

SUPPLEMENTAL MATERIAL- ROBERTS ET AL. 2023

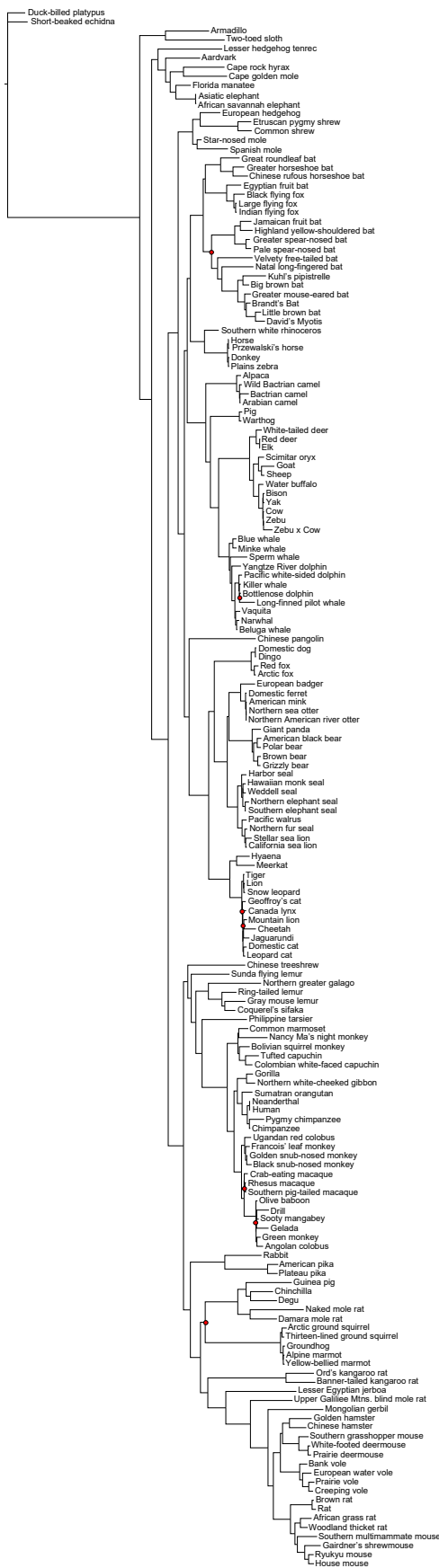


Figure. S1. Phylogenetic analysis of *Zan* DNA sequence divergence. Shown is the gene tree constructed by Bayesian and ML analysis of 170 aligned Eutherian *Zan* sequences and the *ZanL* gene from two Prototherian mammals, Duck-billed platypus and Short-beaked echidna, with the former species used as outgroup. Nodes lacking statistical support are marked with a red dot (only 7/173 nodes unsupported, <5.0%). Note the statistically supported monophyletic grouping of all species into their respective Orders and of all Orders into their respective Superorders. Taxonomy per McKenna and Bell (1997) and Foley et al. (2016).

Figure S2. Comparison of protein zonadhesin sequences and pairwise sequence identities from 17 species representing 17 of the 19 placental Orders. Orthologous segments (Avg. 1280 amino acids) spanning the vWD0-vWD3TIL+E domains of platypus *ZanL*, armadillo, sloth, tenrec, aardvark, manatee, treeshrew, colugo, human, elephant, shrew, little brown bat, horse, dog, pangolin, house mouse, rabbit, and cow were aligned using MegAlign Pro (DNASTAR Lasergene 17). Corresponding pairwise identities between the 17 placental mammal species average 65.7% with the maximum genetic distance between sloth and shrew (52.8%) and the minimum genetic distance between elephant and manatee (93%).

