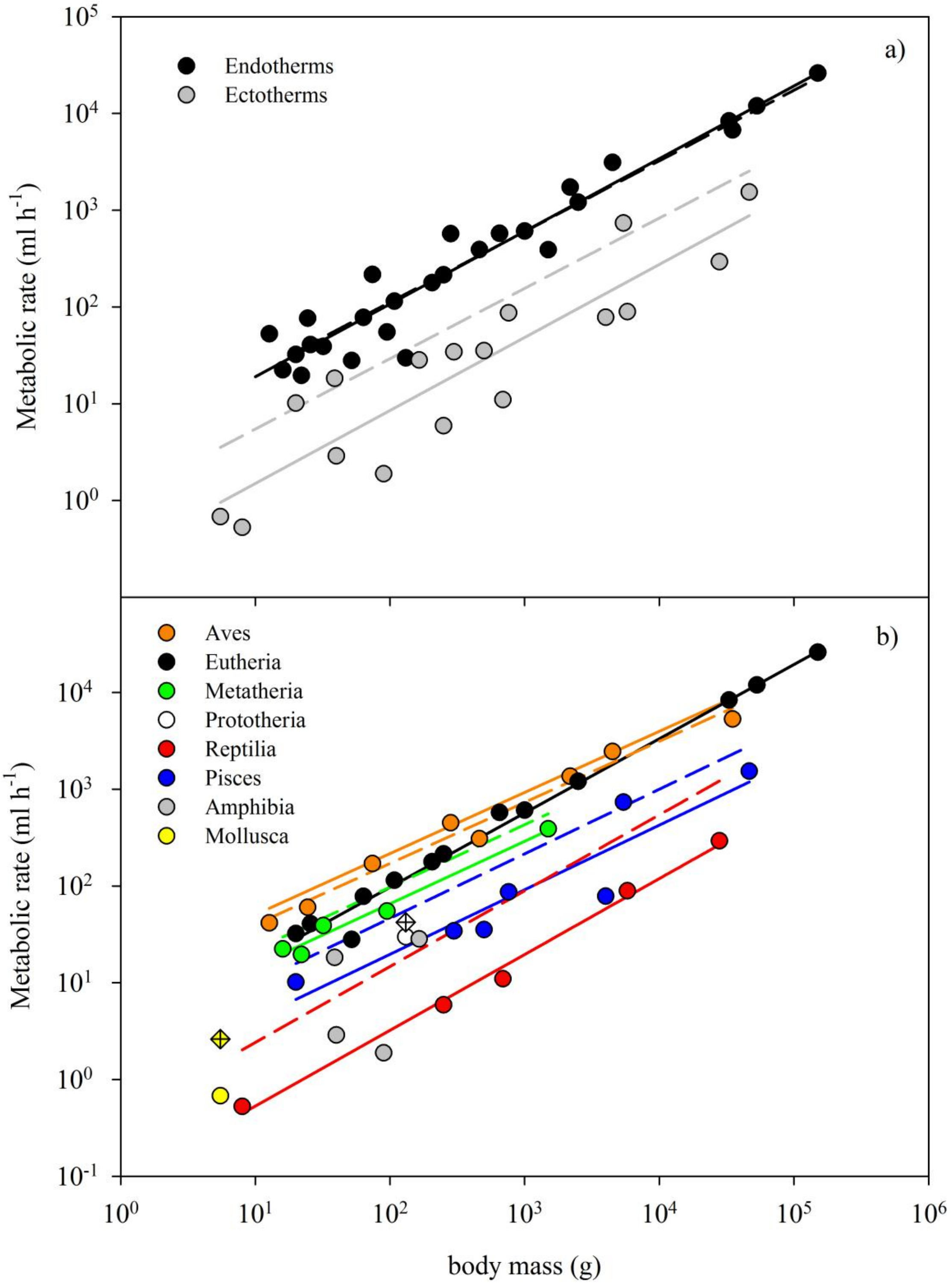
Group	Variable	Figure	*T*	Endotherms
Ectotherms	MR/M	1a	T_{b}	x12.52***
			37°C	x3.86***
	MBPL/M	2a	T_{b}	<6.56kg
			37°C	<1.37kg
	MBPL/MR	3a	T_{b}	<622.3ml h^{-1}

