

## SI Appendix 2

### Results of Sign tests using restricted datasets

Tables SOM2.1 and 3.2 show the results of sign tests which used only those comparisons with both significant variation in substitution rate and at least twofold differences in mass-specific BMR. The significance of the variation in substitution rates was assessed for each comparison pair using a likelihood ratio test. Since tests of this kind are known to have relatively low power to detect rate variation (*1*) significance levels of both 25% (table SOM2.1) and 5% (table SOM2.2) were used to assess the significance of rate variation. While a 5% significance level is likely to be conservative (i.e. exclude a number of comparisons which in fact have significant rate variation), a 25% significance level should be thought of as removing those comparison pairs most likely to yield false negatives. In the sign tests.

The results of these sign tests show no significant association between mass-specific BMR and substitution rate in any of the tests performed.

**Table SOM2.1. Results of Sign Tests Using a restricted dataset.** All tests are one-tailed, and independent comparisons were only included if they showed rate variation significant at the 25% level (see Methods), and at least a two-fold difference in mass-specific BMR. ‘**Taxa**’ indicates the taxa from which phylogenetically independent comparisons were taken for each analysis, ‘**Genes**’ indicates whether genes used in each analysis are of nuclear or mitochondrial origin, or are pooled (marked as ‘all’, see also Table 1). **BL<sub>dN</sub>** indicates non-synonymous branch lengths, **BL<sub>dS</sub>** indicates synonymous branch lengths for protein coding genes, and **BL<sub>T</sub>** indicates total branch. The number of comparisons in which the sign of the difference in substitution rate and mass-specific BMR is the same (as predicted by the metabolic rate hypothesis, indicated as ‘+’) or different (indicated as a ‘-’) is indicated in brackets for each analysis in the (+/-) column. ‘*p*’ indicates the probability that the results of each test could have occurred by chance. ‘n/a’ indicates that comparisons were not available in that particular subset of the data.

Taxa	Genes	Protein Coding Genes				RNA Coding Genes	
		BL <sub>dN</sub>		BL <sub>dS</sub>		BL <sub>T</sub>	
		(+/-)	<i>P</i>	(+/-)	<i>p</i>	(+/-)	<i>p</i>
All	All	(18/19)	0.500	(19/20)	0.500	(16/21)	0.744
	Mitochondrial	(16/17)	0.500	(14/20)	0.942	(11/10)	0.500
Mammalia	Mitochondrial	(5/6)	0.500	(2/9)	0.967	(5/5)	0.500
	Nuclear	(4/4)	0.500	(6/2)	0.145	n/a	n/a
Aves	Mitochondrial	(5/6)	0.500	(2/9)	0.910	n/a	n/a
Mollusca	Mitochondrial	(4/3)	0.500	(4/4)	0.500	(3/3)	0.500
	Nuclear	n/a	n/a	n/a	n/a	(2/5)	0.500
Arthropoda	Mitochondrial	(4/3)	0.500	(3/4)	0.500	(1/5)	0.813
	Nuclear	n/a	n/a	n/a	n/a	(6/3)	0.254

**Table SOM2.2. Results of Sign Tests Using a restricted dataset.** All tests are one-tailed, and independent comparisons were only included if they showed rate variation significant at the 5% level (see Methods), and at least a two-fold difference in mass-specific BMR. ‘**Taxa**’ indicates the taxa from which phylogenetically independent comparisons were taken for each analysis, ‘**Genes**’ indicates whether genes used in each analysis are of nuclear or mitochondrial origin, or are pooled (marked as ‘all’, see also Table 1). **BL<sub>dN</sub>** indicates non-synonymous branch lengths, **BL<sub>dS</sub>** indicates synonymous branch lengths for protein coding genes, and **BL<sub>T</sub>** indicates total branch. The number of comparisons in which the sign of the difference in substitution rate and mass-specific BMR is the same (as predicted by the metabolic rate hypothesis, indicated as ‘+’) or different (indicated as a ‘-’) is indicated in brackets for each analysis in the (+/-) column. ‘*p*’ indicates the probability that the results of each test could have occurred by chance. ‘n/a’ indicates that comparisons were not available in that particular subset of the data. At least 5 datapoints are required to perform a valid one-tailed sign test, so subsets of the data in which 5 independent comparisons were not available are also marked as ‘n/a’.

