

## Supplementary Information

Comparative genomics reveals contraction in olfactory receptor genes in bats

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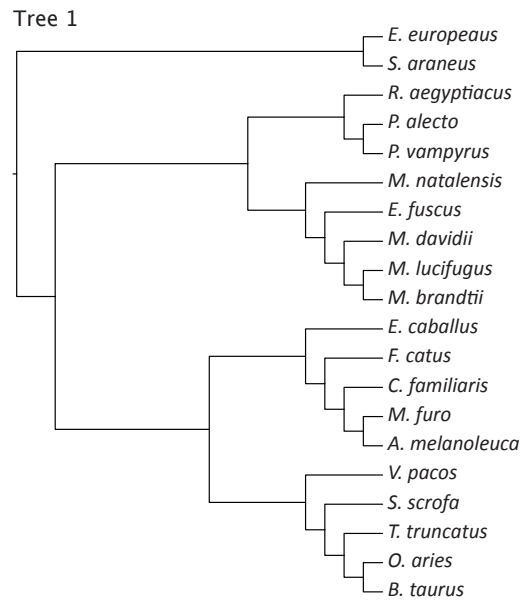
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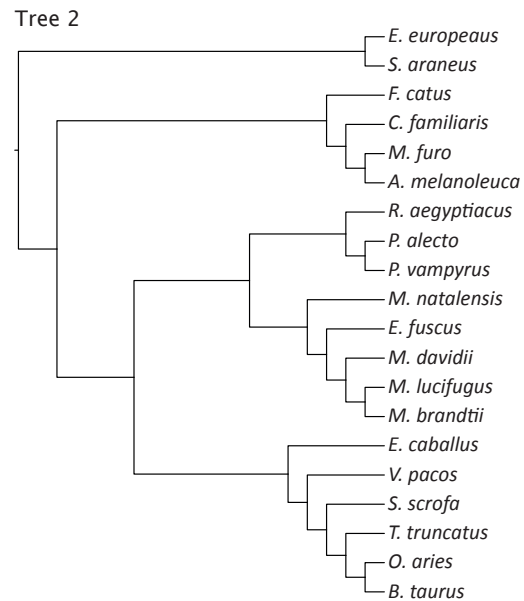
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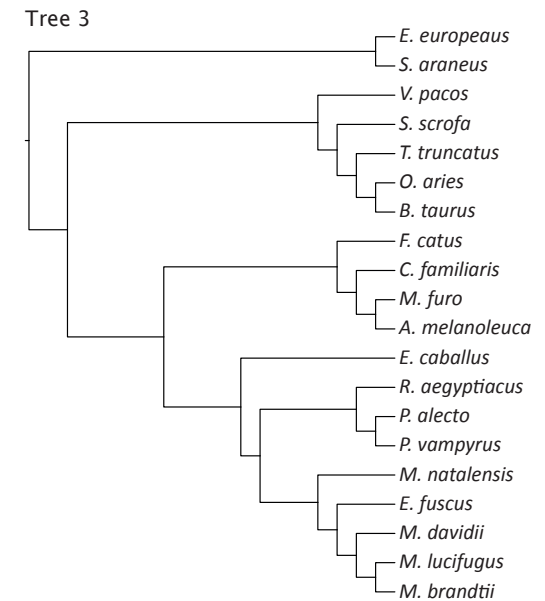
\*Corresponding author



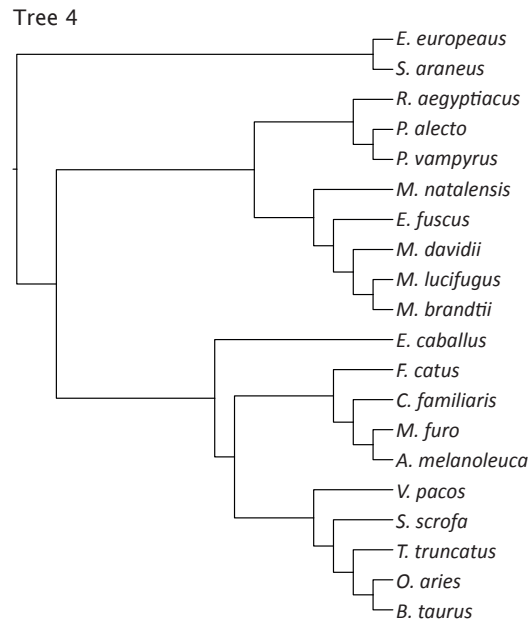
Following Waddell et al 1999, McCormack et al 2011 and O'Leary et al 2013 (Fig 4B)



