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ARTICLE (Discoveries section)

Sexual selection of protamine 1 in mammals

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ABSTRACT

Protamines have a crucial role in male fertility. They are involved in sperm chromatin packaging and influence the shape of the sperm head and, hence, are important for sperm performance. Protamine structure is basic with numerous arginine-rich DNA-binding domains. Postcopulatory sexual selection is thought to play an important role in protamine sequence evolution and expression. Here we analyze patterns of evolution and sexual selection (in the form of sperm competiton) acting on protamine 1 gene sequence in 237 mammalian species. We assessed common patterns as well as differences between the major mammalian subclasses (Eutheria, Metatheria) and clades. We found that a high arginine content in protamine 1 associates with a lower sperm head width, which may have an impact on sperm swimming velocity. Increase in arginine content in protamine 1 across mammals appears to take place in a way consistent with sexual selection. In metatherians, increase in sequence length correlates with sexual selection. Differences in selective pressures on sequences and codon sites were observed between mammalian clades. Our study revealed a complex evolutionary pattern of protamine 1, with different selective constraints, and effects of sexual selection, between mammalian groups. In contrast, the effect of arginine content on head shape, and the possible involvement of sperm competition, was identified across all mammals.

INTRODUCTION

Understanding how evolutionary processes generate the large variation observed among species is one of the fundamental questions in evolutionary biology. Sperm competition, as a form of postcopulatory sexual selection, is a unique evolutionary process which acts on sperm of males competing for the fertilization of ova (Parker 1970). This selective process drives the adaptation of sperm form, function and fertility (reviewed in Birkhead and Møller 1998, Simmons 2001, Birkhead et al. 2009). Uncovering how sperm competition can shape phenotypes through changes in gene and regulatory DNA sequences would allow us to draw conclusions about evolutionary process in general, the evolution of function, and causes underlying medical conditions such as sub- or infertility.

Sperm morphology varies considerably among species (Cummins and Woodall 1985, Roldan et al. 1992, Pitnick et al. 2009). Changes in sperm head size and shape are important determinants of sperm swimming velocity that, in turn, is a key determinant of male fertility. Sperm head morphology seems to be greatly influenced by the condensation of nuclear chromatin (Balhorn 2007, Cree et al. 2011), and variation in the process of histone replacement and in the proteins involved can have a great effect on the shape of the sperm head (Balhorn 2007). Among these proteins, protamines, which are basic arginine-rich sperm nuclear proteins, play a crucial role. They are involved in the train of successive replacements of histones to transition proteins to protamines (Oliva and Dixon 1991). This process results in a tightly packed, efficiently shielded chromatin and an almost complete silencing of expression (Balhorn 2007). Due to the existence of cysteine residues, protamines of eutherian mammals form disulphide bonds within and between protamines stabilizing the chromatin structure (Balhorn et al. 1992, Queralt et al. 1993).

Because protamines are crucial for the process of chromatin condensation, alterations in their expression can affect fertility (Cho et al. 2003, Aoki et al. 2005, Oliva 2006, Carrel et al. 2007). In men, changes in sperm protamine content affects sperm head morphology, as well as sperm motility (Aoki et al. 2005). In mouse models, modification of the protamine content is linked to sperm morphological abnormalities and decreases in sperm motility (Cho et al. 2001). Furthermore, changes in protamine gene sequences and protamine expression ratios affect head size and shape in rodents (Lüke et al. 2014a,b).

Protamines can be found in the sperm of protostomes as well as deuterostomes (Oliva and Dixon 1991). They most likely evolved in chordates from a sperm-specific histone H1 through a shift from a lysine-rich histone H1 to the arginine-rich protamine (Lewis et al. 2004). The selection for an arginine-rich protamine is thought to be driven by selective constraints imposed by internal fertilization (Kansinsky et al. 2011). Arginine richness, as opposed to lysine, results in a higher affinity for the protein to bind to the DNA molecule as well as a greater binding flexibility due to its guanidinium group (Ausio et al. 1984, Cheng et al. 2003). In mammals, two types of protamines have been identified. Protamine 1 is found in all mammals and shows sequence similarities to bird protamine 1 (Oliva and Dixon 1991). Protamine 2 is found in primates and rodents but evidence for the existence of the protamine 2 gene sequence, transcripts and, in some cases, mature protein has been presented for several species of other mammalian clades (Oliva 2006, Balhorn 2007). Mammalian protamines contain three or more DNA anchoring domains which comprise 3-7 arginine residues that are separated by uncharged amino acids (Balhorn et al. 1999). Within mammals in general protamines are thought to be diverse, especially in the C-terminal region, but contain conserved regions that are also found in birds (N-terminal ARYR, SRSRSR phosphorylation site, 3 arginine clusters) (Queralt et al. 1993). In marsupials (and birds) cysteine residues are absent except in the *Planingale* genus.

Several studies claim that protamines are the fastest evolving reproductive proteins, exhibiting high structural heterogenity, when compared to other sperm nuclear basic proteins, (Oliva and Dixon 1991, Wyckoff et al. 2000). However, evidence of purifying selection acting on the maintenance of the high arginine concentration exists, while the position of arginine residues seems to be variable, leading to the conclusion that protamine 1 may be affected by positive and purifying selection alike (Rooney et al. 2000). Evidence of positive selection in the protamine 1 gene sequence has been detected in a small group of primates (Rooney et al. 1999, Wyckoff et al. 2000). Other studies have demonstrated different selective constraints in other mammalian species (Martin-Coello et al. 2009, Lüke et al. 2011). Among cricetid rodents we showed that protamine 1 is under conserved selective constraint with signs of positive selection restricted to specific codon sites. On the other hand, the gene sequence of protamine 2 was shown to be under relaxed constraint on the way to degradation.

There is limited evidence for an effect of sperm competition on coding sequences of sperm proteins. The seminal fluid proteins SEMG2 and SVS, the sperm surface proteins ADAMs 2 and 18, and the acrosomal proteins Zonadhesin and SPAM1 have all been found to be positively affected by level of sperm competition in primates (Dorus et al. 2004, Herlyn and Zischler 2007, Ramm et al. 2008, Finn and Civetta 2010, Prothmann et al. 2012). For butterflies, Walters and Harrison (2011) were able to show a relaxation of purifying selection on seminal fluid proteins in the absense of sperm competition. Similarly, sexual selection on protamine 2 in rodents has been reported to increase selective constraint (Lüke et al. 2011). The evolution of protamine genes and regulatory sequences, as well as their expression, is affected by sperm competition in different groups of rodents. Contrary to expectations, sexual selection could not be detected to act on protamine 1 gene in cricetids or murids. In contrast, sperm competition was shown to reduce the relaxation acting on the gene sequence of protamine 2, resulting in a more conserved state of the gene in species with high levels of sperm competition (Lüke et al. 2011).

Until now sexual selection on protamine gene sequences and its effect on sperm head phenotype has only been studied in rodents. Thus, in this study we aimed to analyze evolutionary patterns of protamine 1 coding sequence and their effects on sperm head dimensions on a broader scale across mammals. The extensive availability of data on protamine 1 sequence in Genbank allowed us to study the evolution and selection of protamine 1 across major mammalian clades. Here we analyze the evolution and selection of protamine 1 in 237 mammal species. We examined the evolutionary rate on whole sequence and site level and tested for sexual selection and possible effect on head dimensions. Additionally, we tested for sexual selection acting on arginine content. We expected to find differences in selective constraints between clades especially in clades with high diversity in levels of sperm competition. We hypothesized that protamine 1 would be sexually selected across mammals and that changes in protamine 1 coding sequence and arginine content may influence head dimensions.

RESULTS

Sequence properties

A summary of all data used in this study is shown in Table S1. Amino acid alignments for all

mammalian species (Eutheria and Metatheria) are shown in Figures S1-S3.

Amino acid frequency and sequence similarity within mammals, as well as within eutherians and marsupials, and within different clades, were compared. Results are shown in Table S2.

Metatheria showed significantly higher relative arginine content than Eutheria (t(219.33)=-17.26, p<0.001). Within Eutheria, a one-way ANOVA revealed significant differences in arginine content between clades (F(4,144)=100, p<0.001). Post-hoc comparisons showed that Chiroptera and Rodentia exhibit significantly higher content of arginine in the coding sequence of protamine 1 (Fig. 1*A*, Table S3). Within Metatheria, Diprotodontia showed a significantly higher arginine content than Dasyuromorpha (t(52.8) = 9.42, p < 0.001)(Fig. 1*B*).

Measures of sequence divergence and amino acid frequency within clades are shown in Table S2. Considering the differences in sequence properties between Eutheria and Metatheria all subsequent analyses which included correlation with arginine content were done separatedly for eutherian and metatherian clades.

Selective pressures across mammals

The phylogenetic tree used in evolutionary analyses is shown in Figure S4. We tested for the general mode of selection acting on protamine 1 in mammals. To obtain the background pressure acting on the whole sequence across all mammals we calculated the evolutionary rate (ω) for the whole tree on the whole protamine 1 sequence (Codeml (PAML4) model M0 as explained in Supplementary materials and methods). The evolutionary rate calculated across mammals in model M0 was ω =0.38.

Comparison of selective pressures

To assess the comparative selective pressures for the entire protamine 1 sequence and the directed selective pressures on codon sites we employed the branch analysis and the branch-site analysis (see Supplementary materials and methods), alternatively marking metatherians as foreground against eutherians as background, and then eutherians as foreground against metatherians as background. The branch analysis comparing Eutheria and Metatheria suggests a significantly stronger selective constraint on metatherians than eutherians (Eutheria: LRT M0 vs MC non significant, M0 ω considered, Metatheria: LRT M0 vs MC non significant, M0 ω considered, Metatheria: LRT M0 vs MC significant, ω is significantly lower than 1) (Table 1). The branch-site test showed no directed selection on codon sites for Eutheria or Metatheria (BSfixed vs BS non significant)(Table 1).

Secondly, we tested for differences between mammalian clades by employing

branch- and branch-site analyses for each group, marking the clade under analysis as foreground against their corresponding eutherian or metatherian background. In the comparison of eutherian clades, we found that Primates, Rodentia and Cetacea showed selective constraints different from the background (M0 vs MC significant, MC ω considered). In contrast, Chiroptera and Artiodactyla showed similar selective constraint as their background (M0 vs MC non significant, M0 ω considered). Primates and Cetacea showed signs of relaxed selective constraint (MCfixed vs MC non significant, ω did not differ significantly from 1), while Rodentia, Chiroptera and Artiodactyla showed evidence for purifying selection (MCfixed vs MC significant, ω is significantly lower than 1). Branch-site analysis revealed evidence for positive selection on codon sites for Chiroptera and Cetacea (BSfixed vs BS significant, PSS detected). For Artiodactyla Bsfixed vs BS is significant while no positively selected sites were detected. This might be interpreted as evidence for high purifying selection on codon sites. Primates and Rodentia showed no evidence for positive selection at the site level (BSfixed vs BS non significant) (see Table 1).

In the comparison of metatherian clades neither Dasyuromorpha nor Diprotodontia showed selective constraints differing from the background. The selective constraint is therefore considered the same for both groups (M0 vs MC non significant, M0 ω considered). Diprotodontia showed evidence of purifying selection acting on the protamine 1 coding sequence (MCfixed vs MC significant, ω is significantly lower than 1) while the likelihood ratio test of MCfixed vs MC was not significant for Dasyuromorpha, likelihood ratio test M0 vs MC shows this clade to be evolving under the same selective constraints as the background and Diprotodontia. We therefore consider purifying selection to be the most probable mode of selection acting on this clade. The branch-site test showed directed positive selection on codon sites for Dasyuromorpha and Diprotontia (BSfixed vs BS significant)(Table 1).

The ω calculated by using COEVOL (see below) for each mammalian species is shown in Table S1.

Relationships with a proxy of sexual selection

Rationale for analyses

To examine the possible associations between relative testes mass (a sperm competition proxy) and protamine 1 coding sequence within all mammals and, subsequently, for each clade, we employed COEVOL (a Bayesian Markov Chain Monte Carlo sampling software for comparative analyses) to test for correlations between genotype and phenotype data.

Furthermore we calculated the clades' median absolute deviation of residual testes mass and arginine content to test for an effect of variability of sperm competition ("median absolute deviation of residual testes mass") on the clades' ω value (Codeml (PAML4) model M3, see Supplementary materials and methods), arginine content, and arginine content

variability ("median absolute deviation of arginine content"). The median absolute deviation (or absolute deviation around the median) is a robust measure of variability. It is calculated as the median of the absolute deviations from the data's median (i.e., the median of each absolute value minus the median) and it is not sensitive to the presence of outliers (Huber 1981).

Relationships with relative testes mass across mammals

COEVOL correlation analyses showed no significant correlations between relative testes mass and protamine 1 ω in mammals. A nearly significant positive correlation was found for arginine content with relative testes mass (Fig. 2, Table S4). Arginine content was significantly and negatively correlated with ω (Fig. 2, Table S4). For a comparison between amino acid substitution according to PAML codeml marginal ancestral reconstruction and evolutionary rate as computed by codeml see Figure S5. For Eutheria a significant positive correlation was found between arginine content and relative testes mass (Fig. 2, Table S4).

Relationships with relative testes mass within clades

COEVOL analysis showed significantly positive correlations between arginine content (as percent of sequence length) and relative testes mass for Rodentia and Cetacea (Fig. 2, Table S4). Tests for sexual selection could not be done for Artiodactyla or Chiroptera due to limited data for testes mass.

Median absolute deviation of relative testes mass for clades correlated positively with the clade's ω (M3) and with the clade's median absolute deviation of arginine frequency. On the other hand, it correlated negatively with the clade's mean arginine frequency (Table S3, Table S4).

Because the sequence of protamine 1 was significantly longer in marsupials than in eutherians (t(227.5) = -72.69, p < 0.001) (Fig. 3A) we tested for possible relationships between relative testes mass and sequence length within this subclass. Due to the presence of distinct sequence length groups we chose to test differences between length groups for corrected testes mass using ANOVA, followed by visual analysis of a trend when plotted against corrected testes mass. We found significant differences between sequence length groups for relative testes mass (F(43)=7.73, p<0.001) in Metatheria. Post-hoc comparisons showed that the lowest sequence length group (183bp) has significantly lower residual testes mass than the two highest sequence length groups (192bp, 195bp), and that the intermediate length group (189bp) has a significantly lower residual testes mass than the 192bp length group (Fig. 3*B*, Table S3).

Associations with sperm head dimensions

In order to test for the effect of changes in the coding sequence of protamine 1 on sperm head dimensions, the evolutionary rate and the arginine content were used in COEVOL post-analysis to test for correlation with relative head length (HL), relative head width (HW) and head elongation (HL/HW). For Chiroptera, Artiodactyla and Dasyuromorpha data available for sperm head dimensions were insufficient for regression analyses. For Primates data available for relative HW were insufficient for regression analysis. We examined possible relationships across mammals and within clades.

Across mammals and within eutherians we found a significant negative correlation between arginine content and relative HW (Fig. 2, Table S4) as well as a trend for positive correlation between arginine content and head elongation. In Eutheria the negative correlation between arginine content and relative HL was nearly significant. In Rodentia COEVOL correlation analysis showed a significant negative correlation between arginine content and relative HW (Fig. 2, Table S4) as well as a negative trend for arginine content with relative HL and a positive trend for head elongation. In addition, a trend for a negative correlation was found in Cetacea for arginine content and HW (Fig. 2, Table S4).

DISCUSSION

In this study we analyzed the evolution of protamine 1 in mammals and the possibility that postcopulatory sexual selection may be an important selective driver for this protein. Our results suggest that postcopulatory sexual selection drives maintenance of high arginine content in protamine 1 amino acid sequences across mammals and, in turn, that higher arginine content associates with reduced sperm head width. There was also evidence that higher protamine 1 evolutionary rates are associated with lower arginine content across mammals. On the other hand, the relationship throughout mammals between postcopulatory sexual selection and the arginine content of protamine 1 could not be confirmed within all the mammalian clades but was observed only in two of them, rodents and cetaceans. Within metatherians, the length of protamine 1 seems to be important as it was positively associated to sperm competition levels. Finally, we found that higher variability in inferred sperm competition levels to be related to a higher evolutionary rate and, in addition, a higher variability in arginine content (see Fig. 4 for a summary of the relationships found).

Protamine sequence length is sexually selected in Metatheria but not in Eutheria

In this study we analyzed protamine 1 gene sequences of 237 mammalian species of eutherians and metatherians. Marsupial protamine 1 differs from the eutherian gene in several important ways and, in fact, is more similar in structure to bird protamines (Retief et al. 1995a). Whereas eutherian protamine 1 contains 5 to 9 cysteine residues, which allow

protamines to form complex tertiary structures by building disulfide bridges between and within protamines (Balhorn 1982, Oliva and Dixon 1991, Balhorn et al. 1995), metatherian protamine sequences, like those of birds and fishes, lack these residues. An exception is the Planigale genus, which seems to have obtained up to 7 cysteine residues by convergent evolution (Retief et al. 1995a). The lack of cysteine residues in most metatherians results in a less stable chromatin which is more easily decondensed compared to that of eutherian mammals (Cummins 1980). Metatherian protamine 1 is significantly longer than eutherian's and contains more serine and tyrosine. N- and C-terminal serine residues, as well as the Cterminal threonine residues, are known phosphorylation targets in protamines (Balhorn 2007). It seems plausible that the higher serine content in metatherian protamine 1 might lead to a higher degree of phosphorylation. Due to these differences, metatherians are likely to bind chromatin in a slightly different manner than eutherians (Balhorn 1985). In fishes and birds the size of protamines was found to be an important factor in chromatin condensation (Oliva et al. 1987, Oliva and Dixon 1991). Since metatherians, as birds and fishes, cannot rely on disulfide bridges to stabilize the chromatin, a longer protamine sequence might be necessary for efficient chromatin condensation. We therefore tested if sexual selection might be affecting protamine 1 sequence length within metatheria and found significant differences in sperm competition levels between sequence length groups across metatherians following a positive trend. Additional analysis including a greater variety of metatherian genera will be necessary to confirm this relationship. However, our results suggest sequence length to be a key factor in stabilizing chromatin in metatherian mammals as shown by its selection towards an increase of sequence length in species with higher levels of sperm competition.

Sperm competition maintains high arginine content of protamine 1 through sequence conservation

Protamine 1 is crucial for correct sperm chromatin condensation. Alterations in protamine content are linked to morphological abnormalities of the sperm head, increases in DNA damage, and decreases in sperm motility (Belokopytova et al. 1993, Cho et al. 2001, Aoki et al. 2005), and strongly affect male fertility (Cho et al. 2003, Aoki et al. 2005, Oliva 2006, Carrel et al. 2007).

It was known that protamines with higher arginine content form more stable chromatin complexes, replace histones more efficiently and are more efficient in chromatin decondensation following fertilization (Ohtsuki et al. 1996). We found arginine content of protamine 1 to be correlated positively with a proxy of sperm competition levels in mammals. This correlation was significant when Metatheria was not included in the analysis; metatherians have a significantly higher arginine content than eutherian mammals and might therefore affect the regression slope. However, when metatherians were included a clear trend was still observed. Species experiencing higher selective pressure through sperm competition showed higher arginine content in the protamine 1 amino acid sequence. Additionally, we found that an elevated evolutionary rate is generally associated with less arginine in mammals. However an association between sperm competition proxy and evolutionary rate was not found within mammalian clades. Sexual selection on protamine 1 therefore seems to be specifically concentrated on arginine content. Rooney et al (2000) described an unusual form of purifying selection on protamine 1. While the arginine content seemed to be conserved throughout mammals the distribution of arginine residues varies to a great extent. Here we were able to provide evidence for sexual selection as a factor in this unusual form of selection acting on protamine 1. Arginine content seems to be under even stronger selective constraint in highly promiscuous species. These relationships are clear throughout mammals, but we are presented with a more complex scenario when we examine these associations within clades. Evidence of sexual selection on arginine content was only found in two eutherian clades (rodents and cetaceans). If high arginine content is beneficial for species with high levels of sperm competition, and high evolutionary rate shows a general trend towards lower arginine content throughout mammals, why do we not find the same trend when clades are examined?