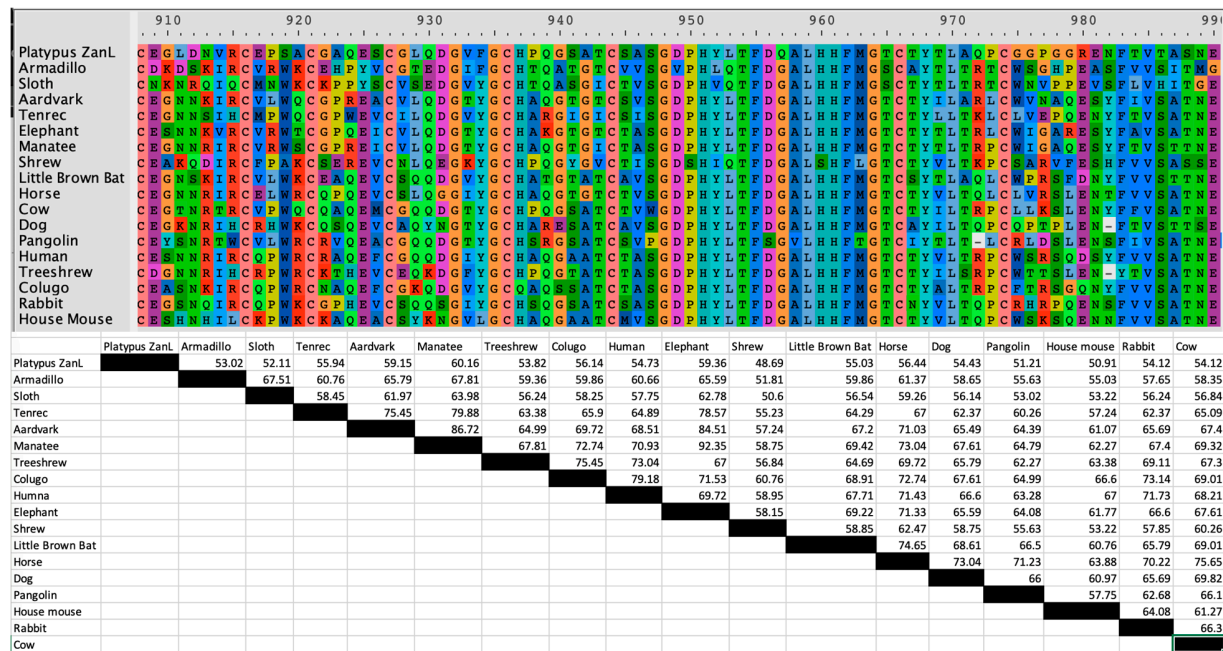


Table S1. Global and Ordinal nucleotide substitution models for *Zan*.

Dataset	<i>Zan</i>	LH	AIC-c
Global	GTR+I+G	-170071.98	189510.77
Afrotheria	GTR+G	-18680.19	37400.52
Carnivora	TVM+G	-27893.03	55937.93
Cetartiodactyla	TVM+G	-24950.64	50028.59
Chiroptera	TVM+G	-29317.30	58721.22
Eulipotyphla	TVM+I	-22344.72	44719.51
Perissodactyla	TVM+I	-9300.33	18630.74
Primates	TVM+G	-39173.01	78477.43
Rodentia	TVM+I+G	-59961.20	120068.20

Table S2. Orders with limited taxon representation and therefore no tanglegrams, and taxonomic designations of included species, listed Xenarthra> Afrotheria> Laurasiatheria> Euarchontoglires.

Order	Family	Species	Common Name
Cingulata	Dasypodidae	<i>Dasytus novemcinctus</i>	Nine-banded armadillo
Tubulidentata	Orycteropodidae	<i>Orycteropus afer</i>	Aardvark
Hyracoidea	Procaviidae	<i>Procavia capensis</i>	Rock hyrax
Sirenia	Trichechidae	<i>Trichechus manatus</i>	West Indian manatee
Proboscidea	Elephantidae	<i>Loxodonta Africana</i>	African savannah elephant
		<i>Elaphus maximus</i>	Asian elephant
Pholidota	Manidae	<i>Manis javanica</i>	Malayan pangolin
Dermoptera	Cynocephalidae	<i>Galeopterus variegatus</i>	Sunda flying lemur
Scandentia	Tupaiidae	<i>Tupaia belangeri</i>	Chinese treeshrew
Lagomorpha	Octodontidae	<i>Octodon degus</i>	Pika
	Leporidae	<i>Oryctolagus cuniculus</i>	Domestic rabbit

Table S3. Global phylogenetic comparisons via Shimodaira-Hasegawa (SH) and Adjusted Unbiased (AU) tests for *Zan*. SH and AU tests evaluated the best unconstrained phylogeny (gene tree) to a constrained phylogeny (Upham [Upham et al. 2019]). Columns are defined: LH, likelihood score; $\Delta(LH)$, difference in the likelihood scores between constrained and unconstrained phylogenies; SH, p-value for SH test; AU, p-value for AU test. When multiple constrained trees were given as output, p-values were adjusted accordingly using the Bonferroni correction. Significant p-values are indicated with an asterisk.

