

## Supplementary Material

### **The egg coat zona pellucida 3 glycoprotein – evolution of its putative sperm-binding region in Old World murine rodents (Rodentia: Muridae)**

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**Table S1.** Codon-based Test of Positive Selection for analysis between ZP exon 6 sequences from rodents.

The probability of rejecting the null hypothesis of strict-neutrality ( $dN = dS$ ) in favor of the alternative hypothesis ( $dN > dS$ ) (below diagonal) is shown. Values of P less than 0.05 are considered significant at the 5% level and are highlighted. The test statistic ( $dN - dS$ ) is shown above the diagonal.  $dS$  and  $dN$  are the numbers of synonymous and nonsynonymous substitutions per site, respectively. The variance of the difference was computed using the analytical method. Analyses were conducted using the Nei-Gojobori method [1]. The analysis involved 31 nucleotide sequences. All ambiguous positions were removed for each sequence pair.

There were a total of 21 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [2].

Mus_musculus		-1.041	-1.539	-0.477	1.010	1.010	1.010	1.443	-2.411	-3.357	-2.411	-1.041	-0.749	-1.041	-1.041	-1.539	0.000	-0.744	-1.539	
Rattus_norvegicus	1.000		-1.979	-1.105	-0.733	-0.733	-0.749	-0.477	-2.411	-3.357	-2.862	-1.539	-1.318	-1.539	-1.041	-1.041	-1.041	-1.312	-1.539	
Dasymys_incomtus	1.000	1.000		-1.598	-1.301	-1.301	-1.318	-1.105	-3.357	-4.609	-3.357	-1.979	-1.785	-1.979	-1.979	-2.411	-1.539	-1.778	-2.411	
Micaelamys_namaquensis	1.000	1.000	1.000		-0.183	-0.183	-0.221	0.022	-2.056	-3.568	-2.518	-1.105	-0.901	-1.105	-1.105	-1.598	-0.477	-0.889	-1.598	
Aethomys_chrysophilus	0.157	1.000	1.000	1.000		0.000	1.444	1.787	-2.202	-3.122	-2.202	-0.733	-0.449	-0.733	-0.733	-1.301	1.010	-0.440	-1.301	
Aethomys_ineptus4SAM	0.157	1.000	1.000	1.000	1.000		1.444	1.787	-2.202	-3.122	-2.202	-0.733	-0.449	-0.733	-0.733	-1.301	1.010	-0.440	-1.301	
Lemnisomys_griselda	0.157	1.000	1.000	1.000	0.076	0.076		1.010	-2.231	-3.182	-2.231	-0.749	-0.477	-0.749	-0.749	-1.318	1.010	-0.468	-1.318	
Rhabdomys_pumilo	0.076	1.000	1.000	0.491	0.038	0.038	0.157		-2.056	-3.012	-2.056	-0.477	-0.221	-0.477	-0.477	-1.105	1.443	-0.208	-1.105	
Hylomyscus_alleni	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-3.357	-2.862	-1.979	-1.785	-1.979	-1.979	-1.979	-2.411	-1.778	-1.539	
Mastomys_natalensis	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-2.862	-3.924	-3.743	-3.924	-2.862	-3.924	-3.357	-3.712	-3.357	
Apodemus_chevrieri	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-2.411	-2.231	-2.411	-2.862	-2.862	-2.411	-2.221	-2.862	
Leopoldomys_edwardsi	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.010	0.000	-1.539	-1.041	-1.041	1.010	-1.041	
Leopoldomys_sabanus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157		1.010	-1.318	-0.749	-0.749	1.443	-0.749	
Niviventer_bukit	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157		-1.539	-1.041	-1.041	1.010	-1.041	
Maxomys_bartelsii	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-1.539	-1.041	-1.312	-1.041	
Maxomys_hellwardi	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-1.539	-0.744	-1.041	
Bandicota_indica	1.000	1.000	1.000	1.000	0.157	0.157	0.157	0.076	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-0.744	-1.539
Bunomys_adspersus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	0.076	0.157	1.000	1.000	1.000		-0.744	
Paruromys_dominator	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
Rattus_exulans	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_steini	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_niobe	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_verecundas	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_mordax	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_praetor	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_leucopus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_fuscipies	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_lutreolus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_sordidus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_tunneyi	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000
Rattus_villosissimus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.157	1.000	1.000	1.000	1.000	1.000	0.157	1.000

1. Nei M. and Gojobori T. (1986). Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Molecular Biology and Evolution* 3:418-426.

2. Tamura K., Stecher G., Peterson D., Filipksi A., and Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.

**Table S2.** Codon-based Test of Positive Selection for analysis between ZP exon 7 sequences from rodents. The probability of rejecting the null hypothesis of strict-neutrality ( $dN = dS$ ) in favor of the alternative hypothesis ( $dN > dS$ ) (below diagonal) is shown. Values of P less than 0.05 are considered significant at the 5% level and are highlighted. The test statistic ( $dN - dS$ ) is shown above the diagonal.  $dS$  and  $dN$  are the numbers of synonymous and nonsynonymous substitutions per site, respectively. The variance of the difference was computed using the analytical method. Analyses were conducted using the Nei-Gojobori method [1]. The analysis involved 31 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 37 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [2].