The gene sequence of protamine 1 seems to be under purifying selective constraint across mammals and within all clades, with the exception of primates and cetaceans, which show evidence of relaxation. We analyzed the evolutionary rate not only on whole sequences but also performed an additional study calculating the possible positive selection at site level. We found signals for positive selection on site level in Chiroptera, Cetacea and in both metatherian clades. Previous studies show that, in rodents, changes in the protamine gene sequences and protamine expression ratio are associated with head size and shape changes (Lüke et al. 2014a,b). Within the coding sequence of protamine 1 in mammals highly variable, as well as highly conserved regions can be found (Oliva and Dixon 1991, Rooney et al. 2000, Wyckoff et al. 2000). In primates the coding sequence was shown to be positively selected (Wyckoff et al. 2000), while within rodents it has been shown to be functionally conserved, with directed positive selection on a few C-terminal sites (Lüke et al. 2011). It seems that the unusual form of selection affecting protamine 1 is driven by adaptations to sexual selection (Oliva and Dixon 1991, Rooney et al. 2000, Wyckoff et al. 2000). Due to the importance in sperm form and function, as well as the protection of sperm DNA, protamine 1 might be affected by different types of selection resulting in a careful balance between conservation of function and adaptations to high sperm competition levels.

The main reason for sexual selection acting on protamine 1 is proposed to be its effect on sperm head shape. Here we found arginine content to be positively associated with slimmer sperm heads including a possible postitive effect on sperm head elongation. Yet

also here the relationships seem to be present as a general trend across mammals but not to be as straightforward when focusing on clades. As an adaptation to high levels of sperm competition protamines might affect the head shape to become more hydrodynamically efficient thus favoring higher sperm velocity. However, which type of sperm head is the most beneficial to ensure high sperm velocity is presently unknown. Considering the high variety of sperm head shapes and sperm sizes in mammals (Cummins and Woodall 1985, Roldan et al. 1992, Pitnick et al. 2009) it is plausible that a complex interplay between sperm metabolism, pattern of flagellar movement, total sperm size, hook shape and nucleus shape determine the ability of the sperm to swim faster (Malo et al. 2006, Gómez-Montoto et al. 2011, Tourmente et al. 2013). For example, while a previous study found that relative reduction in protamine 2 content seems to lead to a more beneficial head shape in mice in terms of reproductive fitness (Lüke et al 2014b), we might not be able to extrapolate this relationship to other groups of species in which, for example, sperm heads do not have a hook or the flagellum shows a different beating pattern. Broader sperm heads might be beneficial for certain sperm morphologies, especially taking into account sperm head thickness. Sexual selection might therefore be affecting the arginine content and evolutionary rate of protamine 1 to varying degrees in different groups of species.

In general terms high evolutionary rate seems to be associated with lower arginine content. We found this relationship across mammals but not within clades. Sexual selection was found to act on cetacean and rodent arginine content. Rodents show high arginine when compared to other eutherian clades which could be proposed to be the result of sexual selection driving high arginine content in this clade. Cetaceans however contradict this theory since this clade shows a comparably low arginine level in protamine 1 sequence. A family level analysis revealing possible differential selection on deeper organizational level might be useful to understand the role sexual selection plays on protamine 1 in this clade. Bats show a high level of arginine when compared to other eutherian mammals and we might expect this to be a result of sexual selection acting to maintain, or promote, a high arginine content. However, not enough data are available on body mass and testes mass in the literature to test for associations in this clade. Thus, further studies are needed to understand the role of protamine 1 in bats.

Less selective constraints in clades with highly variable sperm competition levels?

When comparing selective constraints on protamine coding sequences between clades we observed conserved constraints in all groups except for primates and cetaceans, which showed a comparatively relaxed constraint. This seems to coincide with a comparatively higher variability in inferred levels of sperm competition in both groups. The clades' evolutionary rates correlated positively with clades' variability of sperm competition. While the

clades' variability in arginine content seemed to increase with clades' variability of sperm competition, the clade mean arginine content decreased. Similarly, when looking at eutherians and metatherians in general, the lower selective constraints in metatherians seemed to coincide with lower variability in levels of sperm competition compared to eutherians. A group of species exhibiting big differences in sperm competition therefore also show bigger differences in their coding sequences resulting in the detection of a higher evolutionary rate and less selective constraints in the group. As a result, clades with high variability in sperm competition show higher variability and lower mean arginine content. Therefore, while the effect of sexual selection on evolutionary rate or arginine content seems to be difficult to detect by analyzing the relationship directly through regression analysis, the simple existence of an effect of sexual selection on protamine 1 evolutionary rate might be detected by analyzing levels of variation. This might lead to the conclusion that protamine 1 is sexually selected in primates and cetaceans while the nature and direction of the selection is complex and could be different between primate and cetacean families.

Conclusions

Adaptation to high levels of sperm competition seems be a factor in the unusual form of purifying selection of protamine 1 as described by Rooney et al (2000). We found evidence for sexual selection, in the form of sperm competition, maintaining a high arginine content across mammals, while changes in the protamine 1 coding sequence seem to be associated with less arginine. A higher arginine content seems to be associated with a reduction in sperm head width and was previously found to result in more efficient chromatin condensation. Due to a lack of cysteine residues, metatherian protamine 1 cannot rely on disulfide bridges to stabilize chromatin; this may be compensated by an increase in sequence length, which is sexually selected. Within mammalian clades sexual selection on arginine content was detected within Rodentia and Cetacea, while we found differences in selective pressures on sequences and codon sites between mammalian clades. Due to the wide variety of sperm head morphologies within mammals changes in arginine content and changes in protamine coding sequence might be beneficial in some mammalian groups and disadvantageous in others. This would result in a complex evolutionary pattern of protamine 1 and difficulties in detecting sexual selection within mammalian groups. However, due to general sequence conservation, and the importance of arginine in chromatin condensation and head shaping, a general trend for sexual selection acting on arginine content, and its possible effect of sperm head width, has been identified in mammals. Since the interplay between evolutionary rate, arginine content, sperm head morphology, and swimming speed seems to be very complex, the effect of sexual selection on protamine 1 might not be readily detected in some groups. However, if an effect of sexual selection exists in general, it might

be revealed when testing for an association between the level of variability in sperm competition and evolutionary rate.

MATERIAL AND METHODS

Sequence data and phylogenetic tree

Gene sequences of mammalian protamine 1 for which at least 10 species were available were obtained from NCBI Genbank and previous publications (Lüke et al. 2011) (Table S1), visualized with Geneious 5.5.9 (Biomatters, http://www.geneious.com/) and trimmed to coding sequence based on NCBI Genbank information. Sequences were manually checked to ensure correct trimming. Translation alignments based on the muscle alignment algorithm implemented in Geneious 5.5.9 were performed and checked manually. In addition to an alignent including all 237 mammalian species (translated alignment see Fig. S1) we performed separate alignments for each mammalian clade studied (Primates, Rodentia, Chiroptera, Cetacea, Artiodactyla, Dasyuromorpha, Diprotodontia) as well as separate alignments for eutherians (translated alignment see Figs. S2 and S3). Amino acid frequencies, pairwise percent identity and percentage of identical sites were calculated using Geneious 5.5.9 for each alignment.

The phylogenetic tree of 237 included mammalian species was constructed as a consensus of phylogenies available in literature (Fig. S4 and references therein).

Phenotype data

Data on body mass, testes mass and sperm dimensions were obtained from the literature (Table S1 and references therein). Testes mass corrected for body mass ("relative testes mass", is a well recognized proxy for sperm competition; Birkhead et al. 2009, Gomendio et al. 1998, Soulsbury and Dornhaus 2010). Testes and body mass data were available for 132 of the 237 species for which protamine 1 information was available. Data on sperm head width were found for 65 species, and sperm head length for 87 species. Residual testes mass data were obtained from a regression analysis including body mass as independent and testes mass as dependent variable. Residual testes mass was obtained for each clade separately due to previously reported differences of body mass and testes mass regression slopes between clades (MacLeod 2010). Residual testes mass was used for graphical representation of multiple regression results and comparisons between clades. Because total sperm length varies greatly among-species, and drag resulting from head size should be analyzed taking into account the length of the flagellum (Humphries et al. 2008), sperm head length and head width were each employed in analyses as proportions of total sperm length (TSL) (hereafter, relative HL and relative HW).

Analysis of selective pressures

The nonsynonymous/synonymous substitutions rate ratio ($\omega = dN/dS$) is an indicator of selective pressure at the protein level, with ω =1 indicating neutral evolution, ω <1 purifying selection, and ω >1 diversifying positive selection (Goldman and Yang 1994). To estimate rates of sequence evolution across mammals and within clades we used the application Codeml implemented in PAML 4 (Yang and Rannala 1997, Yang 2007). Applied models are explained in Supplementary materials and methods. Likelihood-ratio-tests (LRT) were performed to test if the alternative model presents a better fit to the dataset against the null model. For the Codeml codon frequency setting, as well as the setting for number of categories, we used the setting with the best fit for each analysis according to the preliminary likelihood-ratio-analysis. Branch lengths calculated in the model M0 "one-ratio" (see Supplementary materials and methods) were used as input for subsequent models. For estimation of rates of sequence evolution per branch for subsequent correlation with phenotypic data we employed COEVOL (Lartillot and Poujol 2011).

Association between evolutionary rate and phenotype data

In order to test for associations between protamine 1 and relative testes mass as well as its effect on sperm head dimensions we employed the program COEVOL a Bayesian Markov Chain Monte Carlo sampling software for comparative analyses, to test for correlation between genotype and phenotype data. It allows for joint estimation of evolutionary rate for the input alignment and changes in the phenotypic input variables. It allows for correlation and multiple regression analyses. Unlike previous approaches this software allows for detection of associations between genotypic and phenotypic data taking into account all parameter estimates of internal nodes. Additionally, COEVOL can be used to estimate ancestral traits and evolutionary rates (Lartillot and Poujol 2011). We ran two independent chains per analysis providing an input nucleotide alignment, data-table of guantitative traits and phylogeny without branch-lengths (Fig. S4). Chains were run until discrepancy between summary statistics was below 0.02 and effective size larger than 300. After runs are completed correlations, divergence times and ancestral traits were estimated in COEVOL. To test for associations between testes mass and other variables, correlations were corrected for body mass computed by COEVOL using a multiple regression approach. For correlation analyses we used a burn-in of 300. Branch lengths were calculated by COEVOL. The program additionally produces trees and tabulated lists of ancestral rates and traits. These data were used for visualization of results (see Fig. 2).

Phyologenetically corrected regression analysis (PGLS)

To test for correlations between clades' evolutionary rates and sperm competition variability

we employed the phylogenetic generalized least squares approach (PGLS) (Freckleton 2002). Associations between genetic and morphometric traits should also take into account that such traits are not independent from their phylogenetic history (Harvey and Pagel 1991). The PGLS approach has been shown to be a powerful tool to detect associations of this kind (Rholf 2001), and it has been used in earlier studies in combination with the root-to tip dN/dS method showing genetic-morphometric associations (Lüke et al. 2011, Montgomery et al. 2011, 2012, Pointer et al. 2012). We performed PGLS analysis using CAPER v0.5 (Orme et al., 2012) package for R (v3.0.1; R Foundation for Statistical Computing 2013).

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FIGURE LEGENDS

Figure 1. Boxplots for arginine content (as percentage of total sequence length). (A) Eutherian clades. (B) Metatherian clades. Clades with comparatively high arginine content within each subclass are shown as hatched bars. Comparisons are statistically significant between all clades except between Artiodactyla and Primates.

Figure 2. Visualization of associations identified using COEVOL. (A) Phylogenetic visualization of COEVOL computed protamine 1 dN/dS reconstruction for mammalian species. (B) Phylogenetic visualization of COEVOL computed reconstruction of protamine 1 arginine content (%) for mammalian species. (C) Scatterplot showing relationship between protamine 1 arginine content and dN/dS including reconstructed ancestral node data. (D) Scatterplot showing correlation between residual testes mass and protamine 1 arginine content (as percentage of total sequence length) in mammals (Eutheria and Metatheria highlighted). (E) Correlations between protamine 1 arginine content (as percentage of total sequence length) and relative sperm head width in mammals (Rodentia and Cetacea highlighted). (F) Correlations between protamine 1 arginine content (as percentage of total sequence length) and relative sperm head width in mammals (Eutheria and Metatheria highlighted). (G). Correlations between protamine 1 arginine content (as percentage of total sequence length) and relative sperm head width in mammals (Rodentia and Cetacea highlighted). (P values are shown for all mammals and for highlighted clades and represent level of significance according to COEVOL analysis.

Figure 3. Sequence length in Metatheria. (A) Boxplot visualizing significant difference in protamine 1 coding sequence length between Eutheria and Metatheria. (B) Visualization of associations between metatherian protamine 1 coding sequence length groups and residual testes mass.

Figure 4. Schematic presentation of relationships found in this study. Arrows represent relationships found across mammals. Dashed arrows represent relationships not found across mammals and proposed to be diverse between groups. Positive correlations are marked with (+) and negative correlations are marked with (-). Diverse or unknown relationships are marked with (?).

												<u>н</u>		
		LR	Ts for		LRTs for bra	nch-site		Pro	portion	of sites	s in ω	ttp:		
		branch	n analysis		analys	is		S	ite clas	ses (B	S):	//mt	inte	erpretation
			2Δ		2Δ							Positively	Selection over	•
Foreground	2Δ		(MCfixed-		(BSfixed-							selected sites	whole	Directed selection
branches	(M0-MC)	Ρ	MC)	Р	BS)	Р	ω	0	1	2a	2b	(BEB = < 0.05)	seqence	on sites
					·							mal		
Primates	13.77	0.01	1.09	ns	3.02	ns	0.791	0.53	0.15	0.25	0.07	s.org	relaxed	non significant PSS
Rodentia	12.38	0.01	71.95	0.00	2.12	ns	0.226	0.65	0.32	0.02	0.01	y by	purifying	no signal
Chiroptera	-0.15	ns	30.26	0.00	15.34	0.00	0.205	0.65	0.27	0.06	0.02	54ୱ 55Y	puriyfing	positive
Artiodactyla	0.28	ns	6.15	0.05	5.48	0.05	0.305	0.68	0.32	0.00	0.00	est c	puriyfing	purified
												5R, 15C, 28C,		
Cetacea	15.57	0.00	-5.70	ns	5.65	0.05	0.94	0.43	0.20	0.26	0.12	32R, 35kg, 55Y,	relaxed	positive
												€0R		
												2, 20		
Dasyuromorphia	-10.37	ns	2.19	ns	18.05	0.00	0.237	0.73	0.24	0.02	0.01	27₩, 28R	purifying	positive
Diprotodontio	9 57	20	100.76	0.00	22.16	0.00	0 007	0.74	0 10	0 10	0.02	41R, 42R, 43R,	nurifying	nonitivo
Diprotodontia	2.57	ns	100.76	0.00	23.10	0.00	0.237	0.74	0.12	U. 1Z	0.02	45R, 47K, 48G	punnying	positive
Eutheria	2.71	ns	134.76	0.00	-2.80	ns	0.376	0.44	0.28	0.17	0.11		purifying	non significant PSS
Metatheria	5.67	0.05	146.75	0.00	-3.87	ns	0.273	0.57	0.26	0.12	0.05		purifying	non significant PSS

Table 1. Results of branch analyses and branch-site analyses for eutherian and metatherian clades, as well as Eutheria and Metatheria.

Downloa

LRT= Likelihood ratio test, ω =clade's ω as calculated by branch analysis; if LRT of M0 versus MC significant MC, ω is reported; if LRT is non significant, M0 ω is reported. PSS=positively selected sites. ω site classes: 0: 0< ω <1 for foreground and background branches, 1: ω =1 for foreground and background branches, 2a: 0< ω <1 for background and ω >1 for foreground, 2b: ω =1 for background and ω >1 for foreground.









Supplementary Information for

Sexual selection of protamine 1 in mammals

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Table S1. Data included in this study: sperm head width, sperm head length, total sperm length, body mass, testes mass; residual testes mass claculated separatedly for each clade.

Figure S1. Multiple sequence alignment for 237 mammalian species based on muscle algorithm.

Figure S2. Multiple sequence alignment for eutherian species based on muscle algorithm.

Figure S3. Multiple sequence alignment for metatherian species based on muscle algorithm

Table S2. Clade descriptive statistics of residual testes mass and arginine content data and amino acid alignment statistics.

Table S3. Results for Tukey HD post-hoc comparisons for one-way anovas.

Figure S4. Phylogenetic tree constructed as consensus of phylogenetic data available in the literature.

Table S4. Results of COEVOL correlation analysis and phylogenetically controlled regression analyses (PGLS).

Figure S5. Comparison of amino acid substitutions along branches and dN/dS branch values.

Supplementary Material and Methods

I. EVOLUTIONARY MODELS APPLIED IN CODEML (PAML4)

A. Branch analysis

In order to obtain the evolutionary rate of clades and groups of species we computed the clade model comparing marked foreground branches against the unmarked background in the analysed phylogenetic tree. Three models were computed: M0 "one ratio" in which all branches were constrained to evolve at the same rate; MCfixed "two-ratio, foreground fixed" where the background clade ω was allowed to be estimated freely while the foreground ω was restrained to a value of $\omega = 1$; and MC "two ratio" model which estimates for both background and the Cricetidae clade a free and independent ω . To test if the foreground evolves at a significantly different rate than the background we compare M0 versus MC by means of LRT. If foreground omega is significantly higher than 1 (LRT significant for MCfixed vs MC and ω >1) we assume positive selection acting on the foreground branches on whole sequence level. If foreground omega is significantly lower than 1 (LRT significant for MCfixed vs MC and ω >1) we report purifying selection acting on the branch on whole sequence level. Relaxed selective constraint for the foreground branch is assumed if foreground evolves at a significantly different ω than the background (M0 vs MC), and this ω was not significantly different from 1 (MCfixed vs MC) (Yang 1998).

B. Branch-site analysis

Similarly, to test evolution along coding sequences and infer amino acids under positive selection for marked foreground branches in contrast to the unmarked background two models were computed. BSfixed "branch-site model A, foreground fixed" in which the codon site ω for background branches is allowed to be computes freely and BS "branch-site model A" in which codon sites in both foreground and background are computed freely (Zhang et al. 2005). Evidence of the existence of positively selected codon sites (PSS) is reported if LRT between BSfixed and BS is significant and sites significantly belonging to the positive selected site category are reported by the model.

II. EXPERIMENTAL DESIGN

A. Comparison of sequence properties

Amino acid frequency and sequence similarity within mammals as well as within eutherian and marsupialian mammals and within the 7 clades were compared and described (Table S1).

B. Selective pressures across mammals

In a first instance we test for the general mode of selection acting on protamine 1 in mammals. To obtain the backround pressure acting on the whole sequence across all mammals we calculate an ω for the whole tree on the whole sequence (M0 as explained above).

C. Comparison of selective pressures

In order to obtain comparative selective pressures for the whole sequence and for directed selective pressure on codon sites we employ the branch analysis and the branch-site analysis as explained above, once marking metatherians as foreground against eutherians as background and once marking eutherians as a foreground against metatherians as background. Secondly we test for differences between mammalian clades by employing a branch- and branch-site analysis for each group marking the respective clade as foreground against either eutherian or metatherian background.

D. Testing for sexual selection

To test for sexual selection on protamine coding sequence within mammals and subsequently in clades, we determined evolutionary rate for each branch in the included pgylogenetic tree followed by a analysis of associations with testes mass corrected for body mass (computed in COEVOL a Bayesian Markov Chain Monte Carlo sampling software for comparative analyses, to test for correlation between genotype and phenotype data. It allows for joint estimation of evolutionary rate for the input alignment and changes in the phenotypic input variables). Additionally we calculated the clades median absolute deviation of residual testes mass and arginine content to test for an effect of variability of sperm competition (median absolute deviation of residual testes mass) on the clades ω (M3), arginine content and arginine content variability (median absolute deviation of arginine content).