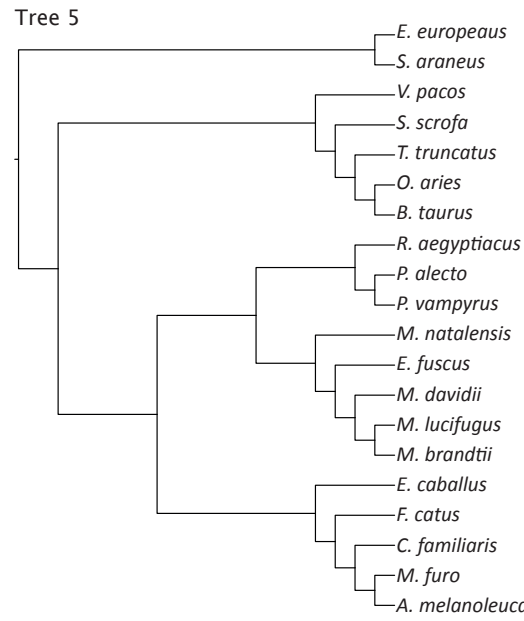
Following Madsen et al 2001 and O'Leary et al 2013



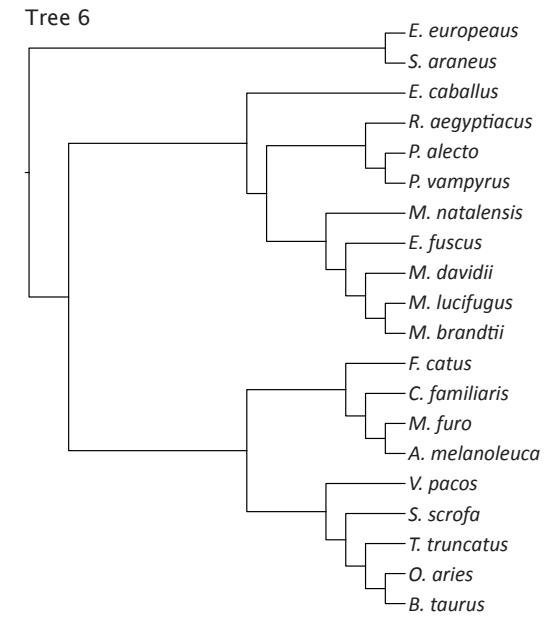
Following Nishihara et al 2006 and Zhang et al 2013



Following Prasad et al 2008



Following McCormack et al 2011 and Lindblad-Toh et al 2011



Following McCormack et al 2011 (Fig 4B)

Figure S1. Alternative laurasiatherian phylogenies used in OMA analyses.