Mus_musculus		-0.222	-0.014	-0.718	-1.287	-1.287	-0.323	-0.351	-1.886	-1.609	-0.513	-1.701	-1.090	-0.882	-1.769	-0.367	-0.559	-0.559	-0.559	-0.210	-0.779
Rattus_norvegicus	1.000		0.808	-0.046	-0.222	-0.222	-0.161	0.294	-1.394	-0.923	0.156	0.385	2.759	2.969	-0.378	1.763	2.047	2.047	2.047	2.048	-0.076
Dasymys_incomtus	1.000	0.210		-0.485	-0.014	-0.014	0.263	0.233	-0.916	-1.180	0.466	-0.531	-0.005	0.248	-1.357	0.835	0.197	0.197	0.197	0.207	0.206
Micaelamys_namaquensis	1.000	1.000	1.000		-0.718	-0.718	-0.493	2.304	-1.356	-0.336	-0.325	-1.054	-0.710	-0.504	-1.762	-0.021	-0.549	-0.549	-0.549	-0.540	-0.463
Aethomys_chrysophilus	1.000	1.000	1.000	1.000		0.000	-0.502	-0.526	-1.757	-1.464	-0.336	-1.536	-0.901	-0.695	-1.769	-0.367	-0.559	-0.559	-0.559	-0.210	-0.779
Aethomys_ineptus4SAM	1.000	1.000	1.000	1.000	1.000		-0.502	-0.526	-1.757	-1.464	-0.336	-1.536	-0.901	-0.695	-1.769	-0.367	-0.559	-0.559	-0.559	-0.210	-0.779
Lemnisomys_griselda	1.000	1.000	0.396	1.000	1.000	1.000		-0.666	-1.581	-1.276	-0.136	-0.863	-0.493	-0.493	-1.444	-0.136	-0.334	-0.334	-0.334	-0.687	-0.417
Rhodomys_pumilo	1.000	0.385	0.408	0.011	1.000	1.000	1.000		-1.338	-0.320	0.037	-0.887	-0.517	-0.310	-1.460	0.323	-0.192	-0.192	-0.192	-0.181	-0.147
Hylomyscus_alleni	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-1.858	-1.455	-2.255	-1.487	-1.591	-1.356	-1.367	-1.252	-1.252	-1.252	-1.239	-1.691
Mastomys_natalensis	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-0.997	-1.985	-1.455	-1.283	-1.312	-0.897	-1.057	-1.057	-1.057	-1.045	-1.239
Apodemus_chevrieri	1.000	0.438	0.321	1.000	1.000	1.000	1.000	0.485	1.000	1.000		-0.872	-0.504	-0.476	-1.302	0.184	-0.008	-0.008	-0.008	0.005	0.021
Leopoldomys_edwardsi	1.000	0.350	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-0.752	-0.480	-1.532	0.197	-0.041	-0.041	-0.041	0.599	2.047
Leopoldomys_sabanus	1.000	0.003	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.006	-1.282	2.539	1.763	1.763	1.763	2.539	0.156
Niviventer_bukit	1.000	0.002	0.402	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.158		-1.071	2.760	2.048	2.048	2.048	2.760	0.397
Maxomys_bartelsii	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		-0.531	-1.094	-1.094	-1.094	-0.540	-1.239
Maxomys_hellwardi	1.000	0.040	0.203	1.000	1.000	1.000	1.000	0.373	1.000	1.000	0.427	0.422	0.006	0.003	1.000		2.303	2.303	2.303	2.048	0.156
Bandicota_indica	1.000	0.021	0.422	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.040	0.021	1.000	0.011		0.000	0.000	2.303	-0.527
Bunomys_adspersus	1.000	0.021	0.422	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.040	0.021	1.000	0.011	1.000		0.000	2.303	-0.527
Paruromys_dominator	1.000	0.021	0.422	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.040	0.021	1.000	0.011	1.000	1.000		2.303	-0.527
Rattus_exulans	1.000	0.021	0.418	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.498	0.275	0.006	0.003	1.000	0.021	0.011	0.011	0.011		0.547
Rattus_steini	1.000	1.000	0.418	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.492	0.021	0.438	0.346	1.000	0.438	1.000	1.000	1.000	0.293	
Rattus_niobe	0.493	0.012	0.125	0.488	0.493	0.493	0.469	0.345	1.000	1.000	0.277	0.438	0.021	0.011	1.000	0.006	0.158	0.158	0.158	0.006	1.000
Rattus_verecundas	0.493	0.012	0.125	0.488	0.493	0.493	0.469	0.345	1.000	1.000	0.277	0.438	0.021	0.011	1.000	0.006	0.158	0.158	0.158	0.006	1.000
Rattus_mordax	1.000	0.006	0.130	0.498	1.000	1.000	0.479	0.355	1.000	1.000	0.287	0.368	0.012	0.006	1.000	0.003	0.078	0.078	0.078	0.012	1.000
Rattus_praetor	1.000	0.006	0.130	0.498	1.000	1.000	0.479	0.355	1.000	1.000	0.287	0.368	0.012	0.006	1.000	0.003	0.078	0.078	0.078	0.012	1.000
Rattus_leucopus	1.000	1.000	0.418	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.492	1.000	0.438	0.346	1.000	0.438	1.000	1.000	1.000	0.293	1.000
Rattus_fuscipes	1.000	0.006	0.280	1.000	1.000	1.000	1.000	0.343	1.000	1.000	0.388	1.000	0.254	0.174	1.000	0.058	0.078	0.078	0.078	0.130	1.000
Rattus_lutreolus	1.000	0.006	0.280	1.000	1.000	1.000	1.000	0.343	1.000	1.000	0.388	1.000	0.254	0.174	1.000	0.058	0.078	0.078	0.078	0.130	1.000
Rattus_sordidus	1.000	0.006	0.280	1.000	1.000	1.000	1.000	0.343	1.000	1.000	0.388	1.000	0.254	0.174	1.000	0.058	0.078	0.078	0.078	0.130	1.000
Rattus_tunneyi	1.000	0.006	0.280	1.000	1.000	1.000	1.000	0.343	1.000	1.000	0.388	1.000	0.254	0.174	1.000	0.055	0.078	0.078	0.078	0.130	1.000
Rattus_villosissimus	1.000	0.006	0.280	1.000	1.000	1.000	1.000	0.343	1.000	1.000	0.388	1.000	0.254	0.174	1.000	0.058	0.078	0.078	0.078	0.130	1.000