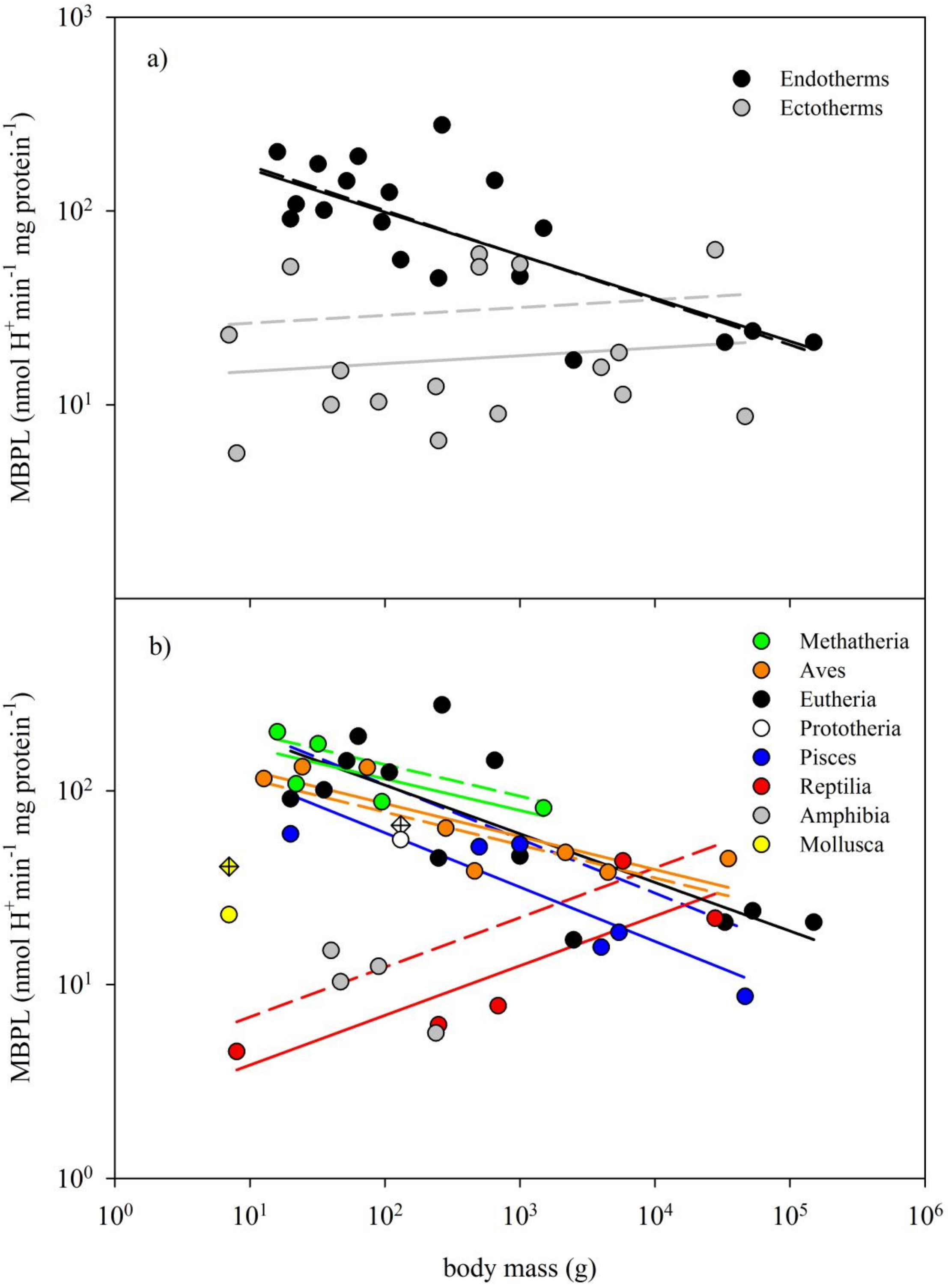
			37°C		<392.6ml h^{-1}		

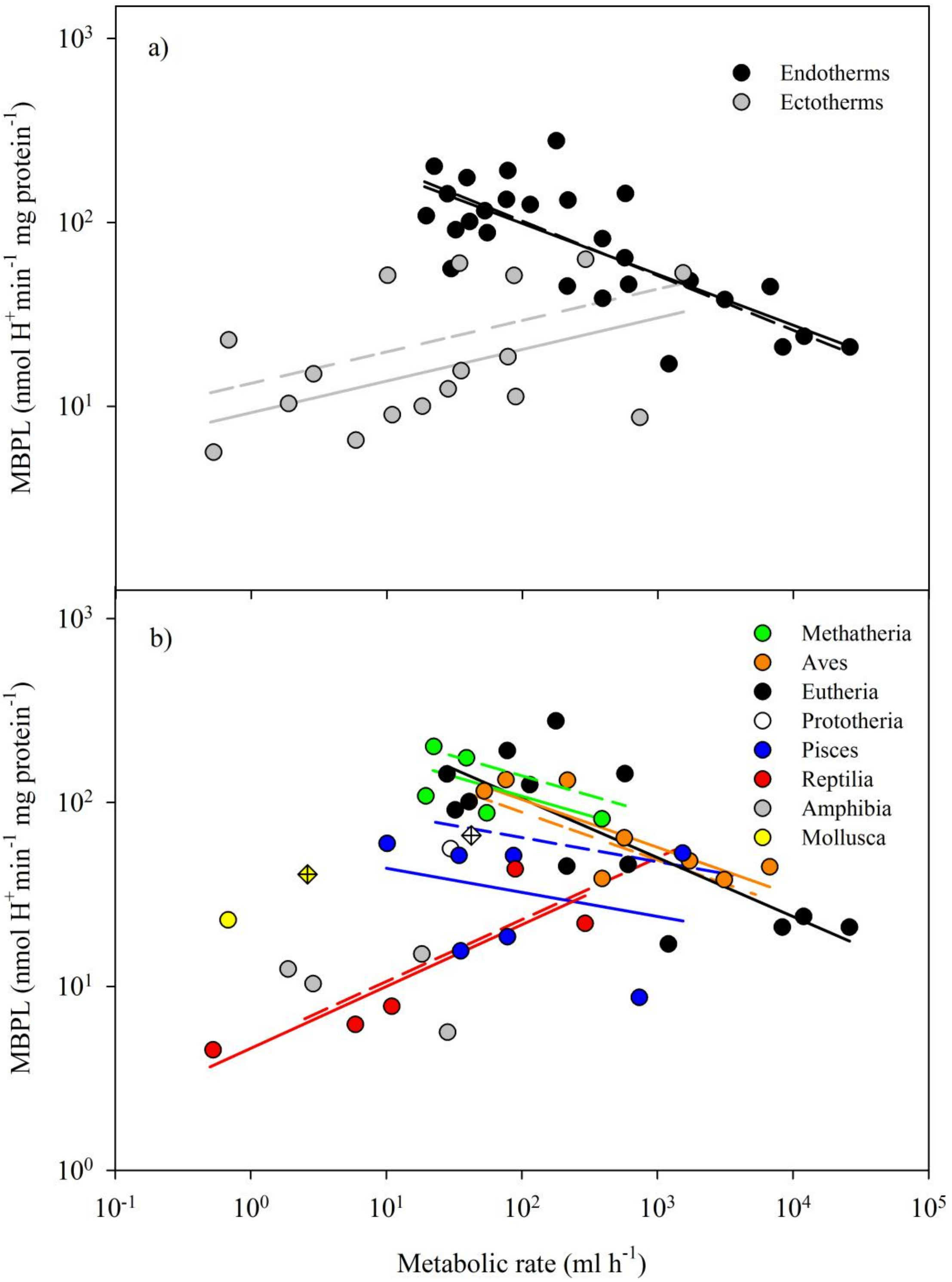
Taxon				Eutheria	Aves	Metatheria	Reptilia
Aves	MR/M	1b	T_b	<3.30kg			
			37°C	<0.75kg			
	MBPL/M	2b	T_b	x1.21			
			37°C	x1.09			
	MBPL/MR	3b	T_b	x0.91			
			37°C	x1.09			
Metatheria	MR/M	1b	T_b	>0.02kg	x3.29***		
			37°C	>6.12kg	x1.74**		
	MBPL/M	2b	T_b	x0.87	x0.74		
			37°C	x0.74	x0.57*		
	MBPL/MR	3b	T_b	x0.95	x0.96		
			37°C	x0.72	x0.63		
Reptilia	MR/M	1b	T_b	x30.04***	<2.36x10^8kg	x18.10***	
			37°C	x6.59***	<6.29x10^5kg	x5.91***	
	MBPL/M	2b	T_b	<3.50kg	<5.00kg	<1.97kg	
			37°C	<1.37kg	<1.46kg	<1.14kg	
	MBPL/MR	3b	T_b	<195.9ml h^{-1}	<287.7ml h^{-1}	<292.2ml h^{-1}	
			37°C	<254.09ml h^{-1}	<283.8ml h^{-1}	<295.6ml h^{-1}	
Pisces	MR/M	1b	T_b	x6.29***	x10.23***	x3.24*	x0.21**
			37°C	x2.68***	x3.34**	x2.06	x0.41*
	MBPL/M	2b	T_b	x1.88	x1.75	x2.15*****	<1.34kg
			37°C	x1.06	x0.89	x1.44	<1.34kg
	MBPL/MR	3b	T_b	x3.11**	x3.06**	x3.66**	<31.3ml h^{-1}
