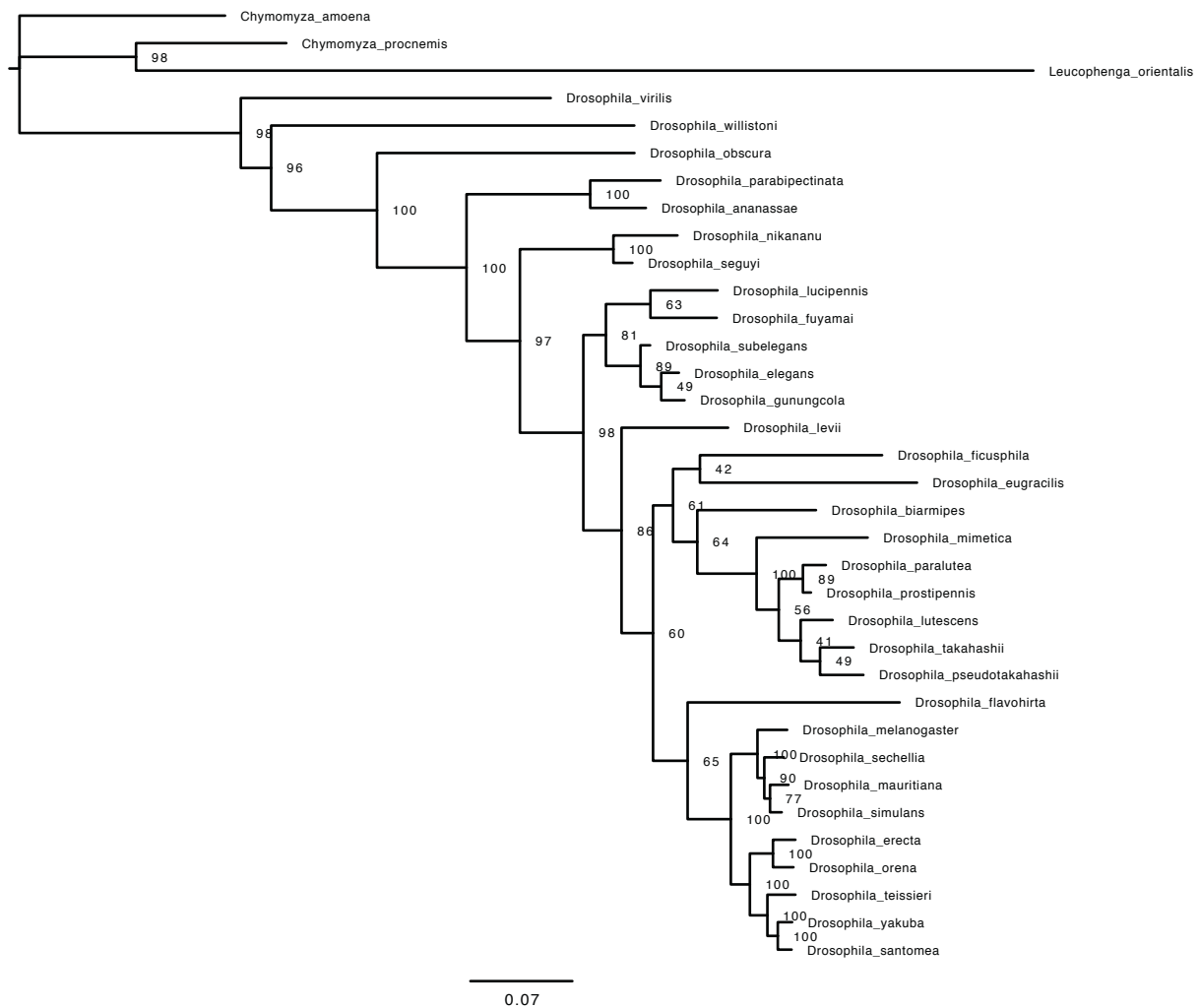
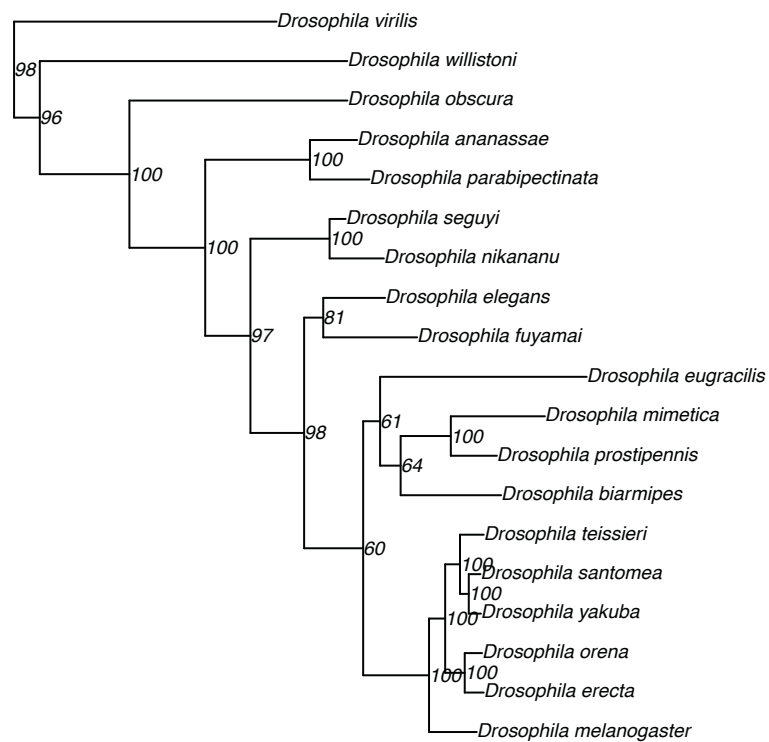