1. Nei M. and Gojobori T. (1986). Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Molecular Biology and Evolution* 3:418-426.

2. Tamura K., Stecher G., Peterson D., Filipksi A., and Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.

0.017	0.017	-0.006	-0.006	-0.779	-0.245	-0.245	-0.245	-0.245	-0.245
2.303	2.303	2.538	2.538	-0.076	2.538	2.538	2.538	2.538	2.538
1.155	1.155	1.131	1.131	0.206	0.586	0.586	0.586	0.586	0.586
0.029	0.029	0.006	0.006	-0.463	-0.233	-0.233	-0.233	-0.233	-0.233
0.017	0.017	-0.006	-0.006	-0.779	-0.245	-0.245	-0.245	-0.245	-0.245
0.017	0.017	-0.006	-0.006	-0.779	-0.245	-0.245	-0.245	-0.245	-0.245
0.077	0.077	0.052	0.052	-0.417	-0.186	-0.186	-0.186	-0.186	-0.186
0.400	0.400	0.372	0.372	-0.147	0.405	0.405	0.405	0.405	0.405
-0.875	-0.875	-0.903	-0.903	-1.691	-0.578	-0.578	-0.578	-0.578	-0.578
-0.654	-0.654	-0.681	-0.681	-1.239	-0.819	-0.819	-0.819	-0.819	-0.819
0.592	0.592	0.563	0.563	0.021	0.286	0.286	0.286	0.286	0.286
0.156	0.156	0.338	0.338	-0.757	-0.163	-0.163	-0.163	-0.163	-0.163
2.047	2.047	2.303	2.303	0.156	0.663	0.663	0.663	0.663	0.663
2.303	2.303	2.538	2.538	0.397	0.943	0.943	0.943	0.943	0.943
-0.919	-0.919	-0.757	-0.757	-1.239	-1.082	-1.082	-1.082	-1.082	-1.082
2.538	2.538	2.758	2.758	0.156	1.584	1.584	1.584	1.610	1.584
1.006	1.006	1.431	1.431	-0.527	1.431	1.431	1.431	1.431	1.431
1.006	1.006	1.431	1.431	-0.527	1.431	1.431	1.431	1.431	1.431
1.006	1.006	1.431	1.431	-0.527	1.431	1.431	1.431	1.431	1.431
2.538	2.538	2.303	2.303	0.547	1.131	1.131	1.131	1.131	1.131
-0.775	-0.775	-0.548	-0.548	-1.492	-0.537	-0.537	-0.537	-0.537	-0.537
	0.000	1.006	1.006	-0.775	1.006	1.006	1.006	1.006	1.006
1.000		1.006	1.006	-0.775	1.006	1.006	1.006	1.006	1.006
0.158	0.158		0.000	-0.548	1.431	1.431	1.431	1.431	1.431
0.158	0.158	1.000		-0.548	1.431	1.431	1.431	1.431	1.431
1.000	1.000	1.000	1.000		-0.537	-0.537	-0.537	-0.537	-0.537
0.158	0.158	0.078	0.078	1.000		0.000	0.000	1.006	0.000
0.158	0.158	0.078	0.078	1.000	1.000		0.000	1.006	0.000
0.158	0.158	0.078	0.078	1.000	1.000	1.000		1.006	0.000
0.158	0.158	0.078	0.078	1.000	0.158	0.158	0.158		1.006
0.158	0.158	0.078	0.078	1.000	1.000	1.000	1.000	0.158	