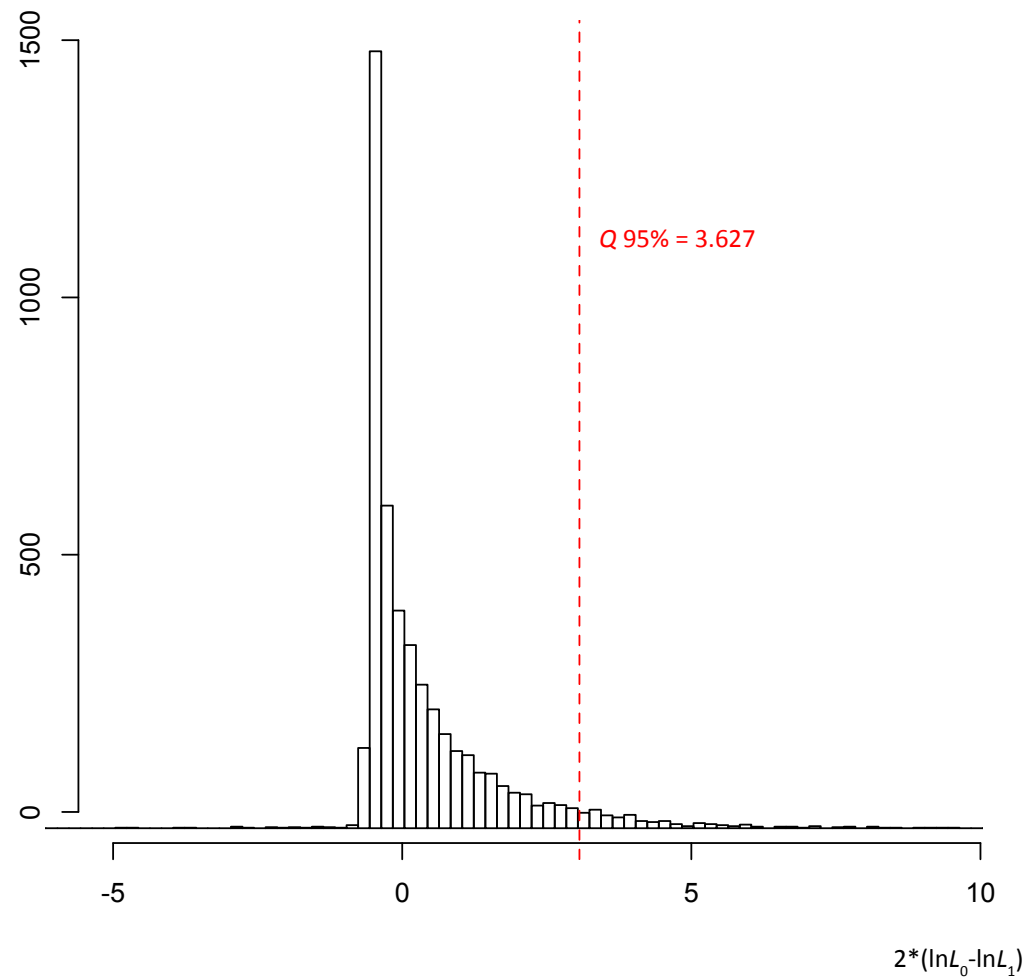


Figure S2. Null distribution of likelihood ratios based on 5,000 simulated datasets under the model of a global lambda versus one with two-lambda values assuming two independent rates, one for bats and one for the other mammals.

Table S1. Assessment of laurasiatherian proteome completeness using Benchmarking Universal Single-Copy Orthologs (BUSCO).

| <b>Species proteome</b>       | <b>Complete</b> | <b>Duplicated</b> | <b>Fragmented</b> | <b>Missing</b> |
|-------------------------------|-----------------|-------------------|-------------------|----------------|
| <i>Pteropus alecto</i>        | 98%             | 0.80%             | 1.00%             | 0.60%          |
| <i>Rousettus aegyptiacus</i>  | 98%             | 1.60%             | 1.00%             | 0.50%          |
| <i>Mustela putorius furo</i>  | 97%             | 1.50%             | 1.70%             | 1.00%          |
| <i>Eptesicus fuscus</i>       | 97%             | 1.60%             | 1.70%             | 0.50%          |
| <i>Bos taurus</i>             | 97%             | 1.90%             | 1.60%             | 0.50%          |
| <i>Ailuropoda melanoleuca</i> | 97%             | 2.10%             | 1.80%             | 0.30%          |
| <i>Canis familiaris</i>       | 97%             | 3.80%             | 1.80%             | 0.40%          |
| <i>Miniopterus natalensis</i> | 96%             | 1.00%             | 2.90%             | 0.60%          |
| <i>Felis catus</i>            | 96%             | 1.20%             | 2.80%             | 0.50%          |
| <i>Ovis aries</i>             | 96%             | 2.40%             | 2.10%             | 0.90%          |
| <i>Equus caballus</i>         | 95%             | 1.00%             | 3.80%             | 0.40%          |
| <i>Tursiops truncatus</i>     | 94%             | 0.80%             | 4.20%             | 1.40%          |
| <i>Pteropus vampyrus</i>      | 93%             | 0.70%             | 5.10%             | 1.50%          |
| <i>Myotis brandtii</i>        | 93%             | 3.30%             | 4.90%             | 1.30%          |
| <i>Myotis lucifugus</i>       | 93%             | 4.10%             | 3.10%             | 3.00%          |
| <i>Myotis davidii</i>         | 92%             | 1.50%             | 4.90%             | 2.00%          |
| <i>Sus scrofa</i>             | 83%             | 6.10%             | 6.80%             | 10.00%         |
| <i>Erinaceus europaeus</i>    | 62%             | 0.70%             | 17.00%            | 19.00%         |
| <i>Vicugna pacos</i>          | 57%             | 0.20%             | 12.00%            | 29.00%         |
| <i>Sorex araneus</i>          | 55%             | 0.50%             | 14.00%            | 29.00%         |