Supplemental Figure 1. Maximum likelihood tree showing phylogenetic relationships among 19 *Drosophila* species. Each ancestral node is numbered. Probabilities of a monomorphic or dimorphic ancestor at each node is shown in Supplemental Table 1.



Supplemental Figure 2. Complete phylogenetic estimate from Maximum Likelihood analysis of all six nuclear genes concatenated (ADH, amyrel, Ddc, Gphd, Sod, and Xdh). Node labels indicate ultrafast bootstrap support values, with a value of 95 or greater indicating a well supported node.

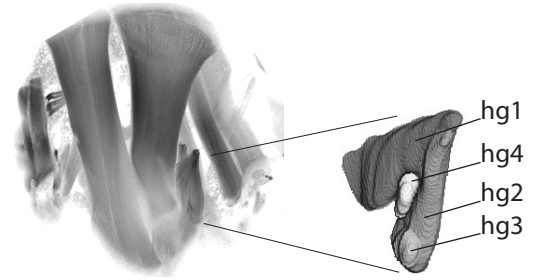
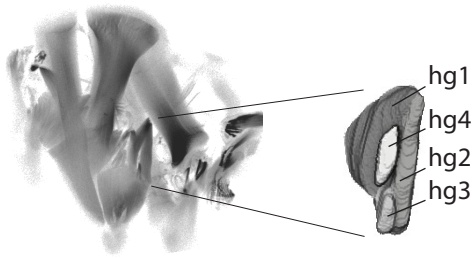


Supplemental Figure 3. Paired down phylogeny used for Ancestral State Reconstructions seen in Figures 3-4. Full phylogeny (Supplemental Figure 2) was paired down to include only species for which muscle volume data was available for the ancestral state reconstruction. Node labels indicate ultrafast bootstrap support values, with a value of 95 or greater indicating a well supported node. a