E. Effect on sperm head dimensions

In order to test for the effect of changes in the coding sequence of protamine 1 on sperm head dimensions, the evolutionary rate or arginine content were examined for associations with relative HL, relative HW and head elongation (HL/HW) by using COEVOL. We tested for effects on sperm head dimensions across mammals and within clades.

Table S1: Data included in study. HW= sperm head width, HL= sperm head length, TSL= total sperm length, BMASS= body mass, TMASS= testes mass, RTM= residual testes mass claculated seperately for each clade.

	Class	Order	Family	Dem 1 a	Length	Of Angleine	LINK	u	TEI	HW/TS	LIL (TEL		BMACC (-)	THASE (-)	Ing (BMACC)		PTM (by slade)	Genbank accession	n Beferensee en ern dimensione	References body and testes man
Caxa	Class	Ulder	ranny	Print W	Prm1	76 Arginine	nw.		IaL	L			BMA33 (g)	IMA33 (g)	IUg(DMA33)	IUY(TMASS)	KTM (by clade)	Prm1	References sperm dimensions	References body and testes mass
Acomys cahirinus	Eutheria	Rodentia	Muridae	1.114	153	59	2.60	7.70	124.00	0.02	0.06	2.96	75.50	0.51	1.88	-0.29	-0.21	Ramm et al. 2008	Gage 1998	Ramm et al 2005
Aeovorymnus rufescens	Metatheria	Diprotodontia	Potoroidae	0.493	186	63	2.60	5.90	106.40	0.02	0.06	2.27	2400.00	4.68	3.38	0.67	-0.03	AF187547	Taggart et al 1995	Taggart et al 1998
Antechinomys laniger	Metatheria	Dasyuromorpha	Dasyuridae	0.326	189	57												AF001587		
Antechinus bellus	Metatheria	Dasyuromorpha	Dasyuridae	0.318	192	58												AF038295		
Antechinus flavipes	Metatheria	Dasyuromorpha	Dasyuridae	0.240	192	58	-						48.80	0.34	1.69	-0.47	0.05	AF038293		Taggart et al 1998
Antechinus leo	Metatheria	Dasyuromorpha	Dasyuridae	0.428	192	58	-			-			103.00	0.20	2.02	-0.71	-0.42	AF038297		raggartet ar 1556
Antechinus minimus	Metatheria	Dasyuromorpha	Dasyuridae	1.495	192	58							51.00	0.46	1.71	-0.34	0.16	AF038294		Taggart et al 1998
Antechinus naso	Metatheria	Dasyuromorpha	Dasyuridae	1.236	192	58		11.40	271.10		0.04		40.00	0.64	1.00	0.10	0.20	AF038301	Commission and Wandall 1005	Terrent et al 1008
Antechinus stuartii Antechinus swainsonii	Metatheria	Dasyuromorpha	Dasyuridae	1 387	189	60		11.40	271.10		0.04		40.00	0.64	1.60	-0.19	0.38	L35335	Cummins and woodall 1985	Taggart et al 1998
Antilocapra americana	Eutheria	Artiodactyla	Antilocapridae	0.310	156	46							71.40	0.05	1.05	-0.20	0.20	EU189418		Taggart et al 1550
Apodemus sylvaticus	Eutheria	Rodentia	Muridae	0.353	159	55	5.32	9.24	126.15	0.04	0.07	1.74	30.43	0.96	1.48	-0.02	0.44	FJ411394	Gómez Montoto et al 2011b	Gómez Montoto et al 2011a
Anvicala capidus	Futboria	Redentia	Crisotidae	0 507	152	E-7	4.02	7 00	115.54	0.02	0.06	1 76	217.67	2.24	2.24	0.25	0.01	Lüke et al. 2011	Cómoz Montoto et al 2011h	L. Gomez-Montoto, E.K.S. Koldan and M.
Arvicola sapidus Arvicola terrestris	Eutheria	Rodentia	Cricetidae	0.353	153	55	4.02	7.05	118.32	0.03	0.00	1.70	91.56	0.41	1.96	-0.39	-0.39	Lüke et al. 2011	Lüke et al. 2014a	Gómez Montoto et al 2011
																			Anderson et al 2005; González-Moreno et al	
Ateles paniscus	Eutheria	Primates	Atelidae	0.153	153	55	3.14	6.40	65.10	0.05	0.10	2.04	9800.00	64.23	3.99	1.81	0.49	AF119242	2000	Dixson and Anderson 2004
Balaena mysticetus Balaenontera acutorostrata	Eutheria	Cetacea	Balaenidae	5.860	150	48		5.20	56.70		0.09		12000000.00	162996.00	7.71	5.21	0.44	EU444938	Plön and Bernard 2006	MacLeod 2010 MacLeod 2010
Balaenoptera bonaerensis	Eutheria	Cetacea	Balaenopteridae	0.137	150	42		5.20	50.70		0.05		12000000.00	0750.00	7.00	5.54	-0.43	EU444934	Thomand Bernard 2000	Macebourzono
Balaenoptera borealis	Eutheria	Cetacea	Balaenopteridae	0.354	150	44							1800000.00	16398.00	7.26	4.21	-0.27	EU444932		MacLeod 2010
Balaenoptera edeni	Eutheria	Cetacea	Balaenopteridae	0.990	150	44	2.13	3.82	56.03	0.04	0.07	1.79	18000000.00	19998.00	7.26	4.30	-0.18	EU444933	Kita et al 2001	MacLeod 2010
Balaenoptera musculus Balaenoptera physalus	Eutheria	Cetacea	Balaenopteridae	0.735	150	44							51000000.00	58293.00	7.71	4.84	-0.13	EU444931 FU444930		MacLeod 2010 MacLeod 2010
Berardius bairdii	Eutheria	Cetacea	Ziphidae	5.070	150	44	1.45	4.28	51.60	0.03	0.08	2.95	11380000.00	10003.02	7.06	4.00	-0.35	GQ368522	Kita et al 2001	MacLeod 2010
Bettongia penicillata	Metatheria	Diprotodontia	Potoroidae	0.401	189	62	2.30	7.50	162.50	0.01	0.05	3.26	872.00	1.67	2.94	0.22	-0.15	AF187546	Taggart et al 1995	Taggart et al 1998
Bos taurus	Eutheria	Artiodactyla	Bovidae	0.288	156	50	4.30	6.77	53.53	0.08	0.13	1.57	680385.00	681.00	5.83	2.83	0.10	NM_174156	Cummins and Woodall 1985; Gage 1998	Kenagy and Trombulak 1986
Capra hircus	Eutheria	Artiodactvla	Bovidae	0.412	156	52		8.27	59.39		0.14		25420.00	156.80	4.41	2.20	0.05	HM773246	Cummins and Woodall 1985	Anderson et al 2004
																			Cummins and Woodall 1985; Gallardo et al	
Cavia porcellus	Eutheria	Rodentia	Caviidae	0.477	147	55	6.60	10.87	114.07	0.06	0.10	1.65	813.30	4.10	2.91	0.61	-0.29	NM_001173006	2002	Kenagy and Trombulak 1986
cepnaiornyncnus	Futheria	Cetacea	Delphinidae	3 886	144	48							78000.00	1220.00	4 89	3.09	0.12	IE505015		Mad and 2010
Chalinolobus beatrix	Eutheria	Chiroptera	Vespertilionidae	3.281	141	60							10000.00	1220.00	4.05	5.05	0.12	AF435944		MacLeou 2010
Chilonatalus micropus	Eutheria	Chiroptera	Natalidae	0.493	153	55												AF435936		
Chionomys nivalis	Eutheria	Rodentia	Cricetidae	0.544	153	55	4.67	8.40	105.20	0.04	0.08	1.80	49.25	0.99	1.69	-0.01	0.25	Lüke et al. 2011	Gómez Montoto et al 2011b	Gómez Montoto et al 2011a
Colobus quereza	Eutheria	Primates	Cerconithecidae	0.311	150	50	3.70	6.73	83.40	0.04	0.08	1.82	25.25	0.43	1.40	-0.37	0.16	ΔF119233	Gomez Montoto et al 2011b	Gomez Montoto et al 2011a
Corynorhinus townsendii	Eutheria	Chiroptera	Vespertilionidae	0.592	147	57												AF435940		
Cricetulus griseus	Eutheria	Rodentia	Cricetidae	0.366	156	56	2.41	13.47	249.28	0.01	0.05	5.59	33.72	1.78	1.53	0.25	0.66	Lüke et al. 2011	Cummins and Woodall 1985	Lüke et al. 2011
Dasycercus cristicauda	Metatheria	Dasyuromorpha	Dasyuridae	1.280	192	58	1.90	9.50				5.00	74.50	0.77	1.87	-0.11	0.27	AF010270	Cummins and Woodall 1985	Taggart et al 1998
Dasyuroides byrnei	Metatheria	Dasyuromorpha	Dasyuridae	1.022	192	59	2.50	12.70	254.80	0.01	0.05	5.08	123.60	1.38	2.09	0.14	0.38	AF010271	Cummins and Woodall 1985	Taggart et al 1998
Dasyurus albopunctatus	Metatheria	Dasyuromorpha	Dasyuridae	0.568	189	60												AF010272		
Dasyurus geoffroii	Metatheria	Dasyuromorpha	Dasyuridae	0.526	189	60												AF010274		-
Dasyurus paculatus	Metatheria	Dasyuromorpha	Dasyuridae	0.580	186	63				-			796.00	2.49	2.90	0.40	0.08	L35341 AE010276		laggart et al 1998
Dasyurus spartacus	Metatheria	Dasyuromorpha	Dasyuridae	5.311	189	60				-								AF010275		
Dasyurus viverrinus	Metatheria	Dasyuromorpha	Dasyuridae	1.431	186	63	1.90	11.00				5.79	1300.00	3.00	3.11	0.48	0.01	L35340	Cummins and Woodall 1985	Taggart et al 1998
Delphinapterus leucas	Eutheria	Cetacea	Monodontidae	3.523	147	43	1.00	3.80				2.05						GQ368517	Miller et al 2002	
Delphinus capensis	Eutheria	Cetacea	Delphinidae	2 369	141	49	2.08	4 29	70.59	0.03	0.06	2.05	121966 79	4010.27	5.09	3.60	0.51	JF505005	Kita et al 2001: Plön and Bernard 2006	MacLeod 2010
Dendrolagus dorianus	Metatheria	Diprotodontia	Macropodidae	1.221	189	59	2.00	1.2.5	10.00	0.00	0.00	2.00	121000.10	INTOLET	0.00	0.00	0.01	AF187536	inta de al 2001, Hon and Bernard 2000	Maceda Eoro
Dendrolagus goodfellowi	Metatheria	Diprotodontia	Macropodidae	1.786	186	63												AF187537		
Desmodus rotundus	Eutheria	Chiroptera	Phyllostomidae	0.368	147	57	2.71	4.71				1.74	33.70	0.08	1.53	-1.08	-0.11	AF435934	Forman 1979 (?)	Wilkinson and McCracken 2003
Dorcopsulus vanheurni	Metatheria	Diprotodontia	Macropodidae	4.451	189	62									-			AF187539		
Elaphodus cephalophus	Eutheria	Artiodactyla	Cervidae	1.419	156	48												DQ299383		
Eptesicus brasiliensis	Eutheria	Chiroptera	Vespertilionidae	3.690	147	57												AF435943		
Eptesicus fuscus	Eutheria	Cetacea	Vespertilionidae	1.281	14/	57	2.00	5.00	72.00	0.03	0.07	2.50	25000000.00	67500.00	1.19	4.83	0.26	AF435942	Cummins and Woodall 2003	Hosken 1997 MacLeod 2010
Eubalaena australis	Eutheria	Cetacea	Balaenopteridae	0.524	150	48				-			23000000.00	07300.00	7.40	4.05	0.20	GQ368526		Macedou 2010
Eubalaena glacialis	Eutheria	Cetacea	Balaenopteridae	0.722	150	48							74000000.00	972000.00	7.87	5.99	1.11	GQ368527		Brownell and Ralls 1986
Eubalaena japonica	Eutheria	Cetacea	Balaenopteridae	0.620	150	48												EU444939		
Galeonterus varienatus	Eutheria	Chirontera	Chirontera	0.375	144	56				-						-		GQ366511 AF435927		
Globicephala	Luciona	chill opticitu	onnopteru	0.210														1000021		
macrorhynchus	Eutheria	Cetacea	Delphinidae	0.193	144	46	2.00	4.55	74.12	0.03	0.06	2.28						GQ368512	Kita et al 2001	
Gorilla gorilla	Eutheria	Primates	Pongidae	0.228	156	44	2.00	4.78	61.17	0.02	0.08	2.24	134000.00	23.20	5.13	1.37	-0.64	AF215709	Cummins and Woodall 1985	Kenagy and Trombulak 1986
Hexaprotodon liberiensis	Eutheria	Artiodactyla	Hippopotamidae	0.570	153	49	2.00	4.47	74.40	0.03	0.00	2.24	400000.00	7000.00	3.00	3.65	0.42	GQ368531		Freeman 1990
																			Cummins and Woodall 1985; Meisner et al	
Hippopotamus amphibius	Eutheria	Artiodactyla	Hippopotamidae	0.562	153	49	2.90	4.60	33.49	0.09	0.14	1.59	1600000.00	650.00	6.20	2.81	-0.07	EU189424	2005	Gage and Freckleton 2003
Homo saniens	Futheria	Primates	Hominidae	0.175	156	46		4.50	56.90	-	0.08		63540.00	50.20	4.80	1.70	-0.11	Y00443	Anderson et al 2005	Kenagy and Trombulak 1986
Hylobates lar	Eutheria	Primates	Hylobatidae	0.182	156	48		7.20	63.30		0.11		5500.00	5.50	3.74	0.74	-0.43	L14588	Anderson et al 2005	Kenagy and Trombulak 1986
Hypsiprymnodon moschatus	Metatheria	Diprotodontia	Hypsiprimmodontidae	2.034	195	62	1.31	8.50	89.25	0.01	0.10	6.49			-			AF187545	Lloyd et al 2002	
Inia geoffrensis	Eutheria	Cetacea	Iniidae	0.658	147	45				-			122000.00	1447.04	5.09	3.16	0.07	G0368529		MacLeod 2010
Kogia breviceps	Eutheria	Cetacea	Physeteridae	0.661	150	44		3.40	50.50		0.07							GQ368525	Plön and Bernard 2006	MacLeod 2010
Kogia sima	Eutheria	Cetacea	Physeteridae	1.735	150	42	1.80	3.50	32.60	0.06	0.11	1.94	350000.00	4000.00	5.54	3.60	0.22	GQ368524	Plön and Bernard 2006; Meisner et al 2005	Freeman 1990
Lagenodelphis hosei	Eutheria	Cetacea	Delphinidae	0.340	141	49	1.25	4.00				3 20	182500.00	298.82	5.26	2 48	-0.73	JF505011 G0368510	Neuenhagen et al 2007	Neuenbagen et al 2007
Lagenorhynchus albirostris	Eutheria	Cetacea	Delphinidae	0.666	144	46	1.2.5	4.00		-		5.20	102300.00	250.02	5.20	2.40	-0.15	JF505014	Nederinagen et al 2007	Nederinagen et al 2007
Lagenorhynchus obliquidens	Eutheria	Cetacea	Delphinidae	0.215	141	49	1.96	4.23	69.26	0.03	0.06	2.16	90000.00	1100.00	4.95	3.04	0.04	JF505013	Kita et al 2001; Miller et al 2002	Freeman 1990
Lagorchestes hirsutus	Metatheria	Diprotodontia	Macropodidae	1.261	189	59	3.30	6.00	88.40	0.04	0.07	1.82						AF18/544	Jonnston et al 2003	
Lipotes vexillifer	Eutheria	Cetacea	Lipotidae	5.129	147	41	1		1	1					1	1	1	JF701667		1
Lissodelphis borealis	Eutheria	Cetacea	Delphinidae	1.041	144	48												GQ368509		
Macaca fascicularis	Eutheria	Primates	Cercopithecidae	2.543	156	54	3.50	5.53	73.63	0.05	0.08	1.58	4787.00	35.70	3.68	1.55	0.42	AB169169	Cummins and Woodall 1985	Kenagy and Trombulak 1986
Macaca mulatta	Futheria	Primates	Cerconithecidae	0 108	156	56	3 20	5 30	74 10	0.04	0.07	1.66	10430.00	76.00	4.02	1.88	0.54	NM 001143822	Anderson et al 2005; Cummins and Woodall	Kenagy and Trombulat 1986
Macropus agilis	Metatheria	Diprotodontia	Macropodidae	0.501	186	63	3.20	7.50	113.20	0.03	0.07	2.34	11400.00	25.64	4.06	1.41	0.20	L35451	Cummins and Woodall 1985	Taggart et al 1998
Macropus eugenii	Metatheria	Diprotodontia	Macropodidae	3.605	189	63	3.38	6.67	106.40	0.03	0.06	1.97	7050.00	32.90	3.85	1.52	0.46	L35450	Cummins and Woodall 1985	Paris et al 2005
Macropus giganteus	Metatheria	Diprotodontia	Macropodidae	0.400	186	63	2.20	7.30	118.90	0.02	0.06	3.32	40720.00	42.02	4.61	1.62	0.00	L35333	Cummins and Woodall 1985	Taggart et al 1998
Macropus parryi Macropus rufogriseus	Metatheria	Diprotodontia	Macropodidae	1.068	189	63	4.89	9.21	119.53	0.04	0.08	1.90	18500.00	54.57	4 27	1 74	0.37	AF18/533	Cummins and Woodall 1985	Rose et al 1997
Macropus rufus	Metatheria	Diprotodontia	Macropodidae	0.658	183	61	3.40	5.10	123.90	0.03	0.04	1.50	39825.00	38.18	4.60	1.58	-0.04	L35447	Cummins and Woodall 1985	Taggart et al 1998