Table S2: HOG size changes along each branch of the laurasiatherian phylogeny.

| Parent Node  | Daughter Node  | # Ancestral genes | # Ancestral genes remaining identical | # Ancestral genes lost | # Ancestral genes expanded | # Multicopy genes |
|--|--|-------------------|---------------------------------------|------------------------|----------------------------|-------------------|
| <b>Scrotifera</b>  | <b>Chiroptera</b>  | <b>20,846</b>     | <b>18,602</b>                         | <b>1,053</b>           | <b>1,191</b>               | <b>2,678</b>      |
| <b>Chiroptera</b>  | <b>Old World fruit bats</b>  | <b>21,411</b>     | <b>18,573</b>                         | <b>2,272</b>           | <b>566</b>                 | <b>1,283</b>      |
| Old World fruit bats   | <i>P. alecto</i> +<br><i>P. vampyrus</i>   | 19,883            | 18,667                                | 994                    | 222                        | 461               |
| <b>Chiroptera</b>  | <b>Yangochiroptera</b>   | <b>21,411</b>     | <b>19,469</b>                         | <b>1,467</b>           | <b>475</b>                 | <b>1,076</b>      |
| Yangochiroptera  | <i>E. fuscus</i> +<br>Myotinae   | 20,632            | 19,766                                | 501                    | 365                        | 796               |
| <i>E. fuscus</i> + Myotinae  | Myotinae   | 20,635            | 19,526                                | 753                    | 356                        | 790               |
| Myotinae   | <i>M. lucifugus</i> +<br><i>M. brandtii</i>  | 20,444            | 19,216                                | 785                    | 443                        | 1,026             |
| <b>Scrotifera</b>  | <b>Ferungulata</b>   | <b>20,846</b>     | <b>19,174</b>                         | <b>409</b>             | <b>1,263</b>               | <b>2,759</b>      |
| Ferungulata  | Carnivora  | 22,544            | 19,160                                | 2,638                  | 746                        | 1,671             |
| Carnivora  | <i>A. melanoleuca</i> +<br><i>C. familiaris</i> +<br><i>M. furo</i>                  | 20,933            | 19,877                                | 807                    | 249                        | 536               |
| <i>A. melanoleuca</i> +<br><i>C. familiaris</i> +<br><i>M. furo</i>                  | <i>A. melanoleuca</i> +<br><i>M. furo</i>  | 20,460            | 19,241                                | 1,024                  | 195                        | 435               |
| Ferungulata  | Euungulata   | 22,544            | 21,009                                | 925                    | 610                        | 1,331             |
| Euungulata   | Cetartiodactyla  | 22,431            | 21,569                                | 591                    | 271                        | 562               |
| <i>B. taurus</i> +<br><i>S. scrofa</i> +<br><i>O. aries</i> +<br><i>T. truncatus</i> | <i>B. taurus</i> +<br><i>O. aries</i> +<br><i>T. truncatus</i>                       | 21,973            | 20,374                                | 1,405                  | 194                        | 408               |
| <i>B. taurus</i> +<br><i>O. aries</i> +<br><i>T. truncatus</i>                       | <i>B. taurus</i> +<br><i>O. aries</i>  | 20,786            | 19,233                                | 1,131                  | 422                        | 1,095             |
| Cetartiodactyla  | <i>B. taurus</i> +<br><i>S. scrofa</i> +<br><i>O. aries</i> +<br><i>T. truncatus</i> | 22,177            | 20,844                                | 859                    | 474                        | 1,046             |