Taxa	Genes	Protein Coding Genes				RNA Coding Genes	
		BL <sub>dN</sub>		BL <sub>dS</sub>		BL <sub>T</sub>	
		(+/-)	<i>p</i>	(+/-)	<i>p</i>	(+/-)	<i>p</i>
All	All	(6/6)	0.500	(6/7)	0.500	(10/14)	0.729
	Mitochondrial	(4/6)	0.623	(4/7)	0.726	(6/9)	0.696
Mammalia	Mitochondrial	n/a	n/a	n/a	n/a	(2/3)	0.500
	Nuclear	n/a	n/a	n/a	n/a	n/a	n/a
Aves	Mitochondrial	n/a	n/a	n/a	n/a	n/a	n/a
Mollusca	Mitochondrial	n/a	n/a	n/a	n/a	n/a	n/a
	Nuclear	n/a	n/a	n/a	n/a	n/a	n/a
Arthropoda	Mitochondrial	n/a	n/a	n/a	n/a	n/a	n/a
	Nuclear	n/a	n/a	n/a	n/a	(6/1)	0.063

## Results of Tortoise/Hare Comparisons

Despite all comparisons having very large differences in BMR values, Tortoise/Hare tests showed no significant association between mass-specific BMR and any of the substitution rate measures examined. Comparisons for synonymous substitution rate for protein coding genes (Table SOM2.3) had an average 16.7-fold difference in mass-specific BMR values, with 8 '+' and 12 '-' scored comparisons (one-tailed sign test,  $p = 0.748$ ). Comparisons for non-synonymous substitution rate for protein coding genes (Table SOM2.3) had an average 15.7-fold difference in mass-specific BMR values, with 9 '+' and 11 '-' scored comparisons (one-tailed sign test,  $p = 0.588$ ). Comparisons for overall substitution rate of RNA coding genes (Table SOM2.4) had an average 19.3-fold difference in mass-specific BMR values, with 10 '+' and 10 '-' scored comparisons (one-tailed sign test,  $p = 0.500$ ). Results of Tortoise/Hare tests are qualitatively identical if the 20 comparisons are also required to show rate variation significant at the 25% and 5% levels.

The distribution of '+' and '-' signs shows no obvious relationship to the size of the ratio in mass-specific BMR (Tables SOM2.1-SOM2.3, comparisons are ordered with respect to mass-specific BMR ratio). Indeed, the distribution is such that no sign tests would be significant for any number of comparisons less than 20 (e.g. a sign test using the 10 comparisons with the largest ratios of mass-specific BMR). Furthermore, the number of negative comparisons in each sign test indicates that it is extremely unlikely that the addition of further comparisons would lead to a significant sign test result. In the most conservative example (10 '+' and 10 '-' comparisons for RNA coding genes, table SOM2.3), a significant sign test result would require the addition

of 10 extra comparisons, all of which would have to be scored as ‘+’ (i.e. support the metabolic rate hypothesis). It is therefore extremely unlikely that the failure to detect an effect with the Tortoise/Hare tests is erroneous.

**Table SOM2.3 Comparisons used in protein coding genes, synonymous substitutions Tortoise/Hare tests.** The two ingroup species of each comparison are shown in **Species 1** and **Species 2**. The ratio of the higher to the lower mass-specific BMR values is shown in  $(B_{high}/B_{low})$ . The log ratios of molecular branch length and mass-specific BMR are shown in the  $\ln(\lambda_1/\lambda_2)$  and  $\ln(B_1/B_2)$  columns respectively. The gene from which the branch lengths were calculated is shown in the **gene** column. The score of each comparison is shown in the **Score** column. A ‘+’ indicates that the comparison supports the metabolic rate hypothesis, and a ‘-’ indicates that the comparison contradicts the hypothesis. Comparisons are ordered in descending order of the msBMR ratio.