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Sigmodon hispidus	Eutheria	Rodentia	Cricetidae	0.345	156	54	3.20	6.10	87.00	0.04	0.07	1.91 223.00	1.73	2.35	0.24	-0.13	EU980395	Cummins and Woodall 1985	Kenagy and Trombulak 1986
Sminthopsis aitkeni	Metatheria	Dasyuromorpha	Dasyuridae	0.449	192	58						18.00	0.11	1.26	-0.96	-0.15	AF089871		Taggart et al 1998
Sminthopsis archeri	Metatheria	Dasyuromorpha	Dasyuridae	0.189	192	58											AF089872		
Sminthopsis bindi	Metatheria	Dasyuromorpha	Dasyuridae	0.166	192	56						15.30	0.11	1.18	-0.97	-0.11	AF089873		Taggart et al 1998
Sminthopsis crassicaudata	Metatheria	Dasyuromorpha	Dasyuridae	0.411	192	58		12.80	264.90		0.05	14.60	0.17	1.16	-0.78	0.09	L32743	Gage 1998	Taggart et al 1998
Sminthopsis dolichura	Metatheria	Dasyuromorpha	Dasyuridae	0.462	192	58						16.00	0.14	1.20	-0.85	0.00	AF089874		Taggart et al 1998
Sminthopsis douglasi	Metatheria	Dasyuromorpha	Dasyuridae	1.010	192	58											AF089875		
Sminthopsis gilberti	Metatheria	Dasyuromorpha	Dasyuridae	0.485	192	58											AF089876		
Sminthopsis granulipes	Metatheria	Dasyuromorpha	Dasyuridae	0.535	192	58						18.50	0.28	1.27	-0.55	0.25	AF089877		Taggart et al 1998
Sminthopsis griseoventer	Metatheria	Dasyuromorpha	Dasyuridae	0.539	192	59						20.00	0.15	1.30	-0.82	-0.04	AF089878		Taggart et al 1998
Sminthopsis hirtipes	Metatheria	Dasyuromorpha	Dasyuridae	0.477	192	58						16.50	0.16	1.22	-0.81	0.03	AF089879		Taggart et al 1998
Sminthopsis leucopus	Metatheria	Dasyuromorpha	Dasyuridae	0.441	192	58						22.50	0.23	1.35	-0.63	0.11	AF089880		Taggart et al 1998
Sminthopsis longicaudata	Metatheria	Dasyuromorpha	Dasyuridae	0.935	189	59											AF089881		
Sminthopsis macroura	Metatheria	Dasyuromorpha	Dasyuridae	0.349	192	58						20.00	0.24	1.30	-0.62	0.16	AF001586		Taggart et al 1998
Sminthopsis murina	Metatheria	Dasyuromorpha	Dasyuridae	0.401	192	58						20.50	0.27	1.31	-0.58	0.20	AF001585		Taggart et al 1998
Sminthopsis ooldea	Metatheria	Dasyuromorpha	Dasyuridae	0.424	192	58						12.00	0.11	1.08	-0.97	-0.04	AF089882		Taggart et al 1998
Sminthopsis psammophila	Metatheria	Dasyuromorpha	Dasyuridae	0.408	192	58											AF089883		
Sminthopsis virginiae	Metatheria	Dasyuromorpha	Dasyuridae	0.407	192	58						31.00	0.25	1.49	-0.60	0.05	AF089884		Taggart et al 1998
Sminthopsis youngsoni	Metatheria	Dasyuromorpha	Dasyuridae	0.315	192	58						11.00	0.12	1.04	-0.94	0.02	AF089885		
Sotalia fluviatilis	Eutheria	Cetacea	Delphinidae	0.597	141	49											JF505012		
Sousa chinensis	Eutheria	Cetacea	Delphinidae	0.530	144	48											JF505010		
Stenella attenuata	Eutheria	Cetacea	Delphinidae	0.506	141	49						80500.00	1029.03	4.91	3.01	0.04	JF505009		MacLeod 2010
Stenella coeruleoalba	Eutheria	Cetacea	Delphinidae	2.229	144	46											EU697409		
Stenella frontalis	Eutheria	Cetacea	Delphinidae	1.209	141	49											JF505008		
Stenella longirostris	Eutheria	Cetacea	Delphinidae	0.507	141	49											JF505007		
Steno bredanensis	Eutheria	Cetacea	Delphinidae	1.129	144	48	2.00	3.60				1.80					EU697407	Meisner et al 2005	
Sus scrofa	Eutheria	Artiodactyla	Suidae	0.168	153	49	5.00	8.50	54.60	0.09	0.16	1.70 39700.00	128.20	4.60	2.11	-0.12	NM_214253	Cummins and Woodall 1985; Gage 1998	Almeida et al 2006
Tasmacetus shepherdi	Eutheria	Cetacea	Ziphidae	2.356	150	42											GQ368521		
Thylacinus cynocephalus	Metatheria	Dasyuromorpha	Thylacinidae	0.374	192	58											U87140		
Thylogale stigmatica	Metatheria	Diprotodontia	Macropodidae	5.803	189	62	2.20	7.20	110.30	0.02	0.07	3.27					AF187534	Cummins and Woodall 1985	
Trachypithecus cristatus	Eutheria	Primates	Cercopithecidae	2.232	156	52											AF294861		
Trachypithecus francoisi	Eutheria	Primates	Cercopithecidae	0.159	156	52											AF119234		
Trachypithecus geei	Eutheria	Primates	Cercopithecidae	0.226	156	52											AF294857		
Trachypithecus johnii	Eutheria	Primates	Cercopithecidae	0.219	156	54											AF294853		
Trachypithecus obscurus	Eutheria	Primates	Cercopithecidae	1.458	156	52											AF119238		
Trachypithecus phayrei																			
crepuscula	Eutheria	Primates	Cercopithecidae	1.291	153	51											AF294858		
Trachypithecus pileatus	Eutheria	Primates	Cercopithecidae	0.212	156	52											AF294856		
Trachypithecus vetulus	Eutheria	Primates	Cercopithecidae	0.360	153	53											AF119236		
Trichosurus vulpecula	Metatheria	Diprotodontia	Phalangeridae	0.183	189	63	2.58	5.53	94.17	0.03	0.06	2.14 3350.00	8.26	3.53	0.92	0.11	L32744	Cummins and Woodall 1985	Taggart et al 1998
Tursiops aduncus	Eutheria	Cetacea	Delphinidae	0.329	144	46											JF505006		
Tursiops truncatus	Eutheria	Cetacea	Delphinidae	5.738	144	46	2.13	4.41	70.16	0.03	0.06	2.07 146250.00	756.40	5.17	2.88	-0.26	GQ368508	Kita et al 2001; Plön and Bernard 2006	MacLeod 2010
Wallabia bicolor	Metatheria	Diprotodontia	Macropodidae	12.746	189	63	3.05	7.80	109.50	0.03	0.07	2.56 31500.00	14.75	4.50	1.17	-0.38	L35328	Cummins and Woodall 1985	Taggart et al 1998
Zinhius cavirostris	Futheria	Cetacea	Delnhinidae	15 363	1150	42					1	300000 00	8001.00	6 4 8	3 90	-0.08	FI1444928		MacLeod 2010

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Figure S1. Multiple sequence alignment for all 237 included mammal species based on muscle algorithm (implemented in Geneious 5.5.9.)

Consensus									84 ★ ★ ₽ ★
consensus									
Sequence Logo		BRA YR	RKR RRRSR		RRK KRSCR	RRRK R	REY SKR	T RCR Y	
Microtus cabrerae	MARYR YRS	KSRSR C	<u>S</u> RR	RRRCR -	RRR RRCCQ	SRKR	- RC - YRRRRS	CTM-RCKRY*	
Clethrionomys glareolus	MARYR CRS	KSRSR C	RRR	R R R <mark>C</mark> R -	- RR RRCCR	RRRR	- RC - YRRRRS	YSI-MCKRY*	
Microtus arvalis	MARYR YRS	KSRSR C	<u>S</u> RG	RRRCR -	RRR RRCCR	RRRR	- RC - CRRRRT	Y	
Microtus agrestis	MARYR CRS	KSRSR C	<u>S</u> RR	RRR <mark>C</mark> R -	RRR RRCCR	RRRR	- RC - CRRRRT	YTM - RCKRY*	
Microtus gerbei	MARYR CRS	KSRSRC	SRR	RRRCR -	RRR RRCCR	RRRR	- RC - YRRRRS	YTM - RCKRY*	
Pitimys lusitanicus	MARYR CRS	KSRSR C	SRR	RRRCR -	RRR RRCCR	RRRR	- RC - YRRRRS	YTM - RCKRY*	
Pitimys duodecimcostatus	MARYR CRS	KSRSR C	SRH	RRRCR -	RRR RRCCR	RRRR	- RC - YRRRRS	YTM - RCKRY*	
Acomys cahirinus	MARYR - CCRS	KSRSR C	RRR	<u>RRRCR</u> -	RRR RRCCR	RRRR	- RC - CRRRR -	YIL-RCRRI*	
Acomys cilcicus	MARYR - CCRS	KSRSRC	RRR	RRRCR -	RRR RRCCR	RRRR	- RC - CRRRR -	YIL-RCRRI*	
Chionomys nivalis	MARYR CRS	QSRSRC	RKK	RRRCR -	RRR RRCCR	RKKK	- RC - YGRRRI	YIL-RCKRY*	
Phodopus campbelli	MARYR CRS	USKSKC	KKK	KKKCK-	KKK KKCCK	KKKK	- KC - YGKKKI	YIL-RCKRY*	
Arvicola sapidus	MARYR CRS	KSKSKC	KKK	KKKCK-	KKKKKCCK	KKKK			
Arvicola terrestris	MARYR CRS	KSRSRC	KHK	KKKCK-	KKKKKUCK	KKKK			
Phodopus sungorus		KSKSKL						ISL-RUKRI*	
Micromyc minutus									
Micromys minutus Mosocricotus auratus								VTI DCKDV*	
Meriopes unquiculatus									
Peromyscus californicus		KSRSR - C						VTL - RCKRV*	
Peromyscus leucopus	MARYR - CCRS	KSRSR - C			RRR RRCCR		-RC - CRRRR -	YTL - RCKRY*	
Peromyscus manicaulatus	MARYR - CCRS	KSRSR C	RRR	RRRCR -	RRR RRCCR	RRRR	-RC - CRRRR -	YTL - RCKRY*	
Peromyscus polionotus	MARYR - CCRS	KSRSR C	RRR	RRRCR -	RRR RRCCR	RRR	- RC - CRRRR -	YTI - RCKRY*	
Cavia porcellus	MARYR - CCRS	PSRSR C	RRR	RRRFY -	RRR RRCHR	RRRR	C - C R R R	YTR - RCKRY*	
Sigmodon hispidus	MARYR - CCRS	KSRSR C	RRR	RRRCR -	RRR RRCCR	RRRR	- RC - CRRRT	YTL - RCKKY*	
Apodemus sylvaticus	MARYR - CCRS	KSRSR C	RRR	RRRCRS	RRR RRCCR	RRRR	- RC - CRRRRS	YTI-RCKRY*	
Mus pahari	MARYR - CCRS	KSRSR C	RRR	RRRCR -	RRR RRCCR	RRRQ	- RC - CRRRRS	YTI-RCKKY*	
Mus famulus	MARYR - CCRS	KSRSR C	RRR	R R R <mark>C</mark> R -	RRR RRCCQ	RRRR	- RC - CRRRRS	YTI-RCKKY*	
Mus cookii	MARYR - CCRS	KSRSR C	RRR	R R R <mark>C</mark> R -	RRR RRCCR	RRRR	- R C - C R R R R S	YTI-RCKKY*	
Mus macedonicus	MARYR - CCRS	KSRSR C	RRR	R R R C R -	RRR RRCCR	RRRR	- RC - CRRRRS	YTI-RCKKY*	
Mus musculus bactrianus	MARYR - CCRS	KSRSR C	RRR	RRR <mark>C</mark> R -	RRR RRCCR	RRRR	- RC - CRRRRS	YTI-RCKKY*	
Mus musculus castaneus	MARYR - CCRS	KSRSR C	RRR	RRR <mark>C</mark> R -	RRR RRCCR	RRRR	- RC - CRRRRS	YTI-RCKKY*	
Mus musculus domesticus	MARYR - CCRS	<u>KSRSR C</u>	RRR	RRRCR -	RRR RRCCR	RRRR	- RC - CRRRRS	YTI - RCKKY*	
Mus musculus musculus	MARYR - CCRS	KSRSR C	RRR	RRRCR -	RRR RRCCR	RRRR	- RC - CRRRRS	YTI-RCKKY*	
Mus spicilegus	MARYR - CCRS	KSRSR C	RRR	RRRCR -	RRR RRCCR	RRRR	- RC - CRRRRS	YTI-RCKKY*	
Mus spretus	MARYR - CCRS	KSRSR C	RRR	RRRCR -	RRR RRCCR	RRRR	- RC - CRRRRS	YTI-RCKKY*	
Rattus norvegicus	MARYR - CCRS	KSRSR C	RRR	<u>RRRCR</u> -	RRR RRCCR	RRRR	- RC - CRRRRS	YIF-RCKRY*	
Macaca fascicularis	MARYR - CCCS	VSRSR - C	CKK	KKKCR-	KKKKKKCR	AKKK A	MRC - CRRR	YKL - KCKRY*	
Macaca mulatta		<u>USKSKL</u>		KKKCK-	KKKKKKCK	AKKKA		IKL - KUKKY*	
Papio cynocephalus					KKKKKKCK				
Colobus guereza				KKKCK-	KKKKUKFK	AKKKA			
Nasalis larvatus		<u> </u>	UKP	KQKCK-	KKK KKSCR	АККК А	<u> МКС-СККК</u>	1 KL - KUKKY*	

Piliocolobus badius	MARYR - CCRS	QSRSR C	C RR	RRRCR -	RRR RQRCR	A R R T A	MRC - CRRR	YRR - RCRRY*
Semnopithecus entellus	MARYR - RCRS	Q S R S R C	<u>CRP</u>	R R R <mark>C</mark> R -	- RR RR <mark>SC</mark> R	ARRR A	TRC - CRRR	YRL - RCRRY*
Trachypithecus cristatus	MARYR - CCRS	QSRSR C	<u>CRP</u>	R R R <mark>C</mark> R -	RRR RR <mark>SC</mark> R	ARRR A	TRC - CRRR	YRL - RSRRY*
Trachypithecus obscurus	MARYR - CCRS	QSRSR C	<u>CRP</u>	R R R C R -	RRR RR <mark>SC</mark> R	ARRR A	TRC - CRRR	YRL - RSRRY*
Trachypithecus phayrei	MARYR - CCRS	QSRSR C	<u>CRP</u>	R R R <mark>C</mark> R -	RRR RR <mark>SC</mark> R	ARRR A	TRC - CRRR	YRL SRRY*
Trachypithecus francoisi	MARYR - CCRS	Q S R S R C	<u>CRP</u>	R R R <mark>C</mark> R -	RRR RR <mark>SC</mark> R	ARRR A	TRC - CRRR	YRL - RCRRY*
Trachypithecus geei	MARYR - CCRS	Q S R S R C	<u>CRP</u>	R R R <mark>C</mark> R -	RRR RR <mark>SC</mark> R	ARRR A	TRC - CRRR	YRL - RCRRY*
Trachypithecus pileatus	MARYR - CCRS	Q S R S R C	<u>CRP</u>	RRR <mark>C</mark> R -	RRR RR <mark>SC</mark> R	ARRR A	TRC - CRRR	YRL - RCRRY*
Trachypithecus vetulus	MARYR - RCRS	Q S R S R C	<u>CRP</u>	RRR <mark>C</mark> R -	- RR RR <mark>SC</mark> R	ARRR A	TRC - CRRR	YRL - RCRRY*
Trachypithecus johnii	MARYR - RCRS	QSRSR C	C R P	R R R C R -	RRR RRSCR	ARRR A	TRC - CRRR	YRL - RCRRY*
Gorilla gorilla	M A R Y R - C C R S	Q S R S R C	YRQ	R Q T S R -	RRR RR <mark>SCQ</mark>	TQRR A	MRC - CRR	RNRLRRRKH*
Homo sapiens	M A R Y R - C C R S	QSRSR Y	YRQ	R Q R <mark>S</mark> R -	RRR RR <mark>SCQ</mark>	TRRR A	MRC - CRPR	YRP - RCRRH*
Pan paniscus	MARYR - CCRS	QSRSRC	YRQ	RRSR -	RRK RQSCQ	TQRR A	MRC - CRR	RSRLRRRRH*
Pan troglodytes	MARYR - CCRS	QSRSR C	YRQ	RQR <mark>S</mark> R -	RR K RQSCQ	TQRR A	MRC - CRR	RSRMRRRRH*
Pongo pygmaeus	M A R Y R - C C R S	Q S Q S R C	CRR	RQRCH -	RRR RRCCQ	TRRR A	MRC - CRRR	YRL - RCRRH*
Hylobates lar	MARYR - CCRS	Q S R S R C	YRR	GQRSR -	RRR RRSCQ	TRRR A	MRC - CRPR	YRL - RRRRH*
Ateles sp.	MARYR - CCRS	RSRSRC	YRQ	R P R C R -	RRR RRSCR	RRRG	SRC - CRRR	YRL - RRRRY*
Saguinus imperator	MARYR - CCRS	QSRSR C	YRQ	RRRGR -	RRR RRTCR	RRRA	SRC - CRRR	YKL - TCRRY*
Saimiri sciureus	M A R Y R - C C R S	R S R S R C	YRR	RRRCR -	TRR RRCCR	RRRA	RRC - CRRR	Y <mark>KL</mark> - RCRRY*
Otolemur garnettii	MARYR - CCRS	QSRSRC	RRR	RRRCR -	RRR RRCCR	RRRR	- RC - CRRR	YRL - RCRRY*
Balaena mysticetus	MARNR - CCRS	QSRSRR	RRP	RQRCR -	SQR RRCCR	RRRR	- V C - C R R	YTTVRCARR*
Eubalaena australis	MARNR - CCRS	Q S R S R R	R R P	RQRCR -	SQR RRCCR	RRRR	- V C - C R R	YTTVRCARR*
Eubalaena glacialis	MARNR - CCRS	Q S R S R R	RRP	RQRCR -	SQR RRCCR	RRRR	- V C - C R R	YTTVRCARR*
Eubalaena japonica	MARNR - CCRS	QSRSRR	RRP	RQRCR -	SQR RRCCR	RRRR	- V C - C R R	YTTVRCARR*
Balaenoptera physalus	MARNS - CCRS	PSRSRR	RRP	RQRRR -	SQR RRCCQ	RRRR	- V C - C R R	YTTVRCARQ*
Balaenoptera musculus	MARNT-CCRS	PSRSRR	RRP	RQRCR -	SQR RRCCR	RRRR	- V C - C R R	YTTVRCARQ*
Balaenoptera acutorostrata	MARNS - CCRS	PSRSRR	RRP	RQRCR -	SQR RRCCR	RRRR	- VC - CRR	YTTVRCAGQ*
Balaenoptera bonaerensis	MARNS - CCRS	PSRSRR	RRP	RQRCR -	SQR RRCCR	RRRR	- VC - CRR	YTTVRCAGQ*
Balaenoptera borealis	MARNS - CCRS	PSRSR R	RRP	RORCR-	SQR RRCCR	RRRR	- VC - CRR	Y I I VRCARQ*
Balaenoptera edeni	MARNS - CCRS	PSRSR R	RRP	RORCR-	SQR RRCCR	RKKK	- VC - CRR	Y I I VRCARQ*
Megaptera novaeangliae	MARNS - CCRS	PSRSR R	RRP		SOR RRCCO	KKKK	- VC - CRR	Y I I V RCARQ*
Eschrichtius robustus	MARNS - CCRS	PSRSR R	RRP	RORCR-	SOR RRCCR	KKKK	- VC - CRR	Y I I V RCARQ*
Caperea marginata	MARNR - CCRS	QSRSR R	KKP		SOR RRCCR	KKKK	- VC - CRR	Y I I V RCARQ*
Cephalorhynchus commersonii	MARNR CRS	PSUSRC	KKP		- KK KKCCK	KKKK	- VC - CRR	YAIIRCARQ*
Delphinus capensis	MARNR CRS	PSUSRC	KKP	KKKCK-		KKKK		Y I I I RCAR - *
Delphinus delphis	MARNR CRS	PSUSRC	RKP	KKKCK-		KKKK		Y I I I RCAR - *
Feresa attenuata	MARNR CRS	PSUSRC	KKP			KKKK	- VC - CRR	Y I I I RCARU*
Globicephala macrorhynchus	MARNR CRS	PSUSRC	KKP	KKKYK-		KKKK		Y I I I KCARU*
Grampus griseus			KKP					YTTTDCAD *
Lagenodelphis nosel								YTTTDCAD *
Lagenornynchus acutus								YTTTDCAD *
Lagenornynchus albirostris								VTTTDCADO*
Lissoueiphis porealis								
Popoposophala alastra								
Providence cressidens								
Estalia fluviatilia								VTTTDCARV*
						NNNN		IIIINCAN-"