Table S3: HOG functional annotation overview.

| Parent  | Scrotifera   | Chiroptera           | Chiroptera      | Scrotifera   | Ferungulata  | Euungulata      |
|---|--------------|----------------------|-----------------|--------------|--------------|-----------------|
| Daughter Node                                       | Chiroptera   | Old World fruit bats | Yangochiroptera | Ferungulata  | Carnivora    | Cetartiodactyla |
| # Ancestral genes                                   | 20,846       | 21,411               | 21,411          | 20,846       | 22,544       | 22,431          |
| # Genes remaining identical                         | 18,602       | 18,573               | 19,469          | 19,174       | 19,160       | 21,569          |
| # Multicopy genes from expansions                   | 2,678        | 1,283                | 1,076           | 2,759        | 1,671        | 562             |
| # HOGs showing expansion                            | <b>1,191</b> | <b>566</b>           | <b>475</b>      | <b>1,263</b> | <b>746</b>   | <b>271</b>      |
| GO annotated based on members using UniProtKB       |              |                      |                 |              |              |                 |
| #HOGs   | 830          | 377                  | 340             | 922          | 561          | 185             |
| % HOGs  | <b>69.7%</b> | <b>66.6%</b>         | <b>71.6%</b>    | <b>73.0%</b> | <b>75.2%</b> | <b>68.3%</b>    |
| GO annotated based on human homologs using GOATOOLS |              |                      |                 |              |              |                 |
| # HOGs  | 1,040        | 486                  | 420             | 1,074        | 610          | 233             |
| % HOGs  | <b>87.3%</b> | <b>85.9%</b>         | <b>88.4%</b>    | <b>85.0%</b> | <b>81.8%</b> | <b>86.0%</b>    |
| # Ancestral genes lost                              | <b>1,053</b> | <b>2,272</b>         | <b>1,467</b>    | <b>409</b>   | <b>2,638</b> | <b>591</b>      |
| # HOGs showing contraction                          | 1,004        | 2,041                | 1,419           | 373          | 2,501        | 546             |
| GO annotated based on members using UniProtKB       |              |                      |                 |              |              |                 |
| #HOGs   | 656          | 1,057                | 630             | 195          | 1,198        | 339             |
| % HOGs  | <b>65.3%</b> | <b>51.8%</b>         | <b>44.4%</b>    | <b>52.3%</b> | <b>47.9%</b> | <b>62.1%</b>    |
| GO annotated based on human homologs using GOATOOLS |              |                      |                 |              |              |                 |
| # HOGs  | 698          | 1,205                | 690             | 238          | 1,401        | 382             |
| % HOGs  | <b>69.5%</b> | <b>59.0%</b>         | <b>48.6%</b>    | <b>63.8%</b> | <b>56.0%</b> | <b>70.0%</b>    |

Table S4: Functional annotation of HOGs showing gene expansion and contraction in the MRCA of bats based on GO terms in UniProtKB.