			37°C	x1.29	x1.43	x2.27	<38.6ml h^{-1}

Highlights

- The mitochondrial proton leak does not universally scale with metabolic rate.
- The reptilian proton leak reaches endotherm levels above a critical body mass.
- The protoendothermic tenrec displays intermediate basal proton leak levels.


a)
Endotherms
Ectotherms
b)
Aves
Eutheria
Metatheria
Prototheria
Reptilia
Pisces
Amphibia
Mollusca
Metabolic rate (ml h^{-1})
10^{-1}
10^0
10^1
10^2
10^3
10^4
10^5
10^0
10^1
10^2
10^3
10^4
10^5
10^6
body mass (g)


a)
Endotherms
Ectotherms
b)
Methatheria
Aves
Eutheria
Prototheria
Pisces
Reptilia
Amphibia
Mollusca
MBPL (nmol H$^+$ min^{-1} mg protein^{-1})
MBPL (nmol H$^+$ min^{-1} mg protein^{-1})
10^3
10^2
10^1
10^0
10^0
10^1
10^2
10^3
10^4
10^5
10^6
body mass (g)


a)
Endotherms
Ectotherms
b)
Methatheria
Aves
Eutheria
Prototheria
Pisces
Reptilia
Amphibia
Mollusca
MBPL (nmol H$^+$ min^{-1} mg protein^{-1})
10^0
10^1
10^2
10^3
Metabolic rate (ml h^{-1})
10^{-1}
10^0
10^1
10^2
10^3
10^4
10^5