Sousa chinensis	MARNR CRS	PSQSR C	RR <mark>P</mark>	RRR <mark>C</mark> R -	- RR RR <mark>CC</mark> R	RRRR	- V C - C R R	YTTTRCARQ*	
Stenella attenuata	MARNR CRS	PSQSRC	R R P	RRRCR -	- RR RR <mark>CC</mark> R	RRRR	- V C - C R R	YTTTRCAR - *	
Stenella frontalis	MARNR CRS	PSQSR C	R R P	RRRCR -	- RR RR <mark>CC</mark> R	RRRR	- V C - C R R	YTTTRCAR - *	
Stenella longirostris	MARNR CRS	PSQSRC	R R P	RRR <mark>C</mark> R -	- RR RR <mark>CC</mark> R	RRRR	- V C - C R R	YTTTRCAR - *	
Stenella coeruleoalba	MARNR CRS	PSQSR C	RR P	RRR <mark>C</mark> R -	- RR RLCCR	RRRR	- V C - C R R	YTTTRCARQ*	
Steno bredanensis	MARNR CRS	PSOSR C	RR P	RRR <mark>C</mark> R -	- RR RR <mark>CC</mark> R	RRRR	- V C - C R R	YTTTRCARO*	
Tursiops aduncus	MARNR CRS	PSOSR C	RR P	RRRCR -	- RR RLCCR	RRRR	- V C - C R R	YTTTRCARO*	
Tursiops truncatus	MARNR CRS	PSOSR C	R R P	RRRCR -	- RR RLCCR	RRRR	- V C - C R R	YTTTRCARO*	
Inia geoffrensis	MARNR CRS	PSOSRG	RRP	RRR YR -	SRR RRCCO	RRRR	- VC - CRR	YTTVRCARO*	
Lipotes vexillifer	MARNR CRS	PSOSR G	RRP	RRKYR -	SRR RRCCO	RRRR	- VC - CRR	YTTMRCAKO*	
Delphinapterus leucas	MARNR CRS	PSOSRG	RRP	RRR YR -	SKR RRCCO	RRRR	- V C - C R R	YTTTRCARO*	
Monodon monoceros	MARTR CRS	PSOSR - G	R R P	RRRYR -	SKR RRCCO	RRRR	- VC - CRR	YTTTRCARO*	
Neophocaena phocaenoides	MARNR CRS	PSOSRG	R C P	RRRYR -	SKR RRCCO	RRRR	- V C - R R R	YTR-RCARO*	
Phocoena phocoena	MARNR CRS	PSOSR G	RCP	RRR YR -	SKR RRCCO	RRRR	- VC - RRR	YTR-RCARO*	
Phocoenoides dalli	MARNR CRS	PSOSR - G	RCP	RRRYR -	SKR RRCCO	RRRR	- VC - RRR	YTR-RCARO*	
Kogia breviceps	MARNR - CCRS	0SRGR - C	RRP	RRR YR -	SPR RRRYO	RRRR	- VC - CRR	STTMRCASO*	
Kogia sima	MARNR - CCRS	0 SRGR C	R R P	R R R C R -	SPK - RRRY0	RRRR	- VC - CRR	SATMRCASO*	
Physeter catodon	MARNR - CCRS	0 SR SR C	R R P	RRRCR -	SPR RRRYO	RRRR	- VC - CRR	YTVTRCARO*	
Platanista minor	MARNR - CCRS	0 S R S R C	R R P	KRGCR -	SRR RRCYO	RRRR	- VC - CRR	YTTIRCAR0*	
Pontoporia blainvillei	MARNR CRS	PSONR - G	R R P	RRR YR -	SRR RRCCO	RRRR	- VC - CRR	YTSVRCAR0*	
Berardius bairdii	MARNR - CCRS	0505R R	R R P		SRR ROCCO	RRRR	- VC - CRR	YTATRCAR0*	
Mesoplodon gravi	MARNT-CCRS	0505R R	R R P	RRR YR -	SRR KÔCCÔ	K RRR	- VC - CRR	YTATRCAR0*	
Mesoplodon bidens	MARNR - CCRS	0505R R	R R P	RRR YR -	SRR - ROCCO	KRRR	- VC - CRR	YTAIRCARO*	
Mesoplodon peruvianus	MARNR - CCRS	0505R R	R R P	RRR YR -	SRR KOCCO	KRRR	- VC - CRR	YTAIRCARO*	
Tasmacetus shepherdi	MARNR - CCRS	0505R R	RRP	RRR YR -	SRR RÔCCÔ	KRRR	- VC - CRR	YTAIRCARO*	
Ziphius cavirostris	MARNR - CCRG	0505R R	RRP	RRR YR -	SRR RÔCCÔ	KRRR	- VC - CRR	YTATRCAR0*	
Hexaprotodon liberiensis	MARYR - CCRS	PSRSR C	RR0	RRRCR -	RRR RRCCR	0 R R R	RVC - CRR	YTMVRCTR0*	
Hippopotamus amphibius	MARYR - CCRS	PSRSR C	RR0	RRRCR -	RRR RRCCR	0RRR	RVC - CRR	YTMVRCTR0*	
Antilocapra americana	MARYR - CCLT	HSRSR CRP	RRR	RRRCR -	KLR RRFCR	RPRR	RVC - CRR	YTAIRCTR-*	
Bos taurus	MARYR - CCLT	HSGSR C	RRR	RRRRCR -	RRR RRSGR	RRRR	RVC - CRR	YTVIRCTR0*	
Capra hircus	MARYR - CCLT	HSRSR C	RRR	RRRRCR -	RRR RRFGR	RRRR	RVC - CRR	YTVVRCTRO*	
Ovis aries	MARYR - CCLT	HSRSR C	RRR	RRRRCR -	RRR RRFGR	RRRR	RVC - CRR	YTVVRCTRO*	
Ovis dalli	MARYR - CCLT	HSRSR C	RRR	RRRRCR -	RRR RRFGR	RRRR	RVC - CRR	YTVVRCTRO*	
Elaphodus cephalophus	MARYR - CCLT	HSRSR C	RRR	RRRRCH -	RRR KRFGR	RRRR	RVC - CRR	YTVVRCTRO*	
Moschus sp. JEG-2007	MARYR - CRLT	HSRSG C	RRR	RRRR <mark>C</mark> R -	RRR RRFCR	RRRR	RVC - CRR	YTVVRCTRO*	
Potamochoerus porcus	MARYR - CCRS	HSRSR C	R P R	RRR <mark>C</mark> R -	RRR RRCCP	RRRR	AVC - CRR	YTVIRCRRC*	
Sus scrofa	MARYR - CCRS	HSRSR C	R P R	RRR <mark>C</mark> R -	RRR RRCCP	RRRR	AVC - CRR	YTVIRCRRC*	
Pteropus hypomelanus	MARYR - CCRS	QSRSR C	RRR	RRR <mark>C</mark> R -	RRR RRCCR	RRRR	- V C - C R R	YTV - RCRRRR	*
Mormoops megalophylla	MARYR - CCRS	P SRSR C	RRR	RRR <mark>C</mark> R -	RRR RR <mark>SC</mark> R	RRRR	- V C - C R R	YTV - RCRRR*	
Pteronotus parnellii	MARYR - CCRS	PSRSR C	RRR	RRR <mark>C</mark> R -	RRR RRCCR	RRRR	- V C - C R R	YTV - RCRRR*	
Chilonatalus micropus	MARYR - CCRS	QSRSR C	RRR	RRR <mark>C</mark> R -	TRR RRCCR	RRRR	RVC - CRR	YTVVRCRRR*	
Natalus stramineus	MARYR - CCRS	QSRSRC	R P R	RRR <mark>C</mark> R -	TRR RRCCR	RRRR	R	YTVVRCRRR*	
Desmodus rotundus	MARYR - CCRS	P SRSR C	RRR	RRR <mark>C</mark> R -	RRR RRCCR	RRRR	- V C - C R R	YTV - RCRRR*	
Monophyllus redmani	MARYR - CCRS	PSRSR C	RRR	RRRCR -	RRR RRCCR	RRRR	- V C - C R R	YTV - RCRRR*	
Hipposideros commersoni	MARYR - CCRS	HSRSR C	RRR	R R R <mark>S</mark> R -	RRR RRSCG	RRRR	- A G - YRR	YTVRYRRRR*	
Rhinolophus ferrumequinum	MARYS - CCRS	HSRSR S	RRR	R Q R C R -	RRR RR <mark>SCG</mark>	RRRR	- A C - YRR	YTV - RYRRRR	RRR*
Rhinopoma hardwickii	MARYR - CCRS	R S R S R C	R P R	R R R C R -	RRR RRCCR	RRRR	- V C - C R R	YSA - RCRRRR	*
Chalinolobus beatrix	MARYR - CCR -	- <mark>S R S R C</mark>	RRR	RRR <mark>S</mark> Y -	RRR RRCCR	RRRR	R V C - C R R	YV RCRRR *	

Convnorhinus townsondii			DDD			DDDD		
Entosicus brasilionsis								
Eptesicus brasiliensis								
Hypsugo savii Muning augletic								
Murina cyclotis								Y I V I K C K K K *
Galeopterus variegatus		- SKSK C	KKK	KKSCK-		RRAR	- KS - CKKK	ISLKUCKKI*
Myotis daubentonii	MARYR-CCR-	- SKSK C	KKK	KKKCY-	KKKKKUCK	KKKKK	RVC - CRR	YS KCKKK*
Myotis lucifugus	MARYR - CCR -	- SKSK C	KKK		KKKKKCCK	KKKKK	RVC - CRR	YS KCKKK*
Neoromicia brunneus	MARYR-RCR-	- SRSR C	<u> </u>		RKKKKCCK	KKKKK	RAC - CRR	Y RCRRR*
Otonycteris hemprichii	MARYR - CCRS	RSRSR C	RRR	RRKCY -	RRR RRCSR	K RRR	RVC - CRR	Y I VMRCRRR*
Plecotus auritus	MARYR - CCRS	QSRSR - C	RRR	RRRCY -	RRR RRCCR	RRRR	RVC - CRR	YTVVRCRRR*
Antechinomys laniger	MARYRRHSRS	RSRSR - Y	RRR RRRRS	RHHNRR I YR -	RSR RHSRR	RRGR R	RGY - SRRR	YSRRGRRRY*
Antechinus bellus	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - SRRR	YSRRGRRRY*
Antechinus flavipes	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	HHNRRRTYR -	RSR - RHSRR	RRGR R	RGY - <u>S</u> RRR	YSRRGRRRY*
Antechinus godmani	MARYRRHSRS	R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY- <u>S</u> RRR	YSRRGRRRY*
Antechinus leo	MARYRRHSRS	R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY- <u>S</u> RRR	YSRRGRRRY*
Antechinus minimus	MARYRRHSRS	R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY- <u>S</u> RRR	YSRRGRRRY*
Antechinus naso	MARYRRHSRS	R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY- <u>S</u> RRR	YSRRGRRRY*
Antechinus stuartii	MARYRRHSRS	RSRSR YRR	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY- <u>S</u> RRR	YSRRGRRRY*
Antechinus swainsonii	MARYRRHSRS	R	RRR RRRSR	GR - RRRTYR -	RSR RHSRR	RRGR R	RGY- <u>S</u> RRR	YSRRGRRRY*
Dasycercus cristicauda	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR-	RSR RHSRR	RRGR R	RGY- <u>S</u> RRR	YSRRGRRRY *
Dasykaluta rosamondae	MARYRRHSRS	R	RRR RRRSR	HRNRRRTYR -	RSR RHSRR	RRGR R	RGY - S RRR	YSRRGRRRY *
Dasyuroides byrnei	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HRNRRRTYR-	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY *
Dasyurus hallucatus	MARYRRRSRS	RSRSR - Y - R	RRR RRRSR	GR - RRRTYR -	RSR RHSRR	RRGR R	RGY-SRRR	YSR - RGRRR*
Dasyurus viverrinus	MARYRRSRS	RSRSR - Y - R	RRR RRRSR	GR-RRRTYR-	RSR RHSRR	RRGR R	RGY-SRRR	YSR-RGRRR *
Dasyurus albopunctatus	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	GR - RRRTYR -	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Dasvurus geoffroii	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	GR - RRRTYR -	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Dasvurus spartacus	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	GR - RRRTYR -	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Dasvurus maculatus	MARYRRRSRS	RSRSR - Y - R	RRR RRRSR	GR - RRRTYR -	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Micromurexia habbema	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Murexchinus melanurus	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Murexia longicaudata	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	HHNRRRTYR-	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Myoictis melas	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	HRNRRTYR-	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Myoictis wallacei	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	HRNRRTYR-	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Neonhascogale lorentzii	MARYRRHSRS	RSRSR - Y-R	RRR RRRSR	GR - RRRTYR -	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Ningaui ridei	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	HHNRRRTYR-	RSR RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Ningaui timealevi	MARYRRHSRS	RSRSR - Y-R	RRR RRRSR	HHNRRRTYR-	RSR - RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Ningaui vyonnae	MARYRRHSRS	RSRSR - Y-R	RRR RRRSR	HHNRRRTYR-	RSR - RHSRR	RRGR R	RGY-SRRR	YSRRGRRRY*
Paramurevia rothschildi	MARYRRHSRS	RSRSR - Y-R	RRR - RRRSR	HHNRRRTYR-	RSR - RHSRR	RRGR R	RGY-SRRR	VSRRGRRRV*
Parantechinus anicalis	MARYRRHSRS							VSPRGPRPV*
Phaseogalo calura								VCDDCDDDV*
Phaseogale tapeatafa								
Phaseolocorox dorsalic								
Planigalo magulata magulata								
Planigale Maculata Maculata								
rianiyale maculata sinualis Planigala gilaci								
Planigale gliesi								
Planigale tenuirostris		KOKOKNULUK	KKKK5	Π INKKK I IK-		KKVKK	KUUSUKK	

Pseudantechinus bilarni	MARYRRHSRS	RSRSR - Y - R	RRR RRRSR	HRNRRRTYR -	RSR RHSRR	R R G R R	RGY - <u>S</u> RRR	Y <mark>SR - RGRRR *</mark>
Pseudantechinus macdonnellensis	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HRNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRRY</mark> *
Pseudantechinus ningbing	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HRNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRRY</mark> *
Pseudantechinus woolleyae	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HRNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRRY</mark> *
Sarcophilus harrisii	MARYRRRSRS	RSRSR Y - R	RRR RRRSR	GR - RRRTYR -	RSR RHSRR	R R G R R	RGY - <mark>S</mark> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis griseoventer	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRRR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis bindi	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HHNRRRTYR -	RSR RHSIR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRRY</mark> *
Sminthopsis longicaudata	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y
Sminthopsis aitkeni	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis archeri	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis crassicaudata	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis dolichura	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis douglasi	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HHNRRRTYR-	RSR RHSRR	RRGR R	RGY - SRRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis gilberti	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis granulipes	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis hirtipes	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis leucopus	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis macroura	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis murina	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis ooldea	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRRY</mark> *
Sminthopsis psammophila	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis virginiae	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Sminthopsis youngsoni	MARYRRHSRS	RSRSR Y - R	RRR RRRSR	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRR</mark> Y*
Myrmecobius fasciatus	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRRY</mark> *
Thylacinus cynocephalus	MARYRRHSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	HHNRRRTYR -	RSR RHSRR	RRGR R	RGY - <u>S</u> RRR	Y <mark>SRRGRRRY</mark> *
Dendrolagus dorianus	? A R Y R - H S R S	R?RSRY-R	RRR R ? R S R	YR <mark>S</mark> ?RRRYR-	GRR RRRSR	RGRR R	RGY-SR?R	YSRRRRRRY*
Dendrolagus goodfellowi	MARYR - HSRS	RSRSR Y - R	RRR RRRSR	YRSRRRYR -	GRR RRRSR	RGRR	RGY - <u>S</u> RRR	YSRRRRRRY*
Dorcopsis veterum	MARYR - HSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	YRSRRRYR -	GRR RRRSR	RGRR R	RGY - <u>S</u> RRR	Y <mark>S</mark> RRRRRRY*
Dorcopsulus vanheurni	MARYR - HSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	YRSRRR?YR-	GRR RRRSR	RGRR R	RGY - <u>S</u> RRR	Y <mark>S</mark> RRRRRRY*
Lagorchestes hirsutus	MARYR - HSRS	R S R S G Y - R	RQR RRRSR	YRSRRRRYR -	RRQ RRSRR	GRRR GYSR	RRY - <mark>S</mark> RRR	Y <mark>S</mark> RRRRRRY*
Lagostrophus fasciatus	MARYR - HSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	YRSRRRRYR -	GSR RSRSR	RRGR R	RGY - SRRR	Y <mark>S</mark> RRRRRRY*
Macropus rufus	MARYR - HSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	YR <mark>S</mark> QRRRYR -	GRR RRRSR	RGRR	RGY - SRRR	Y
Macropus parryi	MARYR - HSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	YRSRRRRYR -	<u>GRR R?RS</u> R	RGRR	RGY - SRRR	Y <mark>S</mark> RRRRRRY*
Macropus agilis	MARYR - HSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	YRSRRRRSR -	GRR RRRSR	RGRR	RGY - SRRR	Y <mark>S</mark> RRRRRRY*
Macropus eugenii	MARYR - HSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	YRSRRRRSR -	GRR RRRSR	RGRR R	RGY - <u>S</u> RRR	Y <mark>S</mark> RRRRRRY*
Macropus rufogriseus	MARYR - HSRS	RSRSR Y - R	RRR RRR <mark>S</mark> R	YRSRRRRYR -	GRR RRRSR	RGRR R	RGY - <u>S</u> RRR	Y <mark>S</mark> RRRRRRY*
Macropus giganteus	MARYR - HSRS	RSRSR Y - R	RRR RRRSR	YRSRRRRYR -	GRR RRRSR	RGRR R	RGY - <u>S</u> RRR	YSR - RRRRY*
Onychogalea fraenata	MARYR - HSRS	RSRS? Y	RRR - ?RRRSR	YRSRRRRYR -	GRR RRRSR	RGRR	RGY - <u>S</u> RRR	YSRRRRRRY*
Onychogalea unguifera	MARYR - HSRS	RSRSR - Y - R	RRR RRRSR	YRSRRRRYR -	GRR RRRSR	RGRR	RGY - <u>S</u> RRR	YSRRRRRRY*
Petrogale concinna	MARYR - HSRS	RSRSR - Y - R	RRR - RRRSR	YRSRRRR?R-	R RRRSR	RGRR R	RGY - SRRR	YSRRRRRRY*
Petrogale xanthopus	MARYR - HS?S	RSRSR - Y - R	RRR RRRSR	YRSRRRRYR -	GRR RRRSR	RGRR R	RGY - SRRR	YSRRRRRRY*
Setonix brachyurus	MARYR-HSRS	RSRSR - Y - R	RRR RRRSR	YRSRRRYR-	GRR RRRSR	RGRR	RGY-SRRR	YSRRRRRRY*
Thylogale stigmatica	MARYR - HSRS	RSRSR - Y - R	RRR RRRSR	YRSRRRRYR -	GRR RRRSR	RGRR R	RGYSCRR	YSRRRRRRY*
Wallabia bicolor	MARYR - HSRS	RSRSR - Y - R	RRR - RRRSR	YRSRRRRYR -	GRR RRRSR	RGRR R	RGY - SRRR	YSRRRRRRY*
Trichosurus vulpecula	MARYR - HSRS	RSRSR - Y	RRRRRRRSR	YRSRRRRYR -	RSR RR - RR	RGRR R	RGY - SRRR	YSRRGRRRY*
Phascolarctos cinereus	MARYR-HSRS	RSRSR - Y - Q	RRR RRRSR	YRSQRRRYR-	RRRGSRRRRR	RGRR	RGY - RRR	YS RRRR Y *
Aepyprymnus rufescens	MARYR - HSRS	RSRSR - Y - R	RRR RRRSR	YRSRRRRYR -	GSR RRRRS	RRRR R	RGY - SRRR	YSR-RRRRY*
Bettongia penicillata	MARYR - HSRS	RSRSR Y - R	KKR RRRSR	YKSRRRRYR -	GSR RRRSR	RRGR R	RGY - SRRR	YSKRRRRRY*
Hypsiprymnodon moschatus	MARYR - HSRS	RSRSR - Y - R	KKR - RRRSR	YKGRRRRYR -	KSR RRRRS	<u> </u>	RGY - YRRRR -	YSRRRRRYY Y*
Potorous longipes	MARYR - HSRS	KSR?R - Y - R	KRR RRRSR	YRSRRRYR -	GSRRSRSR	RRGR R	RGY-SRRR	YSR-RRRRY*
Pseudochirops cupreus	MARYR - HSRS	KSRSR YRR	KRR RRRSR	YRGRRRRYR -	KSR RRRRR	GREEGNCLGR	KGY - KRRR	YSRRRRRRYY *
-	M A R Y R - C C R S	K S K S K C	KKK	KRRCR -	KKK RRCCR	KKKK	KKC - CRRRR -	Y = -RCRRY X X

Figure S2. Multiple sequence alignment for included eutherian species based on muscle algorithm (implemented in Geneious 5.5.9.)