<b>Species 1</b>	<b>Species 2</b>	$(B_{high}/B_{low})$	$\ln(\lambda_1/\lambda_2)$	$\ln(B_1/B_2)$	<b>Gene</b>	<b>Score</b>
Procambarus clarkii	Orconectes virilis	62.29	-4.14	4.13	COXI	-
Asterias rubens	Pycnopodia helianthoides	40.85	7.12	3.71	COXI	+
Ancylus fluviatilis	Patella vulgata	36.64	-3.58	3.60	COXI	-
Dermacentor variabilis	Hyalomma dromedarii	35.71	-2.34	3.58	COXI	-
Enyo ocyptete	Pachylia ficus	18.90	-11.76	2.94	COXI	-
Pinctada margaritifera	Pinctada mazatlantica	17.11	-2.76	2.84	COXI	-
Homarus americanus	Nephrops norvegicus	15.55	-10.27	2.74	H3	-
Lasius alienus	Paraponera clavata	15.47	-1.19	2.74	COXI	-
Ocythoe tuberculata	Joubiniteuthis portieri	13.45	5.34	2.60	COXI	+
Macroderma gigas	Tadarida brasiliensis	12.16	0.23	-2.50	ATP7a	-
Littorina littorea	Littorina obtusata	11.03	-5.27	-2.40	COXI	+
Amphitretus pelagicus	Pareledone charcoti	8.75	-0.33	-2.17	COXI	+
Ophiopholis aculeata	Eucidaris tribuloides	7.69	12.60	-2.04	COXI	-
Bathyteuthis abyssicola	Ctenopteryx siculus	7.51	-7.56	-2.02	COXI	+
Mustela erminea	Taxidea taxus	7.33	-11.50	1.99	CYTB	-
Pseudocheirus peregrinus	Tarsipes rostratus	6.17	1.40	-1.82	ND5	-
Spisula subtruncata	Macoma balthica	6.04	0.76	1.80	COXI	+
Mercenaria mercenaria	Meretrix meretrix	5.98	4.58	-1.79	COXI	-
Lactrodectus mactans	Tidarren sisypoides	5.54	1.85	1.71	H3	+
Myzus persicae	Acyrtosiphon pisum	5.20	11.52	1.65	CYTB	+

**Table SOM2.4 Comparisons used in protein coding genes, non-synonymous substitutions Tortoise/Hare tests.** The two ingroup species of each comparison are shown in **Species 1** and **Species 2**. The ratio of the higher to the lower mass-specific BMR values is shown in  $(B_{high}/B_{low})$ .

The log ratios of molecular branch length and mass-specific BMR are shown in the  $\ln(\lambda_1/\lambda_2)$  and  $\ln(B_1/B_2)$  columns respectively. The gene from which the branch lengths were calculated is shown in the **gene** column. The score of each comparison is shown in the **Score** column. A '+' indicates that the comparison supports the metabolic rate hypothesis, and a '-' indicates that the comparison contradicts the hypothesis.

Comparisons are ordered in descending order of the msBMR ratio.

<b>Species 1</b>	<b>Species 2</b>	$(B_{high}/B_{low})$	$\ln(\lambda_1/\lambda_2)$	$\ln(B_1/B_2)$	<b>Gene</b>	<b>Score</b>
Procambarus clarkii	Orconectes virilis	62.29	-0.73	4.13	COXI	-
Asterias rubens	Pycnopodia helianthoides	40.85	-2.83	3.71	COXI	-
Ancylus fluviatilis	Patella vulgata	36.64	0.52	3.60	COXI	+
Dermacentor variabilis	Hyalomma dromedarii	35.71	-0.04	3.58	COXI	-
Pinctada margaritifera	Pinctada mazatlanica	17.11	1.06	2.84	COXI	+
Lasius alienus	Paraponera clavata	15.47	0.78	2.74	COXI	+
Ocythoe tuberculata	Joubiniteuthis portieri	13.45	1.77	2.60	COXI	+
Macroderma gigas	Tadarida brasiliensis	12.16	-0.23	-2.50	ATP7a	+
Littorina littorea	Littorina obtusata	11.03	3.14	-2.40	COXI	-
Amphitretus pelagicus	Pareledone charcoti	8.75	1.17	-2.17	COXI	-
Ophiopholis aculeata	Eucidaris tribuloides	7.69	1.79	-2.04	COXI	-
Bathyteuthis abyssicola	Ctenopteryx siculus	7.51	-0.67	-2.02	COXI	+
Mustela erminea	Taxidea taxus	7.33	-0.48	1.99	CYTB	-
Pseudocheirus peregrinus	Tarsipes rostratus	6.17	-0.26	-1.82	ND5	+
Spisula subtruncata	Macoma balthica	6.04	-0.88	1.80	COXI	-
Mercenaria mercenaria	Meretrix meretrix	5.98	-0.24	-1.79	COXI	+
Lactrodectus mactans	Tidarren sisyphoides	5.54	-3.40	1.71	H3	-
Myzurus persicae	Acyrtosiphon pisum	5.20	-0.53	1.65	CYTB	-
Pagurus bernhardus	Munida quadrispina	4.61	-1.01	1.53	COXI	-
Octopus californicus	Octopus vulgaris	4.55	-1.44	-1.51	COXI	+