Tree	LH	$\Delta(LH)$	SH	AU
Unconstrained	214290.33	(best)		
Constrained #1	214290.33	0.00	0.99	0.67
Constrained #2-54	214290.34,	0.02,	0.99,	0.62,
	214310.53	20.21	0.50	0.03
Constrained #55	216101.34	1811.01	~0.000*	~0.000*

Table S4. Ordinal phylogenetic comparisons via Shimodaira-Hasegawa (SH) and Approximately Unbiased (AU) tests for *Zan*.

Gene	Order	Tree	LH	$\Delta(LH)$	SH	AU
<i>Zan</i>	Afrotheria	Unconstrained	-	(best)		
			18680.32			
		Constrained	-	57.92	0.0075*	0.0026*
		#1	18738.24			
	Eulipotyphla	Unconstrained	-	(best)		
			22345.91			
		Constrained	-	0.00	0.50	0.50
		#1	22345.91			
	Carnivora	Unconstrained	-	(best)		
			27898.83			
		Constrained	-	35.19	0.0182*	0.0005*
		#1	27934.02			
	Cetartiodactyla	Unconstrained	-	(best)		
			24952.84			
		Constrained	-	14.94	0.11	0.06
		#1	24967.79			
	Chiroptera	Unconstrained	-	(best)		
			29319.35			
		Constrained	-	166.84	0.0006*	<0.0001*
		#1	29486.18			
	Perissodactyla	Unconstrained	-9300.43	(best)		
			-9300.43	0.00	0.50	0.50
		Constrained	-			
		#1				
	Primates	Unconstrained	-	(best)		
			39180.25			
		Constrained	-	0.00	0.98	0.60
		#1	39180.25			
		Constrained	-	2.42	0.93	0.36
		#2	39182.66			
		Constrained	-	6.11	0.78	0.07
		#3	39186.36			
		Constrained	-	8.54	0.70	0.04
		#4	39188.78			
		Constrained	-	8.77	0.68	0.08
		#5	39189.01			
		Constrained	-	11.19	0.62	0.04
		#6	39191.43			
		Constrained	-	11.52	0.61	<0.0001*
		#7	39191.76			
		Constrained	-	15.56	0.55	0.07
		#8	39195.80			

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Rodentia	Constrained #9	- 39201.90	21.65	0.49	0.01*
	Constrained #10	- 39204.57	24.33	0.47	0.008*
	Constrained #11	- 39207.30	27.06	0.45	<0.0001*
	Unconstrained	- 59977.50	(best)		
	Constrained #1	- 59981.20	3.70	0.41	0.38

Table S5. PAML summary results for *Zan*. Shown are log likelihood scores (LH) and parameter estimates for variable dN/dS (ω) models within the F3×4 codon frequency method. The test statistic 2(Δ LH), equals double the difference in log likelihood values between models being tested with degrees of freedom = 2. Significant p-values are indicated by an asterisk. Sites under positive selection ($\omega > 1$) with ≥ 0.95 Bayes Empirical Bayes statistical support are listed.

Model	Parameter Estimates	LH	2(Δ LH)	P-value
M7: beta	$\omega = 0.159 f = 0.200$ $\omega = 0.331 f = 0.200$ $\omega = 0.662 f = 0.200$ $\omega = 0.921 f = 0.200$ $\omega = 0.998 f = 0.200$	-206036.22		
M8: beta & $\omega > 1$	$\omega = 0.064 f = 0.156$ $\omega = 0.261 f = 0.156$ $\omega = 0.599 f = 0.156$ $\omega = 0.816 f = 0.156$ $\omega = 0.980 f = 0.156$ $\omega = 8.487 f = 0.220$	-205049.35	986.87	<<0.0001*