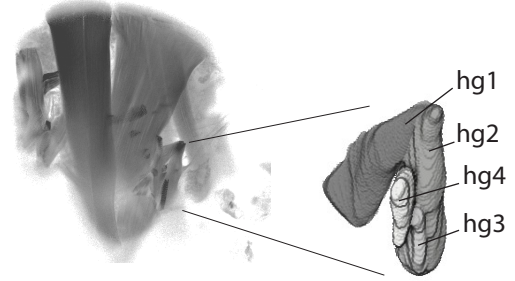
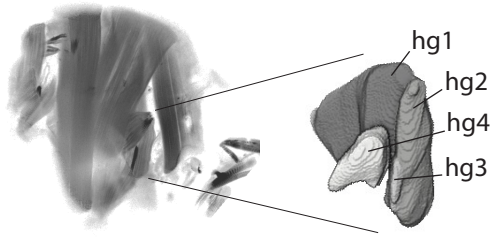
male

female

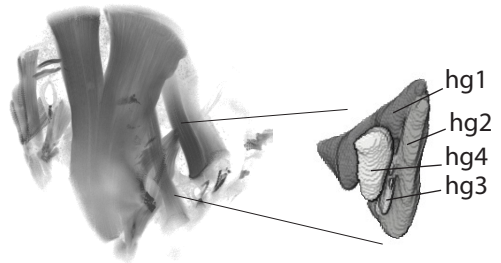
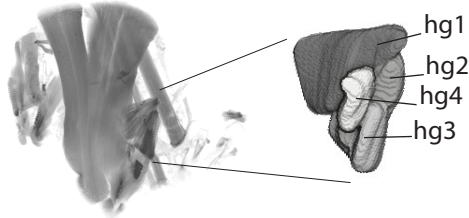
D. orena



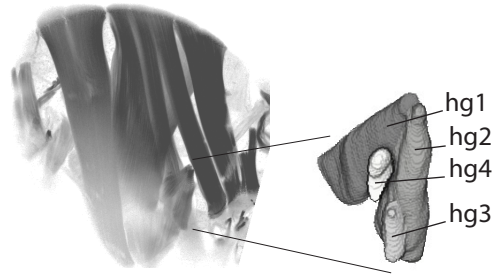
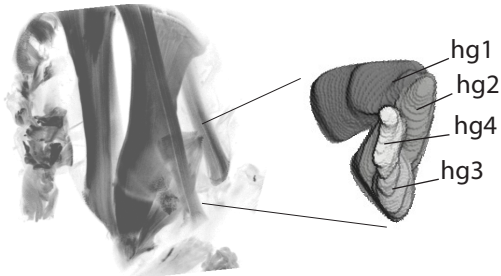
D. erecta



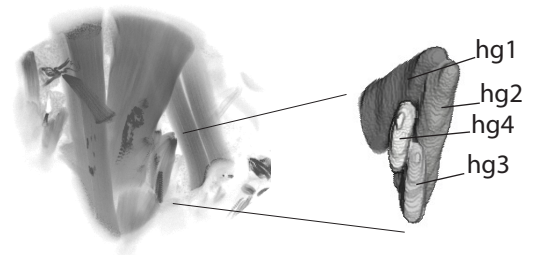
D. yakuba



D. santomea



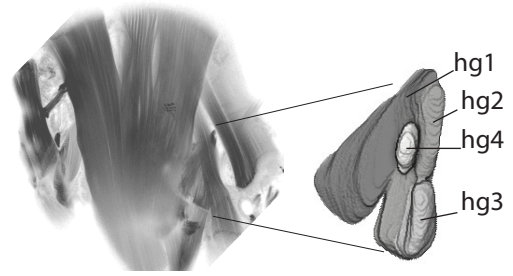
D. teissieri



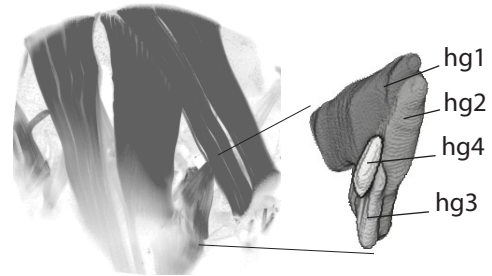
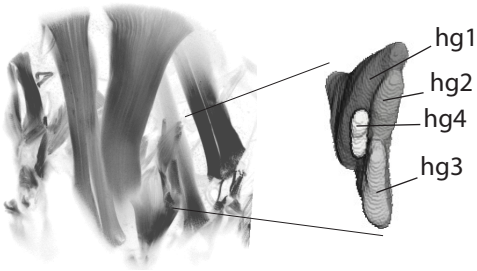
male