	1 10	20	30	40	50	60	62
Consensus	MARYRCCRSP	SRSRCRPRRR	RRR C R S R R R R	RCCRRRRRXM	VCCRRRRSYT	TTRCARY**Ŕ	R×
Sequence Logo		CRCRC RR	RRRCR RRRR	RCCRRRRR	VCCRR _D VT		
Cavia porcellus	MARYRCCRSP	SRSRC RRR	RRRFY-RRRR	RCHRRRRR	-CCRRRYT	R - RCKRY*	
Siamodon hispidus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRTYT	L - RCKKY*	
Apodemus sylvaticus	MARYRCCRSK	SRSRC RRR	RRRCRSRRRR	RCCRRRRR	RCCRRRRSYT	I - RCKRY*	
Mus pahari	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRO	RCCRRRRSYT	I - RCKKY*	
Mus cookii	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRRSYT	I - RCKKY*	
Mus famulus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCORRRR	RCCRRRRSYT	I - RCKKY*	
Mus macedonicus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRRSYT	I - RCKKY*	
Mus musculus bactrianus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRRSYT	I - RCKKY*	
Mus musculus castaneus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRRSYT	I - R <mark>C K K</mark> Y *	
Mus musculus domesticus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRRSYT	I - R <mark>C K K</mark> Y *	
Mus musculus musculus	MARYRCCRSK	SRSRC RRR	RRR <mark>C</mark> R - RRRR	RCCRRRRR	R C C R R R R S Y T	I - R <mark>C K K</mark> Y *	
Mus spicilegus	MARYRCCRSK	SRSRC RRR	RRR <mark>C</mark> R - RRRR	RCCRRRRR	R C C R R R R S Y T	I - R <mark>C K K</mark> Y *	
Mus spretus	MARYRCCRSK	SRSRC RRR	RRR <mark>C</mark> R - RRRR	RCCRRRRR	R C C R R R R S Y T	I - R <mark>C K K</mark> Y *	
Rattus norvegicus	MARYRCCRSK	SRSRC RRR	RRR <mark>C</mark> R - RRRR	RCCRRRRR	R C C R R R R S Y T	F - R <mark>C K</mark> R Y *	
Macaca fascicularis	MARYRCCCSQ	SRSRC CRR	RRR <mark>C</mark> R - RRRR	RRCRARRAM	RCCRRR YR	L - R C R R Y *	
Macaca mulatta	MARYRCCRSQ	SRSRC CRR	RRR <mark>C</mark> R - RRRR	RRCRARRAM	RCCRRR YR	L - R C R R Y *	
Papio cynocephalus	MARYRCCRSQ	SRSRC CRR	RRR <mark>C</mark> R - RRRR	RRCRARRAM	RCCRRR YR	L - R C R R Y *	
Colobus guereza	MARYRCRRSQ	SRSRC CRQ	RRR <mark>C</mark> R - RRRR	QRFRARKRAM	RCCHRR YR	L - R C R R Y *	
Nasalis larvatus	MAKSRCCGSQ	SRSRC CRP	RQRCR - RRRR	RSCRARRAM	RCCRRR YR	L - R C R R Y *	
Piliocolobus badius	MARYRCCRSQ	SRSRC CRR	RRR <mark>C</mark> R - RRRR	QRCRARRTAM	RCCRRR YR	R - R <mark>C</mark> R R Y *	
Semnopithecus entellus	M A R Y R R C R S Q	SRSRC CRP	RRR <mark>C</mark> R RRR	R <mark>S C</mark> R A R R R A T	RCCRRR YR	L - R C R R Y *	
Trachypithecus cristatus	MARYRCCRSQ	SRSRC CRP	RRR <mark>C</mark> R - RRRR	RSCRARRRAT	RCCRRR YR	L - R	
Trachypithecus francoisi	MARYRCCRSQ	SRSRC - CRP	RRR <mark>C</mark> R - RRRR	R <mark>S C</mark> R A R R R A T	RCCRRR YR	L - R C R R Y *	
Trachypithecus geei	MARYRCCRSQ	SRSRC - CRP	RRR <mark>C</mark> R - RRRR	R S C R A R R R A T	RCCRRR YR	L - R C R R Y *	
Trachypithecus johnii	MARYRRCRSQ	SRSRC - CRP	RRRCR - RRRR	RSCRARRRAT	RCCRRR YR	L - R C R R Y *	
Trachypithecus obscurus	MARYRCCRSQ	SRSRC - CRP	RRR <mark>C</mark> R - RRRR	R <mark>S C</mark> R A R R R A T	RCCRRR YR	L - R S R R Y *	
Trachypithecus phayrei	MARYRCCRSQ	SRSRC - CRP	RRRCR - RRRR	RSCRARRRAT	RCCRRR YR	L <u>S R R Y</u> *	
Trachypithecus pileatus	MARYRCCRSQ	SRSRC - CRP	RRRCR - RRRR	RSCRARRAT	RCCRRR YR	L - RCRRY*	
Trachypithecus vetulus	MARYRRCRSQ	SRSRC CRP	RRRCR RRR	RSCRARRAT	RCCRRR YR	L - RCRRY*	
Gorilla gorilla	MARYRCCRSQ	SRSRC YRQ	RQTSR-RRRR	RSCQTQRRAM	RCCRRR NR	L-RRRKH*	
Homo sapiens	MARYRCCRSQ	SRSRY - YRQ	RQRSR - RRRR	RSCQTRRRAM	RCCRPR - YR	P - RCRRH*	
Pan paniscus	MARYRCCRSQ	SRSRC YRQ	R - RSR - RRKR	QSCQTQRRAM	RCCRRR SR	L - RRRRH*	
Pan troglodytes	MARYRCCRSQ	SRSRC YRQ	RORSR - RRKR	QSCQTQRRAM	RCCRRR SR	M - RRRRH *	
Pongo pygmaeus	MARYRCCRSQ	SQSRC - CRR	RQR <mark>CH</mark> - RRRR	RCCQTRRRAM	RCCRRR YR	L - RCRRH *	

Hylobates lar	MARYRCCRSQ	SRSRC YRR	GQRSR - RRRR	RSCQTRRRAM	RCCRPR YR	L-RRRRH*
Ateles sp.	MARYRCCRSR	SRSRC YRQ	RPRCR-RRRR	RSCRRRRGS -	RCCRRR YR	L-RRRRY*
Saguinus imperator	MARYRCCRSQ	SRSRC YRQ	RRRGR - RRRR	RTCRRRRAS -	RCCRRR YK	L - TCRRY*
Saimiri sciureus	MARYRCCRSR	SRSRC YRR	RRRCR - TRRR	RCCRRRRAR -	RCCRRR YK	L-RCRRY*
Otolemur garnettii	MARYRCCRSQ	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRR YR	L-RCRRY*
Balaena mysticetus	MARNRCCRSQ	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRRYT	TVRCARR*
Eubalaena australis	MARNRCCRSQ	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRRYT	TVRCARR*
Eubalaena glacialis	MARNRCCRSQ	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRRYT	TVRCARR *
Eubalaena japonica	MARNRCCRSQ	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRRYT	TVRCARR*
Balaenoptera acutorostrata	MARNSCCRSP	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRRYT	TVRCAGQ*
Balaenoptera bonaerensis	MARNSCCRSP	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRRYT	TVRCAGQ*
Balaenoptera borealis	MARNSCCRSP	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRRYT	TVRCARQ*
Balaenoptera edeni	MARNSCCRSP	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRRYT	TVRCARQ*
Balaenoptera musculus	MARNTCCRSP	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRRYT	TVRCARQ*
Balaenoptera physalus	MARNSCCRSP	SRSRR RRP	RQRRR - SQRR	RCCQRRRR	VCCRR YT	TVRCARQ*
Megaptera novaeangliae	MARNSCCRSP	SRSRR RRP	RQRRR - SQRR	RCCQRRRR	VCCRR YT	TVRCARQ*
Eschrichtius robustus	MARNSCCRSP	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRR YT	TVRCARQ*
Caperea marginata	MARNRCCRSQ	SRSRR RRP	RQRCR - SQRR	RCCRRRRR	VCCRR YT	TVRCARQ*
Cephalorhynchus commersonii	MARNR - CRSP	SQSRC RRP	RRRCR RRR	RCCRRRRR	VCCRR YA	TTRCARQ*
Delphinus capensis	MARNR - CRSP	SQSRC RRP	RRRCR RRR	RCCRRRRR	VCCRR YT	TTRCAR*
Delphinus delphis	MARNR - CRSP	SQSRC RRP	RRRCR RRR	RCCRRRRR	VCCRR YT	TTRCAR*
Feresa attenuata	MARNR - CRSP	SQSRC RRP	RRRYR RRR	RCCRRRRR	VCCRR YT	TTRCARQ*
Globicephala macrorhynchus	MARNR - CRSP	SQSRC RRP	RRRYR RRR	QCCRRRRR	VCCRR YT	TTRCARQ*
Grampus griseus	MARNR - CRSP	SQSRC RRP	RRRYR RRR	QCCRRRRR	VCCRR YT	TTRCARQ*
Lagenodelphis hosei	MARNR - CRSP	SQSRC RRP	RRRCR RRR	RCCRRRRR	VCCRR YT	TTRCAR*
Lagenorhynchus acutus	MARNR - CRSP	SQSRC RRP	RRRCL - RRR	RCCRRRRR	VCCRR YT	TTRCAR*
Lagenorhynchus albirostris	MARNR - CRSP	SQSRC RRP	RRRCR RRR	RCCRRRRR	VCCRRYT	TTRCASQ*
Lagenorhynchus obliquidens	MARNR - CRSP	SQSRC RRP	RRRCR RRR	RCCRRRRR	VCCRRYT	TTRCAR*
Lissodelphis borealis	MARNR - CRSP	SQSRC RRP	RRRCR RRR	RCCRRRRR	VCCRRYT	TIRCARQ*
Orcaella heinsohni	MARNR - CRSP	SQSRC RRP	RRRCR RRR	RCCRRRRR	VCCRRYT	TTRCARQ*
Orcinus orca	MARNR - CRSP	SQSRC RRP	RRRCR RRI	RCCRRQRR	VCCRRYI	TIRCARQ*
Peponocephala electra	MARNR - CRSP	SUSRC RRP	KKKYK KKK	RCCRRRRR	VCCRRYI	
Pseudorca crassidens	MARNR - CRSP	SUSRC RRP	KKKYK KKK	QCCRRRRR	VCCRRYI	
Sotalia fluviatilis	MARNR - CRSP	SUSRC RRP	KKKCK KKK	RCCRRRRR	VCCRRYI	
Sousa chinensis	MARNR - CRSP	SUSRC RRP	KKKCK KKK	RCCRRRRR	VCCRRYI	
Stenella attenuata	MARNK-CRSP	SUSKC KKP	KKKCKKKK	KCCKKKKK	VCCRRY	
Stenella coeruleoalba	MARNK - CRSP	SUSKC KKP	KKKCK KKK	LCCKKKKK	VCCRRY	
Stenella frontalis	MARNR - CRSP	SUSKC KKP	KKKCKKKK	RCCRRRRR	VCCRRY	
Stenella longirostris	MARNR-CRSP	SUSKC KKP	KKKCKKKK	RCCRRRRR	VCCRRY	
Steno bredanensis	MARNR-CRSP	SUSKC KKP	KKKCKKKK	KCCKKKKKK	VCCRRY	TTRCARU*
Tursiops aduncus	MARNR - CRSP	SUSKC KKP	KKKCKKKK	LCCRRRRR	VCCRRYI	
iursiops truncatus	MADND CDCD	SUSKU KKP	KKKCK KKK	LCCKKKKK	VCCRR YI	
	MADND CDCD	SUSKG KKP		RCCORDER	VCCRR YI	
Lipotes Vexiliirer	MADND CDCD	SUSKG KKP	KKKKK - SKKK	RCCORDER	VCCRR Y	
Deiphinapterus ieucas	MARTR CRSP	SUSKG KKP		RCCORDER	VCCRR YI	
Monodon monoceros	MADND CDCD	SUSKG KKP		RCCORDER	VCCKKY	
Neophocaena phocaenoides	MAKNK - CKSP	SUSKG - KCP	KKK I K - 5 K K K	KUUUKKKK	VCKKKYI	K-KCAKU*

Phocoena phocoena	MARNR - CRSP	SQSRG RCP	RRRYR - <mark>SK</mark> RR	RCCQRRRR	VCRRR YT	R - R C A R Q *	
Phocoenoides dalli	MARNR - CRSP	SOSRG RCP	RRRYR - SKRR	RCCORRR	VCRRR YT	R - RCARO*	
Kogia breviceps	M A R N R C C R S Q	SRGRC RRP	RRRYR - SPRR	RRYORRRR	VCCRR ST	TMRCASO*	
Kogia sima	MARNRCCRSQ	SRGRC RRP	RRRCR - SPKR	RRYORRRR	V C C R R S A	TMRCASO*	
Physeter catodon	MARNRCCRSQ	SRSRC RRP	RRRCR - SPRR	RRYORRRR	VCCRR YT	VTRCARQ*	
Platanista minor	MARNRCCRSQ	SRSRC RRP	KRGCR - SRRR	RCYQRRRR	VCCRRYT	TIRCARQ*	
Pontoporia blainvillei	MARNR - CRSP	SQNRG RRP	RRRYR - SRRR	RCCQRRRR	VCCRRYT	SVRCARQ*	
Berardius bairdii	MARNRCCRSQ	SQSRR RRP	RRRNR - SRRR	QCCQRRRR	VCCRRYT	AIRCARQ*	
Mesoplodon bidens	MARNRCCRSQ	SQSRR RRP	RRRYR - <mark>S</mark> RRR	QCCQKRRR	VCCRRYT	AIRCARQ*	
Mesoplodon grayi	MARNTCCRSQ	SQSRR RRP	RRRYR - <mark>S</mark> RRK	Q C C Q K R R R	VCCRR YT	AIRCARQ*	
Mesoplodon peruvianus	M A R N R C C R S Q	<mark>S Q S</mark> R R R R P	RRRYR - <mark>S</mark> RRK	QCCQKRRR	VCCRR YT	AIRCARQ*	
Tasmacetus shepherdi	M A R N R C C R S Q	SQSRR RRP	RRRYR - SRRR	Q C C Q K R R R	V C C R R Y T	AIRCARQ*	
Ziphius cavirostris	M A R N R C C R G Q	<mark>S Q S</mark> R R R R P	RRRYR - <mark>S</mark> RRR	Q C C Q K R R R	VCCRR YT	A T R C A R Q *	
Hexaprotodon liberiensis	M A R Y R C C R S P	SRSRC RRQ	RRRCR - RRRR	R C C R Q R R R R -	V C C R R Y T	MVRCTRQ *	
Hippopotamus amphibius	MARYRCCRSP	SRSRC RRQ	RRRCR - RRRR	R C C R Q R R R R -	VCCRR YT	MVRCTRQ*	
Antilocapra americana	MARYRCCLTH	S R S R C R P RRR	RRRCR - KLRR	RFCRRPRRR -	V C C R R Y T	AIRCTR*	
Bos taurus	MARYRCCLTH	SGSRCR - RRR	RRRCR - RRRR	R	VCCRR YT	VIRCTRQ*	
Capra hircus	MARYRCCLTH	SRSRCR - RRR	RRRCR - RRRR	RFGRRRRR -	V C C R R Y T	VVRCTRQ*	
Ovis aries	MARYRCCLTH	SRSRCR - RRR	RRRCR - RRRR	RFGRRRRR -	VCCRRYT	VVRCTRQ*	
Ovis dalli	MARYRCCLTH	SRSRCR - RRR	RRR <mark>C</mark> R - RRRR	RFGRRRRR -	VCCRR YT	VVRCTRQ*	
Elaphodus cephalophus	MARYRCCLTH	SRSRCR - RRR	RRR <mark>CH</mark> - RRRK	RFGRRRRR -	VCCRR YT	VVRCTRQ*	
Moschus sp.	MARYRCRLTH	SRSGCR - RRR	RRR <mark>C</mark> R - RRRR	RFCRRRRR -	VCCRR YT	VVRCTRQ*	
Potamochoerus porcus	MARYRCCRSH	SRSRC RPR	RRR <mark>C</mark> R - RRRR	RCCPRRRRA -	VCCRR YT	VIRCRRC*	
Sus scrofa	MARYRCCRSH	SRSRC RPR	RRR <mark>C</mark> R - RRRR	RCCPRRRRA -	VCCRR YT	VIRCRRC*	
Pteropus hypomelanus	M A R Y R C C R S Q	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	V C C R R Y T	V - RCRRRR *	
Mormoops megalophylla	M A R Y R C C R S P	SRSRC RRR	RRR <mark>C</mark> R - RRRR	R <mark>SC</mark> RRRRR	V C C R R Y T	V - RCRRR *	
Pteronotus parnellii	M A R Y R C C R S P	SRSRC RRR	RRR <mark>C</mark> R - RRRR	RCCRRRRR	V C C R R Y T	V - RCRRR *	
Chilonatalus micropus	MARYRCCRSQ	SRSRC RRR	RRRCR - TRRR	R <mark>CC</mark> RRRRRR -	VCCRR YT	VVRCRRR*	
Natalus stramineus	M A R Y R C C R S Q	SRSRC RPR	RRRCR - TRRR	RCCRRRRR -	V C C R R Y T	VVRCRRR*	
Desmodus rotundus	MARYRCCRSP	SRSRC RRR	RRR <mark>C</mark> R - RRRR	RCCRRRRR	VCCRR YT	V - RCRRR *	
Monophyllus redmani	MARYRCCRSP	SRSRC RRR	RRR <mark>C</mark> R - RRRR	RCCRRRRR	VCCRR YT	V - RCRRR *	
Hipposideros commersoni	MARYRCCRSH	SRSRC RRR	RRR <mark>S</mark> R - RRRR	R <mark>S C G</mark> R R R R	AG YRR YT	V - R Y R R R *	
Rhinolophus ferrumequinum	MARYSCCRSH	SRSRS RRR	RQRCR - RRRR	R	ACYRR YT	V-RYRRRRR R	{*
Rhinopoma hardwickii	MARYRCCRSR	<u>SRSRC RPR</u>	RRR <mark>C</mark> R - RRRR	RCCRRRRR	VCCRR YS	A - RCRRRR *	
Chalinolobus beatrix	M A R Y R C C R	SRSRC RRR	RRR <mark>SY-</mark> RRRR	RCCRRRRR -	V C C R R Y V	RCRRR *	
Corynorhinus townsendii	MARYRCCRSQ	SRSRC RRR	RRR <mark>C</mark> Y-RRRR	RCCRRRRR -	V C C R R Y T	RYRRR *	
Galeopterus variegatus	MARYRCCR	SRSRC - RRR	R R <mark>S C</mark> R R R R	RCRRRRAR	RSCRRR - YS	LRCCRRY*	
Eptesicus brasiliensis	MARYRCCR	SRSRC RRR	RRR <mark>CY-</mark> RRRR	RCCRRRRR -	VCCRR YT	VIRCRRR*	
Eptesicus fuscus	MARYRCCR	SRSRC - RRR	RRR <mark>C</mark> Y - RRRR	RCCRRRRR -	V C C R R Y T	VIRCRRR*	
Hypsugo savii	MARYRRCR	SRSRC RRR	RRRCH - RRRR	RCCRRRRRR	ACCRR Y -	RCRRR *	
Murina cyclotis	MARYRCCR	SRSRC RRR	RRRCH - RRRR	RCSRRRRR -	VCCRR YT	VIRCRRR*	
Myotis daubentonii	MARYRCCR	SRSRC RRR	RRRCY-RRRR	RCCRRRRRR	VCCRR YS	RCRRR *	
Myotis lucifugus	MARYRCCR	SRSRC RRR	RRRCY-RRRR	RCCRRRRRR	VCCRR YS	RCRRR *	
Neoromicia brunneus	MARYRRCR	SRSRC RRR	RRRCH - RRRR	RCCRRRRRR	ACCRR Y -	RCRRR *	
Otonycteris hemprichii	MARYRCCRSR	SRSRC RRR	RR <mark>KC</mark> Y-RRRR	RCSRKRRRR -	VCCRR YT	VMRCRRR*	