| <b>GO term</b>   | <b>Expansions<br/>Annotated 830/1,191</b> | <b>Contractions<br/>Annotated 656/1,004</b> | <b>All member loss<br/>119/201</b> |
|--|---|---|------------------------------------|
| Cellular process   | 629                                       | 546   | 0                                  |
| Single-organism process  | 535                                       | 455   | 97                                 |
| Biological regulation  | 500                                       | 403   | 92                                 |
| Regulation of biological process                               | 476                                       | 383   | 92                                 |
| Metabolic process  | 366                                       | 312   | 27                                 |
| Response to stimulus   | 294                                       | 289   | 83                                 |
| Multicellular organismal process                               | 271                                       | 229   | 77                                 |
| Single organism signaling                                      | 211                                       | 214   | 76                                 |
| Developmental process  | 191                                       | 122   | 7                                  |
| Cellular component organisation or biogenesis                  | 182                                       | 157   | 11                                 |
| Localization   | 179                                       | 144   | 8                                  |
| Immune system process  | 80  | 51  | 4                                  |
| Multi-organism process   | 59  | 29  | 3                                  |
| Locomotion   | 44  | 22  | 2                                  |
| Reproduction   | 43  | 20  | 2                                  |
| Reproductive process   | 43  | 20  | 2                                  |
| Cell adhesion  | 41  | 30  | 1                                  |
| Behavior   | 21  | 10  | 1                                  |
| Growth   | 14  | 16  | 2                                  |
| Rhythmic process   | 10  | 2   | 0                                  |
| Presynaptic process involved in chemical synaptic transmission | 4   | 2   | 0                                  |
| Detoxification   | 3   | 2   | 0                                  |
| Cell killing   | 2   | 0   | 0                                  |
| Cellular oxidant detoxification                                | 0   | 2   | 0                                  |
| Cartilage condensation   | 0   | 1   | 0                                  |
| Antibody-dependent cellular cytotoxicity                       | 0   | 1   | 0                                  |
| Developmental growth   | 0   | 0   | 2                                  |
| Locomotory behavior  | 0   | 0   | 1                                  |

Table S5: Functional annotation of HOGs showing gene expansion and contraction in the MRCA of bats based on keyword terms in UniProtKB.

| <b>Keyword</b>          | <b>Expansions<br/>Annotated 192/1,191</b> | <b>Contractions<br/>Annotated 206/1,004</b> | <b>Lost in MRCA Bats<br/>73/201</b> |
|-------------------------|---|---|-------------------------------------|
| Olfaction               | 0   | 92  | 68                                  |
| Transport               | 51  | 36  | 1                                   |
| Transcription           | 29  | 23  | 0                                   |
| mRNA splicing           | 8   | 12  | 0                                   |
| Ubl conjugation pathway | 6   | 7   | 1                                   |
| Differentiation         | 2   | 7   | 1                                   |
| Lipid metabolism        | 4   | 6   | 0                                   |
| Cell cycle              | 6   | 5   | 0                                   |
| Cell adhesion           | 11  | 4   | 0                                   |
| DNA repair              | 6   | 4   | 0                                   |
| Apoptosis               | 4   | 3   | 0                                   |
| DNA replication         | 4   | 2   | 0                                   |
| Autophagy               | 2   | 2   | 0                                   |
| Glycolysis              | 1   | 2   | 0                                   |
| Myogenesis              | 0   | 2   | 1                                   |
| Neurogenesis            | 2   | 1   | 0                                   |
| Heme biosynthesis       | 2   | 1   | 0                                   |
| DNA recombination       | 1   | 1   | 0                                   |
| Purine biosynthesis     | 1   | 1   | 0                                   |
| Wnt signaling pathway   | 1   | 1   | 0                                   |
| Peroxisome biogenesis   | 1   | 1   | 0                                   |
| Sensory transduction    | 43  | 0   | 0                                   |
| Immunity                | 5   | 0   | 0                                   |
| Protein biosynthesis    | 5   | 0   | 0                                   |
| Chemotaxis              | 3   | 0   | 0                                   |
| Antiviral defense       | 2   | 0   | 0                                   |
| Exocytosis              | 2   | 0   | 0                                   |
| Carbohydrate metabolism | 2   | 0   | 0                                   |