Sites with $p(\omega > 1) > 0.95$ Bayes Empirical Bayes

3 P 1.00	17 E 1.00	24 G 0.99	52 F 0.96	59 K 0.96	62 A 0.96
76 W 0.99	85 I 0.98	92 T 1.00	96 P 1.00	105 P 0.98	108 T 0.99
112 L 0.99	117 P 1.00	119 Y 0.95	124 G 1.00	125 R 0.99	126 H 1.00
128 G 1.00	130 M 1.00	132 K 1.00	136 I 0.96	139 Q 0.99	152 T 0.99
153 A 0.99	167 S 0.98	192 R 0.99	198 I 0.96	216 R 1.00	231 Q 1.00
233 T 0.96	258 L 0.96	269 E 0.99	273 N 1.00	278 A 0.98	283 Q 0.98
286 Q 0.96	291 A 1.00	298 A 0.99	301 N 0.98	303 M 0.96	308 F 0.95
311 R 0.96	316 R 0.99	321 A 0.99	330 S 0.95	334 T 1.00	335 A 1.00
337 F 0.99	341 P 1.00	355 A 0.96	385 R 0.96	392 P 0.98	400 G 0.99
404 M 0.95	407 Q 0.99	436 L 0.96	438 P 0.99	441 G 0.99	463 N 0.98
467 R 0.96	477 T 1.00	487 S 1.00	494 A 1.00	497 T 0.95	525 P 0.96
527 W 0.96	531 Q 0.95	574 M 0.95	568 I 0.99	583 G 1.00	643 E 1.00
647 R 0.99	653 A 0.96	666 P 0.99	668 S 0.97	679 K 0.96	684 Q 0.97
686 N 0.99	700 M 0.99	711 G 1.00	717 S 0.99	720 A 0.96	728 P 1.00
733 T 0.96	734 L 1.00	735 T 1.00	740 D 1.00	749 Q 0.97	754 P 0.99
768 P 0.97	770 N 1.00	774 P 0.96	784 L 0.96	791 D 0.99	794 A 0.96
825 D 1.00	832 P 0.97	845 R 0.99	858 G 1.00	871 L 0.99	879 A 1.00
886 R 0.96	892 H 0.98	895 D 1.00	899 S 0.99	887 I 0.98	894 V 0.95
901 I 0.95	904 R 0.95	932 K 0.99	952 T 1.00	961 R 0.96	968 Q 0.99
970 T 1.00	986 G 0.98	1004 N 0.99	1010 R 1.00	1013 R 1.00	1018 T 0.97
1038 R 1.00	1065 D 1.00	1086 N 1.00	1091 Q 0.97	1096 G 1.00	1109 D 0.96
1130 T 1.00	1151 P 1.00	1153 P 1.00	1154 A 1.00	1165 T 1.00	1166 Q 1.00
1172 L 1.00	1177 G 1.00	1181 A 1.00	1188 G 0.95	1192 P 0.99	1196 A 0.99
1223 A 0.99	1226 G 0.99	1236 S 1.00	1249 A 0.95	1256 R 0.99	1267 D 0.99
1270 N 0.99	1272 N 0.96	1280 Q 0.99	1282 S 0.99	1283 V 0.99	1284 Y 0.99

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1288 H 1.00	1290 P 0.96	1295 L 0.99	1296 S 0.99	1305 R 1.00	1322 V 0.96
1333 W 0.99	1357 E 0.99	1367 I 0.963	1387 E 1.00	1394 G 0.99	1399 R 0.95
1403 T 0.99	1404 S 0.99	1408 S 0.99	1416 S 0.99	1423 K 0.99	1442 T 0.99
1434 K 0.99	1445 T 0.99	1451 T 0.98	1453 D 0.96	1464 E 0.97	1474 L 0.98
1478 S 0.99	1490 S 0.97	1499 G 0.99	1502 R 0.99	1510 T 0.98	1519 E 0.99
1522 L 0.99	1532 K 0.97	1692 D 0.98	1698 X 0.99	1699 X 0.99	1701 X 0.97
1702 X 1.00	1728 X 0.99	1776 X 0.99	1778 G 0.99	1779 A 0.99	1783 P 1.00
1784 X 0.99	1785 X 0.99	1789 X 0.95	1790 X 1.00	1848 T 0.99	1870 K 1.00
1886 S 0.99	1908 A 0.98	1986 P 0.99	2050 E 1.00	2055 X 0.99	2056 E 1.00
2059 L 0.98					