Plecotus auritus	MARYRCCRSQ	SRSRC RRR	RRRCY-RRRR	RCCRRRRRR -	VCCRR YT	VVRCRRR*
Acomys cahirinus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRR - YT	L-RCRRI*
Acomys cilcicus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRR - YT	L-RCRRI*
Arvicola sapidus	MARYR - CRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCYRRRRTYT	M - RCKRY*
Arvicola terrestris	MARYR - CRSK	SRSRC RHR	RRRCR - RRRR	RCCRRRRR	RCYRRRRTYT	M - RCKRY*
Chionomys nivalis	MARYR - CRSQ	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCYGRRRTYT	L - RCKRY*
Clethrionomys glareolus	MARYR - CRSK	SRSRC RRR	RRRCR RRR	RCCRRRRR	RCYRRRRSYS	I-MCKRY*
Cricetulus griseus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCYRRRRTYT	L - RCKRY*
Meriones unguiculatus	MARYRCCRSK	SRSRC RRR	RRRCR RRR	RCCRRRRR	RCCRRRRRYT	L - RCKRY*
Mesocricetus auratus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRRTYT	L - RCKRY*
Micromys minutus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRRSYT	L - RCKRY*
Microtus agrestis	MARYR - CRSK	SRSRC SRR	RRRCR - RRRR	RCCRRRRR	RCCRRRRTYT	M - RCKRY*
Microtus arvalis	MARYR - YRSK	SRSRC SRG	RRRCR - RRRR	RCCRRRRR	RCCRRRRTYT	V - RCKRY*
Microtus cabrerae	MARYR - YRSK	SRSRC SRR	RRRCR - RRRR	RCCQSRKR	RCYRRRRSCT	M - RCKRY*
Microtus gerbei	MARYR - CRSK	SRSRC SRR	RRRCR - RRRR	RCCRRRRR	RCYRRRRSYT	M - RCKRY*
Peromyscus californicus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRR - YT	L - RCKRY*
Peromyscus leucopus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRR - YT	L - RCKRY*
Peromyscus manicaulatus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRR - YT	L - RCKRY*
Peromyscus polionotus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRR - YT	L - RCKRY*
Phodopus campbelli	MARYR - CRSQ	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCYGRRRTYT	L - RCKRY*
Phodopus roborovski	MARYRCCRSK	SRSRC RRR	RRRCR - SRRR	RCCRRRRR	RCCRRRR - YT	L - RCKRY*
Phodopus sungorus	MARYRCCRSK	SRSRC RRR	RRRCR - RRRR	RCCRRRRR	RCCRRRR - YS	L - RCKRY*
Pitimys duodecimcostatus	MARYR - CRSK	SRSRC SRH	RRRCR - RRRR	RCCRRRRR	RCYRRRRSYT	M - RCKRY*
Pitimys lusitanicus	MARYR - CRSK	SRSRC SRR	RRRCR - RRRR	RCCRRRRR	RCYRRRRSYT	M - RCKRY*

Figure S3. Multiple sequence alignment for included metatherian species based on muscle algorithm (implemented in Geneious 5.5.9.)

	1 10	20	30	40	50	60	70	74
Consensus	MARYRRHSRS	RSRSRYRRR	RRRRSRHRNR	RRTYRRSRGR	HSRRRRGRGX	XLGRRGYYSR	RRYSRRGRRR	Y * X *
			ה הסתהתה				מתה, תחסזתה	T 7
Sequence Logo			KKKKKKHRMK		HCKKKKCK		KK KKCKKK	V
					REFINITED I			1
								-
Antechinomys laniger		KSKSKI-KKK	KKKKSKHHN-	KKIYKKSK-K	HSKKKKGK		KKYSKKGKKK	Υ* Υ*
Antechinus stuartii			RKKKSKHHNK	KKIIKKSK-K			KK I SKKGKKK	I [™] V¥
Antechinus godmani		KSKSKI-KKK	KKKKSKHHNK	KKIIKKSK-K	HSKKKKGK		KK I SKKGKKK	Υ* \
Antechinus leo	MARYRRHSRS	KSKSKY-KKK	KKKKSKHHNK	KKIYKKSK-K	HSKKKKGK	KKGY-SK	RRYSRRGRRR	Y↑ V¥
Antechinus flavipes	MARYRRHSRS	KSKSKY-KKK	KKKKSKHHNK	KKIYKKSK-K	HSKKKKGK	KKGY - SK	RRYSRRGRRR	Υ ↑
Antechinus naso	MARYRRHSRS	RSRSRY-RRR	RERESENTER	RRTYRRSR-R	HSKKKKGK	KKGY - SK	RRYSRRGRRR	Υ *
Antechinus minimus	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR-R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Y *
Antechinus bellus	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RR I YRRSR - R	HSRRRRGR	RRG Y - SR	RRYSRRGRRR	Y *
Antechinus swainsonii	MARYRRHSRS	RSRSRY - RRR	RRRRSRGR - R	RRTYRRSR - R	HSRRRRGR	RRGY - <mark>S</mark> R	RRYSRRGRRR	Y *
Dasycercus cristicauda	MARYRRHSRS	R	RRRRSRHHNR	RRTYRRSR - R	HSRRRGR	RRGY - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Dasykaluta rosamondae	MARYRRHSRS	R	R R R R <mark>S</mark> R H R N R	RRTYRRSR - R	HSRRRRGR	RRGY - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y*
Dasyuroides byrnei	MARYRRHSRS	R	R R R R <mark>S</mark> R H R N R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Dasyurus hallucatus	MARYRRRSRS	R	RRRR <mark>S</mark> R G R - R	R R T Y R R <mark>S</mark> R - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	*
Dasyurus viverrinus	MARYRRRSRS	R	R R R R <mark>S</mark> R G R - R	RRTYRRSR - R	HSRRRRGR	RRGY - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	*
Dasyurus geoffroii	MARYRRHSRS	R	R R R R <mark>S</mark> R G R - R	R R T Y R R <mark>S</mark> R - R	HSRRRRGR	RRGY - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Dasyurus albopunctatus	MARYRRHSRS	R <mark>S</mark> R <mark>S</mark> R Y - RRR	RRRR <mark>S</mark> R G R - R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Dasyurus spartacus	MARYRRHSRS	R <mark>S</mark> RSRY-RRR	RRRR <mark>S</mark> R G R - R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Dasyurus maculatus	MARYRRRSRS	R <mark>S</mark> RSRY-RRR	RRRR <mark>S</mark> R G R - R	RRTYRRSR-R	HSRRRRGR	R R G Y - <mark>S</mark> R	RR Y <mark>S</mark> R R G R R R	Y *
Micromurexia habbema	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR-R	HSRRRRGR	RRGY - SR	RR Y <mark>S</mark> RR G R R R	Y *
Murexchinus melanurus	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR-R	HSRRRRGR	R R G Y - <mark>S</mark> R	RR YSRRGRRR	Y *
Murexia longicaudata	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR-R	HSRRRRGR	R R G Y - S R	RRYSRRGRRR	Y *
Myoictis wallacei	MARYRRHSRS	RSRSRY-RRR	RRRRSRHRNR	RRTYRRSR-R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Y*
Myoictis melas	MARYRRHSRS	RSRSRY-RRR	RRRRSRHRNR	RRTYRRSR-R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Y*
Neophascogale lorentzii	MARYRRHSRS	RSRSRY-RRR	RRRRSRGR-R	RRTYRRSR - R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Y *
Ningaui vyonnae	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR - R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Ý*
Ningaui timealevi	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR - R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Ý*
Ningaui ridei	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR - R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Ý*
Paramurexia rothschildi	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR-R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Ý*
Parantechinus apicalis	MARYRRHSRS	RSRSRY-RRR	RRRRSRHRNR	RRTYRRSR - R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Ϋ́*
Phascogale tapoatafa	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR - R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Ϋ́*
Phascogale calura	MARYRRHSRS	RSRSRY-RRR	RRRSRHHNR	RRTYRRSR-R	HSRRRGR	RRGY - SR	RRYSRRGRRR	Ϋ́*
Phascolosorex dorsalis	MARYRRHSRS	RSRSRY-RRR	RRRRSRGR - R	RRTYRRSR-R	HSRRRRGR	RRGY - SR	RRYSRRGRRR	Ϋ́*
Planigale maculata maculata	MARYRRHSRS	RSRSRY-RRR	RRRSRHHNR	RRTYRRSR-R	HSRRRGR	RRGY - SR	RRYSRRGRRR	Ϋ́*
Planigale maculata sinualis	MARCRRHSRS	RSRSRN - 0C0	RRRRRY-NR	RRTYRRSR-R	HSRRRGR	RRGC - SR	RRYSRRGRRR	Ϋ́*
Planigale tenuirostris	MARCRRHTRS	RSRSRN-0C0	RRRRSHYNR	RRTYRRSR-R	HSRRRVR	RRGC - SC	RRCSRRRRR	· *
Planigale gilesi	MARCRRHSRS	RSRSRN-000	RRRR-RHYNR	RRTYRRSR-R	HSRRRVR	RRGC - SC	RRCSRRRRR	č*
Pseudantechinus hilarni	MARYRRHSRS	RSRSRY-RRR	RRRRSRHRNR	RRTYRRSR-R	HSRRRRGR		RRYSRRGRRR	*
Pseudantechinus woollevae	MARYRRHSRS	RSRSRY-RRR	RRRRSRHRMR	RRTYRRSR-R	HSRRRGR	RRGY - SR	RRYSRRGRRR	γ*
Pseudantechinus ninghing	MARYRRHSRS	RSRSRY_RRR	RRRRSRHRNR	RRTYRRSR-R	HSRRRGR		RRYSRRGRRR	γ*
Pseudantechinus macdonnellensis	MARYRRHCPC	RSRSRY_RPP	RRRRSRHRMP	RRTYRRCR_P	HSRRRRGR -		RRYSRRGRPP	γ*
Sarconhilus harrisii	MARVERECOC				HSPRPRCR			v*
Sarcophilus harrish							NUL 2 UUQUUU	1.1

Sminthopsis griseoventer	MARYRRHSRS	R <mark>S</mark> RSRY-RRR	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Sminthopsis bindi	MARYRRHSRS	R <mark>SRS</mark> RY-RRR	RRRRSRHHNR	RRTYRRSR - R	HSIRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Sminthopsis longicaudata	MARYRRHSRS	R <mark>S</mark> RSRY-RRR	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRYSRR - RRR	Y *
Sminthopsis virginiae	MARYRRHSRS	RSRSRY-RRR	RRRRSRHHNR	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Sminthopsis douglasi	MARYRRHSRS	R <mark>SRS</mark> RY-RRR	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Sminthopsis hirtipes	MARYRRHSRS	R <mark>S R S R Y -</mark> R R R	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Sminthopsis youngsoni	MARYRRHSRS	R <mark>S R S R Y -</mark> R R R	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Sminthopsis psammophila	MARYRRHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Sminthopsis aitkeni	MARYRRHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Sminthopsis macroura	MARYRRHSRS	R	R R R R <mark>S</mark> R H H N R	RRTYRR <mark>S</mark> R - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Sminthopsis dolichura	MARYRRHSRS	R	R R R R <mark>S R H H N</mark> R	RRTYRR <mark>S</mark> R - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Sminthopsis murina	MARYRRHSRS	R	R R R R <mark>S</mark> R H H N R	RRTYRR <mark>S</mark> R - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Sminthopsis ooldea	MARYRRHSRS	R	R R R R <mark>S</mark> R H H N R	RRTYRR <mark>S</mark> R - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R <mark>G</mark> R R R	Y *
Sminthopsis leucopus	MARYRRHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Sminthopsis archeri	MARYRRHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Sminthopsis granulipes	MARYRRHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Sminthopsis gilberti	MARYRRHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S R H H N</mark> R	RRTYRR <mark>S</mark> R - R	HSRRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R G R R R	Y *
Sminthopsis crassicaudata	MARYRRHSRS	R <mark>SRS</mark> RY-RRR	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Myrmecobius fasciatus	MARYRRHSRS	R <mark>S R S R Y -</mark> R R R	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Thylacinus cynocephalus	MARYRRHSRS	R <mark>S R S R Y -</mark> R R R	R R R R <mark>S R H H N</mark> R	RRTYRRSR - R	HSRRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR G RRR	Y *
Dendrolagus dorianus	? A R Y - R H S R S	R ? R <mark>S</mark> R Y - R R R	RR?RSRYRS?	RRRYRGRR - R	RRSRRGRR	R R G Y - <mark>S</mark> R	? RY <mark>S</mark> RRRRR	Y *
Dendrolagus goodfellowi	MARY - RHSRS	R <mark>SRS</mark> RY-RRR	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYRGRR - R	RR <mark>S</mark> RR G RR	R - G Y - <mark>S</mark> R	RR <mark>Y S</mark> R R R R R R	Y *
Dorcopsis veterum	MARY - RHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	RR <mark>S</mark> RR G RR	R R G Y - <mark>S</mark> R	RR <mark>Y S</mark> R R R R R R	Y *
Dorcopsulus vanheurni	MARY - RHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RR?YRGRR-R	RR <mark>S</mark> RR G RR	R R G Y - <mark>S</mark> R	RR <mark>Y S</mark> R R R R R R	Y *
Lagorchestes hirsutus	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYRRRQ - R	R <mark>S</mark> R R G R R R G Y	<mark>S RRRY - S</mark> R	RR <mark>Y S</mark> R R R R R R	Y *
Lagostrophus fasciatus	MARY - RHSRS	RSRSRY-RRR	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYRGSR - R	SRSRRRGR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R R R R R	Y *
Macropus rufus	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S Q</mark>	RRRYR <mark>G</mark> RR - R	RR <mark>S</mark> RRGRR	R - G Y - <mark>S</mark> R	RRY <mark>S</mark> RR - RRR	Y *
Macropus parryi	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	? R <mark>S</mark> R R G R R	R - G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R R R R R	Y *
Macropus agilis	MARY - RHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRR <mark>S</mark> RGRR - R	RR <mark>S</mark> RR G RR	R - G Y - <mark>S</mark> R	RR <mark>YS</mark> R R R R R R	Y *
Macropus eugenii	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRR <mark>S</mark> R G RR - R	RR <mark>S</mark> RR G RR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R R R R R	Y *
Macropus rufogriseus	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	R R <mark>S</mark> R R G R R	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R R R R R	Y *
Macropus giganteus	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	RR <mark>S</mark> RRGRR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR - RRR	Y *
Onychogalea fraenata	MARY - RHSRS	R <mark>S R S ?</mark> Y - R R R	? R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	R R <mark>S</mark> R R G R R	R - G Y - <mark>S</mark> R	RR <mark>YS</mark> R R R R R R	Y *
Onychogalea unguifera	MARY - RHSRS	R <mark>S R S</mark> R Y - R R R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	RR <mark>S</mark> RR G RR	R - G Y - <mark>S</mark> R	RR <mark>YS</mark> R R R R R R	Y *
Petrogale concinna	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRR?RR-R	RR <mark>S</mark> RR G RR	R R G Y - <mark>S</mark> R	RR Y S R R R R R R	Y *
Petrogale xanthopus	MARY - RHS ? S	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	RR <mark>S</mark> RR G RR	R R G Y - <mark>S</mark> R	RR Y <mark>S</mark> R R R R R R	Y *
Setonix brachyurus	MARY-RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	RR <mark>S</mark> RR G RR	R - G Y - <mark>S</mark> R	RRYSRRRRR	Y *
Thylogale stigmatica	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	R R <mark>S</mark> R R G R R	R R G Y - <mark>S C</mark>	R R Y <mark>S</mark> R R R R R R	Y *
Wallabia bicolor	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>G</mark> RR - R	RR <mark>S</mark> RR G RR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R R R R R	Y *
Trichosurus vulpecula	MARY - RHSRS	R <mark>S R S</mark> R Y R R R R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYRR <mark>S</mark> R	RRRRR <mark>G</mark> RR	R R G Y - <mark>S</mark> R	R R Y <mark>S</mark> R R <mark>G</mark> R R R	Y *
Phascolarctos cinereus	MARY - RHSRS	R <mark>S</mark> R <mark>S</mark> RY-QRR	R R R R <mark>S</mark> R Y R <mark>S Q</mark>	R R R Y R R R R G S	RRRRRR <mark>G</mark> R	R R G Y R	RRY <mark>S</mark> RR RR	Y *
Aepyprymnus rufescens	MARY - RHSRS	R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYR <mark>GS</mark> R - R	RRR <mark>S</mark> RRRR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR - RRR	Y *
Bettongia penicillata	MARY - RHSRS	R <mark>S R S R Y -</mark> R R R	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYRGSR - R	RR <mark>S</mark> RRR <mark>G</mark> R	R R G Y - <mark>S</mark> R	RR <mark>YS</mark> RR R R R R	Y *
Hypsiprymnodon moschatus	MARY - RHSRS	R <mark>S</mark> RSRY-RRR	R R R R <mark>S</mark> R Y R G R	RRRYRR <mark>S</mark> R - R	R R R <mark>S</mark> R R G	RRGYYRR	RR <mark>YS</mark> RR R R R R	Y Y Y *
Potorous longipes	MARY-RHSRS	R <mark>S</mark> R?RY-RRR	R R R R <mark>S</mark> R Y R <mark>S</mark> R	RRRYRGSR - R	SRSRRRGR	R R G Y - <mark>S</mark> R	RRY <mark>S</mark> RR - RRR	Y *
Pseudochirops cupreus	MARY - RHSRS	R <mark>S</mark> R <mark>S</mark> R Y R R R R	R R R R <mark>S</mark> R Y R G R	RRRYRR <mark>S</mark> R - R	R R R R G R R R G N	CLGRRGY-RR	RR <mark>YS</mark> RR R R R R	Y

	Evolutionary rate	volutionary Residual testes mass					Arginine content (% of sequence length)						Amino acid frequencies (% of clade alignment)									:	statistics for clade amino acid alignments							
Clade	ω (M3)	n	mean	stdev	median	mad	range	n	mean	stdev	median	mad	range	A	С	FG	н	ΙK	LN	чN	ΙP	QF	≀ s	6 т	VΥ	,	n	% of identical sites	pairwise sequence identity	mean sequence length
Primates	0.791	17	-0.26	0.46	-0.10	0.56	1.46	25	51.14	4.10	51.92	4.30	15.77	5	16	0 0	1	01	2 3	30	1	4 5	18	32	0 6		38	44.40	90.50	51.40
Rodentia	0.226	38	0.17	0.35	0.19	0.34	1.45	37	54.87	1.91	54.90	1.57	9.80	2	16	0 0	0	14	1 2	20	0	0 5	57	2	0 6		25	30.80	83.30	51.60
Chiroptera	0.362	4	-0.04	0.41	-0.01	0.44	0.90	22	57.45	2.84	57.14	1.51	10.89	3	17	0 0	1	0 0	0 2	20	1	1 5	77	2	3 5		21	53.60	85.60	49.30
Artiodactyla	0.452	5	-0.08	0.37	0.04	0.38	0.92	11	49.82	1.92	49.02	1.45	5.77	3	16	1 1	2	10	1 2	20	1	2 5	0 5	55	5 4		11	54.70	84.40	51.60
Cetacea	0.942	26	0.08	0.48	0.35	0.59	1.54	55	45.46	2.70	45.83	3.09	10.94	4	15	01	0	01	0 2	22	4	6 4	58	35	3 3		55	38.00	83.90	48.80
Diprotodontia	0.198	10	-0.38	0.31	-0.39	0.26	1.00	26	61.90	1.51	61.90	1.67	5.20	2	0	05	2	0 0	0 2	20	0	0 6	2 1	50	0 11	1	26	52.70	90.80	62.90
Dasyuromorpha	0.391	38	-0.08	0.30	-0.04	0.19	1.02	61	58.44	1.70	57.81	0.00	9.78	2	0	05	5	0 0	0 2	21	0	0 5	8 14	42	0 9		61	67.70	96.10	63.70

Table S2. Clade descriptive statistics of residual testes mass and arginine content data and amino acid alignment statistics (Geneious 5.5.9). Stdev= standard deviation, mad= median absolute deviation.