|                                      |   |   |   |
|--------------------------------------|---|---|---|
| Iron storage                         | 1 | 0 | 0 |
| Mast cell degranulation              | 1 | 0 | 0 |
| GPI-anchor biosynthesis              | 1 | 0 | 0 |
| Neurotransmitter biosynthesis        | 1 | 0 | 0 |
| Porphyrin biosynthesis               | 1 | 0 | 0 |
| DNA synthesis                        | 1 | 0 | 0 |
| Inflammatory response                | 1 | 0 | 0 |
| Digestion                            | 1 | 0 | 0 |
| Branched-chain amino acid catabolism | 1 | 0 | 0 |
| tRNA processing                      | 1 | 0 | 0 |
| Ubiquinone biosynthesis              | 1 | 0 | 0 |
| Biological rhythms                   | 1 | 0 | 0 |
| Cilium biogenesis/degradation        | 1 | 0 | 0 |
| Transcription regulation             | 0 | 0 | 3 |

Table S6-S13: See separate .xls spreadsheet

Table S14: Functional annotation of HOGs showing gene expansion and contraction in the MRCA of Old World fruit bats and Yangochiroptera based on GO terms in UniProtKB.

| GO term  | Expanded  |  | Contracted  |  |
|--|---|--|---|--|
|  | MRCA<br>Old World fruit bats<br>Annotated 377/566 | MRCA<br>Yangochiroptera<br>Annotated 340/475 | MRCA<br>Old World fruit bats<br>Annotated 1,057/1,263 | MRCA<br>Yangochiroptera<br>Annotated 630/746 |
| Cellular process   | 280   | 256  | 762   | 465  |
| Biological regulation  | 261   | 213  | 622   | 401  |
| Single-organism process  | 254   | 206  | 659   | 404  |
| Regulation of biological process                               | 250   | 201  | 590   | 390  |
| Response to stimulus   | 173   | 127  | 362   | 251  |
| Metabolic process  | 137   | 179  | 458   | 211  |
| Multicellular organismal process                               | 135   | 105  | 313   | 217  |
| Single organism signaling                                      | 127   | 75   | 241   | 198  |
| Developmental process  | 80  | 81   | 212   | 96   |
| Localization   | 76  | 68   | 207   | 100  |
| Cellular component organisation or biogenesis                  | 73  | 79   | 237   | 90   |
| Immune system process  | 32  | 29   | 100   | 44   |
| Multi-organism process   | 24  | 23   | 76  | 41   |
| Locomotion   | 23  | 20   | 39  | 20   |
| Reproductive process   | 20  | 19   | 47  | 25   |
| Reproduction   | 20  | 19   | 47  | 25   |
| Cell adhesion  | 18  | 17   | 0   | 0  |
| Behavior   | 9   | 9  | 22  | 13   |
| Growth   | 8   | 7  | 20  | 5  |
| Rhythmic process   | 4   | 4  | 8   | 7  |
| Presynaptic process involved in chemical synaptic transmission | 3   | 1  | 6   | 2  |
| Cartilage condensation   | 1   | 0  | 2   | 0  |
| T cell mediated cytotoxicity                                   | 1   | 0  | 0   | 0  |

|                                 |   |   |    |    |
|---------------------------------|---|---|----|----|
| Biological adhesion             | 0 | 0 | 46 | 18 |
| Cell killing                    | 0 | 0 | 2  | 0  |
| Cellular oxidant detoxification | 0 | 0 | 2  | 0  |
| Iodide peroxidase activity      | 0 | 1 | 0  | 0  |
| Detoxification                  | 0 | 0 | 2  | 5  |
| Leukocyte mediated cytotoxicity | 0 | 0 | 0  | 1  |
| Menstruation                    | 0 | 0 | 0  | 1  |

Tables S15-S21: See separate .xls spreadsheet

Table S22. Birth-and-death (BD) model fit on 18,698 HOGs present in the MRCA of Scrotifera (Bats + Carnivores + Perissodactyla + Cetartiodactyla).