**Table SOM2.5 Comparisons used in RNA coding genes Tortoise/Hare tests.** The two ingroup species of each comparison are shown in **Species 1** and **Species 2**. The ratio of the higher to the lower mass-specific BMR values is shown in  $(B_{high}/B_{low})$ . The log ratios of molecular branch length and mass-specific BMR are shown in the  $\ln(\lambda_1/\lambda_2)$  and  $\ln(B_1/B_2)$  columns respectively. The gene from which the branch lengths were calculated is shown in the **gene** column. The score of each comparison is shown in the **Score** column. A '+' indicates that the comparison supports the metabolic rate hypothesis, and a '-' indicates that the comparison contradicts the hypothesis. Comparisons are ordered in descending order of the msBMR ratio..

<b>Species 1</b>	<b>Species 2</b>	$(B_{high}/B_{low})$	$\ln(\lambda_1/\lambda_2)$	$\ln(B_1/B_2)$	<b>Gene</b>	<b>Score</b>
Peropteryx macrotis	Saccolpteryx bilineata	102.21	0.44	4.63	12S	+
Procambarus clarkii	Orconectes virilis	62.29	-10.41	4.13	16S	-
Ancylus fluviatilis	Patella vulgata	36.64	0.75	3.60	16S	+
Dermacentor variabilis	Hyalomma dromedarii	35.71	0.03	3.58	12S	+
Homarus americanus	Nephrops norvegicus	15.55	1.69	2.74	28S	+
Lasius alienus	Paraponera clavata	15.47	0.29	2.74	28S	+
Ocythoe tuberculata	Joubiniteuthis portieri	13.45	4.26	2.60	18S	+
Macroderma gigas	Tadarida brasiliensis	12.16	0.66	-2.50	12S	-
Littorina littorea	Littorina obtusata	11.03	-1.97	-2.40	18S	+
Caenorhabditis briggsae	Spirinia parasitifera	9.93	1.69	2.30	18S	+
Mormoops megalophylla	Pteronotus quadridens	9.68	1.73	-2.27	12S	-
Hiatella arctica	Mya arenaria	9.21	1.11	-2.22	28S	-
Capitella capitata	Lumbricus terrestris	9.18	-1.17	2.22	18S	-
Chalinolobus gouldii	Miniopterus schreibersi	8.09	0.77	-2.09	12S	-
Bathyteuthis abyssicola	Ctenopteryx siculus	7.51	0.43	-2.02	16S	-
Spisula subtruncata	Macoma balthica	6.04	-1.26	1.80	18S	-
Lactroedectus mactans	Tidarren sisyphoides	5.54	0.65	1.71	16S	+
Glycera americana	Aphrodita aculeate	5.53	-0.27	1.71	18S	-
Metaphidippus vitis	Plectreurus tristis	5.49	-0.27	1.70	16S	-
Idotea balthica	Glyptonotus antarcticus	5.39	1.00	1.68	18S	+

1. L. Bromham, D. Penny, A. Rambaut, M. D. Hendy, *J Mol Evol* **50**, 296 (2000).