Table S3. Results for Tukey HD post-hoc comparisons for one-way anovas. Diff = difference in the observed means, lwr = lower end point of the interval, upr = upper end point, p adj = p-value after adjustment for the multiple comparisons.

	Comparison	diff	lwr	upr	p adj
	Chiroptera-Rodentia	0.859	0.165	1.552	0.007
	Artiodactyla-Rodentia	-1.685	-2.569	-0.800	0.000
	Primates-Rodentia	-1.244	-1.911	-0.577	0.000
	Cetacea-Rodentia	-3.138	-3.686	-2.591	0.000
Arginine content (% of sequence length) /	Artiodactyla-Chiroptera	-2.543	-3.494	-1.592	0.000
Clade (Eutheria)	Primates-Chiroptera	-2.103	-2.856	-1.350	0.000
	Cetacea-Chiroptera	-3.997	-4.647	-3.347	0.000
	Primates-Artiodactyla	0.440	-0.492	1.372	0.688
	Cetacea-Artiodactyla	-1.454	-2.304	-0.603	0.000
	Cetacea-Primates	-1.894	-2.515	-1.273	0.000
	186bp-183bp	0.390	-0.124	0.904	0.215
	189bp-183bp	0.404	-0.072	0.880	0.130
	192bp-183bp	0.648	0.200	1.097	0.002
	195bp-183bp	1.002	0.249	1.754	0.004
Residual testes mass / Protamine sequence	189bp-186bp	0.015	-0.322	0.351	1.000
length group (Metatheria)	192bp-186bp	0.259	-0.038	0.556	0.114
	195bp-186bp	0.612	-0.061	1.285	0.090
	192bp-189bp	0.244	0.020	0.468	0.027
	195bp-189bp	0.598	-0.047	1.242	0.081
	195bp-192bp	0.354	-0.271	0.978	0.498

Figure S4. Phylogenetic tree constructed as consensus of phylogenetic data available in the literature. Branch lengths represent protamine 1 nucleotide substitution rates (PAML4, M0). References are given below.



Pan paniscus





•Sminthopsis hirtipes Sminthopsis psammophila Sminthopsis youngsoni ¹-Sminthopsis ooldea •Sminthopsis archeri •Sminthopsis dolichura Sminthopsis gilberti Sminthopsis leucopus Sminthopsis murina Phascogale tapoatafa Phascogale calura Antechinus naso Murexia longicaudata Murexchinus melanurus •Paramurexia rothschildi •Micromurexia habbema Antechinus swainsonii •Antechinus minimus •Antechinus godmani Antechinus stuartii •Antechinus flavipes Antechinus leo Antechinus bellus Dasykaluta rosamondae Myoictis melas Myoictis wallacei Pseudantechinus ningbing •Pseudantechinus bilarni Parantechinus apicalis Pseudantechinus macdonnellensis •Dasycercus cristicauda •Pseudantechinus woolleyae Neophascogale İorentzii Phascolosorex dorsalis Dasyuroides byrnei Sarcophilus harrisii Dasyurus hallucatus Dasyurus maculatus Dasyurus viverrinus •Dasyurus albopunctatus Dasyurus geoffroii Dasyurus spartacus

0.31

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Table S4. Results of COEVOL correlation analysis and phylogenetically controlled regression analyses (PGLS). Correlations with relative testes mass are corrected for body mass (see material and methods section), ω =dN/dS substitution rate computed by COEVOL, superscripts following the λ value indicate significance levels (n.s., p > 0.05; * p < 0.05) in likelihood ratio tests against models with λ = 0 (first superscript) and λ = 1 (second superscript), n = number of species in analysis, significant and nearly significant results are shown in boldface.

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Decide a If any of a set	Cetacea	relative head length	ω	15	0.00	0.00	0.50			
Prelative head width relative head width relative head width arginine content11 relative to head width arginine content11 relative to head width relative testes mass0.01 relative testes width relative testes mass0.06 relative testesDasyuromorpha ω arginine contentrelative testes mass38 relative testes width0.00 relative testes0.00 relative testesDasyuromorpha ω arginine content arginine contentrelative testes mass38 relative testes0.00 relative testes0.01 relative testes ω arginine content relative testes mass10 relative testes mass0.00 relative testes0.01 relative testes0.11 relative ω arginine content relative testes mass10 relative testes0.00 relative testes0.01 relative testes0.02 relativeDiprotodontiarelative head width relative head length wdth15 relative testes0.00 relative head length relative testes0.01 relative testes0.02 relativePGLSdependent variableindependent variable relative testes mass variabilityn relative testes mass variability relative testes14 relative testes mass variability relative testes mass variability relative testes0.011 relative testes0.011 relative testesPGLSdependent variable relative testes mass variability relative testes mass variability relative testes mass variability relative testes mass variability relative testes0.03 relative testesMammalian cladesclade ω (M3) mean arginine content	Celacea	Head elongation	ω ω	11	-0.01	-0.10	0.33			
$PGLS = \begin{bmatrix} 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 &$		relative head width	arginine content	11	-0.01	-0.10	0.40			
Head elongation arginine content 14 0.01 0.24 0.17 ω relative testes mass 38 -0.55 -0.14 0.31 arginine content relative testes mass 38 0.00 -0.11 0.24 arginine content w relative testes mass 38 0.00 -0.11 0.24 arginine content w relative testes mass 38 0.00 -0.11 0.24 arginine content w relative testes mass 10 0.01 0.19 0.25 arginine content relative testes mass 10 0.01 0.19 0.25 arginine content w 26 0.00 -0.01 0.49 relative head width w 15 0.00 0.0272 0.52 Head elongation w 15 0.00 0.0255 0.55 relative head width arginine content 15 0.00 0.0486 0.54 Head elongation arginine content 15		relative head length	arginine content	15	0.00	-0.24	0.00	•		
$\begin{array}{ c c c c c c } \hline & & relative testes mass & 38 & -0.55 & -0.14 & 0.31 \\ \hline & arginine content & relative testes mass & 38 & 0.00 & -0.11 & 0.24 \\ \hline & arginine content & \omega & & 61 & 0.07 & 0.18 & 0.35 \\ \hline & \omega & relative testes mass & 10 & 0.00 & -0.02 & 0.48 \\ \hline & arginine content & relative testes mass & 10 & 0.01 & 0.19 & 0.25 \\ \hline & & arginine content & \omega & 26 & 0.00 & -0.01 & 0.49 \\ relative head width & \omega & 15 & 0.00 & 0.016 & 0.51 \\ relative head length & \omega & 15 & 0.00 & 0.0272 & 0.52 \\ Head elongation & \omega & 15 & 0.00 & 0.0129 & 0.51 \\ relative head length & arginine content & 15 & 0.00 & 0.0486 & 0.54 \\ Head elongation & arginine content & 15 & 0.00 & 0.0486 & 0.54 \\ Head elongation & arginine content & 15 & 0.00 & -0.00829 & 0.49 \\ \hline & PGLS & \hline & dependent variable & independent variable & n & slope & F & R2 & \lambda & p \\ \hline & & clade \omega (M3) & relative testes mass variability & 7 & 1.40 & 2.91 & 0.11 & 0(ns,ns) & 0.03 & * \\ \hline & & mean arginine content & relative testes mass variability & 7 & -29.16 & -2.88 & 0.63 & 0(ns,ns) & 0.03 & * \\ \hline & & arginine content variability & relative testes mass variability & 7 & 9.93 & 4.82 & 0.82 & 1(ns,ns) & 0.01 & * \\ \hline \end{array}$		Head elongation	arginine content	14	0.01	0.24	0.17			
Dasyuromorphaarginine content arginine contentrelative testes mass38 0.00 -0.11 0.24 0.18 0.35 ω relative testes mass10 0.00 -0.02 0.48 arginine contentrelative testes mass10 0.01 0.19 0.25 arginine content ω 26 0.00 -0.01 0.49 relative head width ω 15 0.00 0.016 0.51 relative head length ω 15 0.00 0.0129 0.51 relative head length ω 15 0.00 0.0272 0.52 Head elongation ω 15 0.00 0.0486 0.54 relative head lengtharginine content15 0.00 0.0486 0.54 Head elongation ω 15 0.00 0.0486 0.54 PGLSdependent variableindependent variable n $slope$ F $R2$ λ p Mammalian cladesrelative testes mass variability 7 1.40 2.91 0.11 $0(ns,ns)$ 0.03 *mean arginine contentrelative testes mass variability 7 9.93 4.82 0.82 $1(ns,ns)$ 0.01 *		ω	relative testes mass	38	-0.55	-0.14	0.31			
arginine content ω 61 0.07 0.18 0.35 ω relative testes mass10 0.00 -0.02 0.48 arginine contentrelative testes mass10 0.01 0.19 0.25 arginine content ω 26 0.00 -0.01 0.49 relative head width ω 15 0.00 0.016 0.51 relative head length ω 15 0.00 0.0272 0.52 Head elongation ω 15 0.00 0.0625 0.55 relative head lengtharginine content15 0.00 0.0486 0.54 Head elongationarginine content15 0.00 0.0486 0.54 Head elongationarginine content15 0.00 0.00829 0.49 PGLSdependent variableindependent variablenslopeFR2 λ pMammalian cladesrelative testes mass variability7 1.40 2.91 0.11 $0(ns,ns)$ 0.03 *mean arginine contentarginine contentrelative testes mass variability7 -29.16 -2.88 0.63 $0(ns,ns)$ 0.03 *	Dasyuromorpha	arginine content	relative testes mass	38	0.00	-0.11	0.24			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		arginine content	ω	61	0.07	0.18	0.35			
Diprotodontiaarginine content arginine content elative head width relative head length ω relative testes mass ω 100.010.190.25 0.00DiprotodontiaDiprotodontia ω 150.000.0160.51 0.51Diprotodontiarelative head length Head elongation ω 150.000.02720.52 0.52Head elongation relative head width relative head width Head elongation ω 150.000.01290.51 0.00PGLSdependent variable mean arginine content arginine contentindependent variablenslopeFR2 λ pMammalian cladesclade ω (M3) mean arginine content arginine content arginine content variabilityrelative testes mass variability relative testes mass variabil		ω	relative testes mass	10	0.00	-0.02	0.48			
Diprotodontiaarginine content relative head width relative head length w ω 26 0.00 -0.01 0.49 0.51 Diprotodontiarelative head width relative head length relative head length relative head length relative head width relative head width relative head width relative head width relative head width arginine content15 0.00 0.016 0.51 0.00 Diprotodontia ω 15 0.00 0.0272 0.52 Head elongation relative head width Head elongationarginine content15 0.00 0.0625 0.55 relative head width Head elongationarginine content arginine content15 0.00 0.0486 0.54 PGLSdependent variableindependent variablenslopeF $R2$ λ pMammalian cladesclade ω (M3) mean arginine content arginine content variabilityrelative testes mass variability relative testes mass variability relat		arginine content	relative testes mass	10	0.01	0.19	0.25			
Diprotodontiarelative head width relative head length relative head length w ω 15 0.00 0.016 0.51 0.00272 0.52 0.52 Head elongation relative head length relative head width relative head width Head elongation ω 15 0.00 0.0129 0.51 0.00272 PGLSdependent variable mean arginine content arginine content relative testes mass variability n $slope$ F $R2$ λ p Mammalian cladesrelative content variable relative testes mass variability relative testes mass variability <br< td=""><td></td><td>arginine content</td><td>ω</td><td>26</td><td>0.00</td><td>-0.01</td><td>0.49</td><td></td><td></td><td></td></br<>		arginine content	ω	26	0.00	-0.01	0.49			
Diprotodontiarelative head length Head elongation ω 150.000.02720.52Head elongation ω 150.000.01290.51relative head length relative head width Head elongationarginine content150.000.06250.55relative head width Head elongationarginine content150.000.04860.54PGLSdependent variableindependent variablenslopeFR2 λ pMammalian cladesclade ω (M3) mean arginine contentrelative testes mass variability relative testes mass variability relative testes mass variability relative testes mass variability71.402.910.110(ns,ns)0.03*Mammalian cladesarginine content arginine content variabilityrelative testes mass variability relative testes mass variability0.821(ns,ns)0.01*		relative head width	ω	15	0.00	0.016	0.51			
Head elongation relative head length relative head length relative head length relative head width Head elongation ω 15 0.00 0.0129 0.51 0.0025 0.55 0.554 PGLSdependent variableindependent variablen $slope$ F $R2$ λ pMammalian cladesclade ω (M3) mean arginine content arginine content relative testes mass variability7 1.40 2.91 0.11 0.11 $0(ns,ns)$ 0.03 *Mammalian cladesindependent variability7 7 9.93 4.82 0.82 $1(ns,ns)$ 0.01 *	Diprotodontia	relative head length	ω	15	0.00	0.0272	0.52			
InclusionInclusionarginine content150.000.06250.55Inclusionarginine content150.000.04860.54Inclusionarginine content150.00-0.008290.49PGLSdependent variableindependent variablenslopeFR2 λ pInclusionclade ω (M3)relative testes mass variability71.402.910.110(ns,ns)0.03*Inclusionarginine contentrelative testes mass variability7-29.16-2.880.630(ns,ns)0.03*Inclusionarginine contentrelative testes mass variability79.934.820.821(ns,ns)0.01*		Head elongation	ω	15	0.00	0.0129	0.51			
Inclasionarginine content arginine content15 0.00 0.0486 0.54 PGLSdependent variableindependent variablenslopeFR2 λ pMammalian cladesclade ω (M3) mean arginine contentrelative testes mass variability relative testes mass variability71.402.910.110(ns,ns)0.03*Mammalian cladesindependent variabilityrelative testes mass variability7-29.16-2.880.630(ns,ns)0.03*		relative head length	arginine content	15	0.00	0.0625	0.55			
PGLSdependent variableindependent variablenslopeFR2λpMammalian cladesclade ω (M3) mean arginine content arginine content variabilityrelative testes mass variability relative testes mass variability71.402.910.110(ns,ns)0.03*Mammalian cladesrelative testes mass variability arginine content variabilityrelative testes mass variability relative testes mass variability79.934.820.821(ns,ns)0.01*		relative head width	arginine content	15	0.00	0.0486	0.54			
PGLSdependent variableindependent variablenslopeFR2λpMammalian cladesclade ω (M3)relative testes mass variability71.402.910.110(ns,ns)0.03*mean arginine content arginine content variabilityrelative testes mass variability7-29.16-2.880.630(ns,ns)0.03*Mammalian cladesrelative testes mass variability79.934.820.821(ns,ns)0.01*		Head elongation	arginine content	15	0.00	-0.00829	0.49	-		
Mammalian cladesclade ω (M3)relative testes mass variability71.402.910.110(ns,ns)0.03*Mammalian cladesmean arginine contentrelative testes mass variability7-29.16-2.880.630(ns,ns)0.03*arginine content variabilityrelative testes mass variability79.934.820.821(ns,ns)0.01*	PGLS	dependent variable	independent variable	n	slope	F	R2	λ	р	
Mammalian clades mean arginine content relative testes mass variability 7 -29.16 -2.88 0.63 0(ns,ns) 0.03 * arginine content variability relative testes mass variability 7 9.93 4.82 0.82 1(ns,ns) 0.01 *		clade ω (M3)	relative testes mass variability	7	1.40	2,91	0.11	()(ns ns)	0.03	*
arginine content variability relative testes mass variability 7 9.93 4.82 0.82 1 (ns,ns) 0.01 *	Mammalian clades	mean arginine content	relative testes mass variability	7	-29.16	-2.88	0.63	0(ns.ns)	0.03	*
		arginine content variability	relative testes mass variability	7	9.93	4.82	0.82	1 (ns,ns)	0.01	*

Figure S5: Comparison of amino acid substitution along branches (according to PAML, CodeML marginal ancestral reconstruction) and dN/dS branch values (according to PAML CodeML branch model).





Tursiops_truncatus Tursiops_aduncus Stenella frontalis Stenella coeruleoalba Delphinus_capensis Delphinus_delphis Stenella_longirostris Sousa_chinensis Lagenodelphis_hosei Stenella attenuata Sotalia fluviatilis Globicephala macrorhynchus Peponocephala electra Feresa_attenuata Pseudorca_crassidens Grampus_griseus Steno_bredanensis Orcaella_heinsohni Cephalorhynchus commersonii Lagenorhynchus_obliquidens Lissodelphis_boreali Orcinus_orca Lagenorhynchus_albirostris Lagenorhynchus_acutus Phocoenoides_dalli Phocoena_phocoena Neophocaena_phocaenoides Monodon_monoceros Delphinapterus_leucas Pontoporia blainville Inia_geoffrensis Lipotes_vexillifer Platanista_minor Mesoplodon_grayi Mesoplodon_peruvianus 6.44394 Mesoplodon bidens Berardius bairdii Ziphius cavirostris Tasmacetus shepherd Kogia_breviceps Balaenoptera_edeni Balaenoptera borealis Balaenoptera musculus Eschrichtius_robustus Megaptera_novaeangliae Balaenoptera_physalus Balaenoptera acutorostrata Balaenoptera bonaerensis Caperea marginata Eubalaena_japonica Eubalaena_australis Eubalaena_glacialis 0.661188 Balaena_mysticetus Hippopotamus_amphibius Hexaprotodon liberiensis Capra_hircus Elaphodus_cephalophus <u>0.218941</u> Moschus_sp <u>1.42528</u> Antilocapra_americana ³⁵ Potamochoerus_porcus Equus_asinus Plecotus auritus Corynorhinus townsendii Otonycteris hemprichii Eptesicus_fuscus Eptesicus_brasiliensis Chalinolobus_beatrix Neoromicia brunneus Hvpsugo savii Natalus stramineus Chilonatalus_micropus Myotis_lucifugus Myotis_daubentoni Monophyllus redmani Desmodus_rotundus Mormoops_megalophylla Pteronotus_parnellii Rhinolophus_ferrumequinum Hipposideros_commersoni Rhinopoma_hardwickii Pteropus_hypomelanus Galeopterus variegatus





Erinaceus europaeus Pitimys_duodecimcostatus Pitimys_lusitanicus Microtus_gerbei Microtus_arvalis Microtus cabrerae Microtus agrestis Chionomys_nivalis Arvicola_terrestris Arvicola sapidus Clethrionomys glareolus Phodopus roborovski Phodopus campbelli Phodopus_sungorus Mesocricetus_auratus Cricetulus griseus Peromyscus maniculatus Peromyscus polionotus Peromyscus_leucopus Peromyscus californicus Sigmodon hispidus Mus musculus castaneus Mus musculus bactrianus Mus musculus domesticus Mus musculus musculus Mus_spicilegus Mus macedonicus Mus spretus Mus famulus Mus_cookii Mus pahari Apodemus sylvaticus Micromys minutus Rattus norvegicus Acomys cilcicus Acomys_cahirinus Meriones unguiculatus Cavia porcellus Oryctolagus_cuniculus Trachypithecus phayrei Trachypithecus obscurus Trachypithecus_cristatus Trachypithecus francoisi Trachypithecus pileatus Trachypithecus deei Trachypithecus_johnii Semnopithecus_entellus Trachypithecus_vetulus Nasalis larvatus Piliocolobus badius Colobus guereza Macaca mulatta Macaca fascicularis Papio_cynocephalus Pan troglodytes Pan_paniscus Homo sapiens Gorilla_gorilla Pongo_pygmaeus Hylobates_lar Saimiri sciureus Saguinus imperator Ateles sp Otolemur garnettii Loxodonta africana





Pseudantechinus_macdonnellensis