| BD Model  | $\lambda_0$ | $\lambda_1$ | $\mu_0$ | $\mu_1$ | -lnL       | AIC        |
|---|-------------|-------------|---------|---------|------------|------------|
| <b>No error correction</b>  |             |             |         |         |            |            |
| 1-lambda  | 0.0017      | -           | -       | -       | 184,044.80 | 368,091.60 |
| 2-lambda, 1=Bats  | 0.0017      | 0.0018      | -       | -       | 184,028.60 | 368,061.20 |
| 2-lambda, 1=Carnivora   | 0.0019      | 0.0011      | -       | -       | 182,933.79 | 365,871.57 |
| 2-lambda, 1=Cetartiodactyla   | 0.0015      | 0.0023      | -       | -       | 182,934.14 | 365,872.27 |
| 1-lambda <sub>mu</sub>  | 0.0004      | -           | 0.0030  | -       | 167,963.90 | 335,931.81 |
| 2-lambda <sub>mu</sub> , 1=Bats   | 0.0003      | 0.0004      | 0.0029  | 0.0031  | 167,925.19 | 335,858.38 |
| 2-lambda <sub>mu</sub> , 1=Carnivora  | 0.0004      | 0.0002      | 0.0034  | 0.0019  | 166,880.44 | 333,768.87 |
| 2-lambda <sub>mu</sub> , 1=Cetartiodactyla                                    | 0.0003      | 0.0004      | 0.0025  | 0.0040  | 166,891.10 | 333,790.21 |
| <b>Global error correction, <math>\epsilon=0.0635</math></b>                  |             |             |         |         |            |            |
| 1-lambda  | 0.0017      | -           | -       | -       | 184,044.80 | 368,091.60 |
| 2-lambda, 1=Bats  | 0.0017      | 0.0018      | -       | -       | 184,028.60 | 368,061.20 |
| 2-lambda, 1=Carnivora   | 0.0019      | 0.0011      | -       | -       | 182,933.79 | 365,871.57 |
| 2-lambda, 1=Cetartiodactyla   | 0.0015      | 0.0023      | -       | -       | 182,934.14 | 365,872.27 |
| 1-lambda <sub>mu</sub>  | 0.0004      | -           | 0.0030  | -       | 167,963.90 | 335,931.81 |
| 2-lambda <sub>mu</sub> , 1=Bats   | 0.0003      | 0.0004      | 0.0029  | 0.0031  | 167,925.19 | 335,858.38 |
| 2-lambda <sub>mu</sub> , 1=Carnivora  | 0.0004      | 0.0002      | 0.0034  | 0.0019  | 166,880.44 | 333,768.87 |
| 2-lambda <sub>mu</sub> , 1=Cetartiodactyla                                    | 0.0003      | 0.0004      | 0.0025  | 0.0040  | 166,891.10 | 333,790.21 |
| <b>Individual species error correction, <math>\epsilon=[0, 0.4126]</math></b> |             |             |         |         |            |            |
| 1-lambda  | 0.0008      | -           | -       | -       | 162,987.48 | 326,008.97 |
| 2-lambda, 1=Bats  | 0.0008      | 0.0008      | -       | -       | 162,987.36 | 326,010.73 |
| 2-lambda, 1=Carnivora   | 0.0008      | 0.0007      | -       | -       | 162,954.29 | 325,944.58 |
| 2-lambda, 1=Cetartiodactyla   | 0.0008      | 0.0007      | -       | -       | 162,954.29 | 325,944.58 |
| 1-lambda <sub>mu</sub>  | 0.0001      | -           | 0.0016  | -       | 155,133.69 | 310,303.38 |
| 2-lambda <sub>mu</sub> , 1=Bats   | 0.0001      | 0.0001      | 0.0017  | 0.0015  | 155,107.15 | 310,254.29 |
| 2-lambda <sub>mu</sub> , 1=Carnivora  | 0.0001      | 0.0001      | 0.0017  | 0.0014  | 155,033.81 | 310,107.63 |
| 2-lambda <sub>mu</sub> , 1=Cetartiodactyla                                    | 0.0001      | 0.0001      | 0.0016  | 0.0019  | 155,027.65 | 310,095.30 |