female

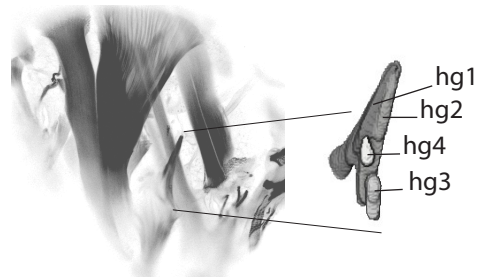
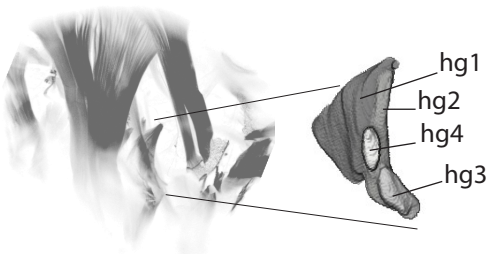
D. biarmipes



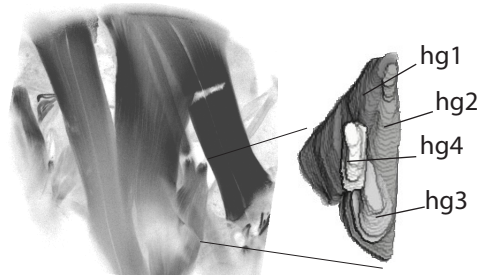
D. prostipennis



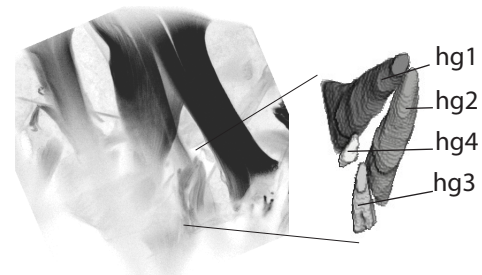
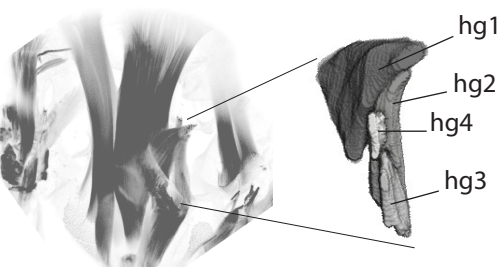
D. mimetica



D. eugracilis



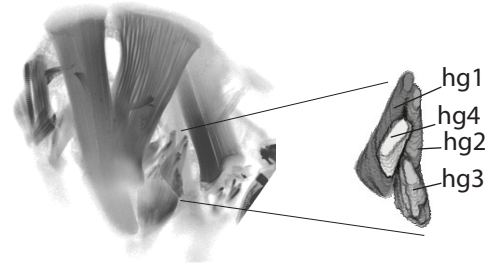
D. fuyamai



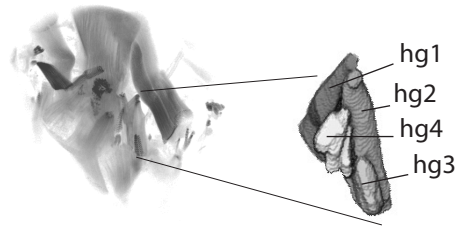
male

female

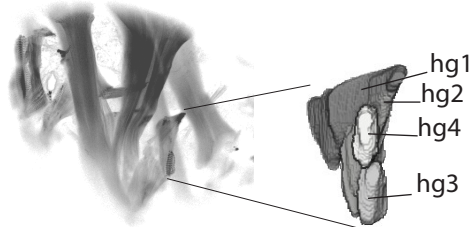
D. elegans



D. nikananu



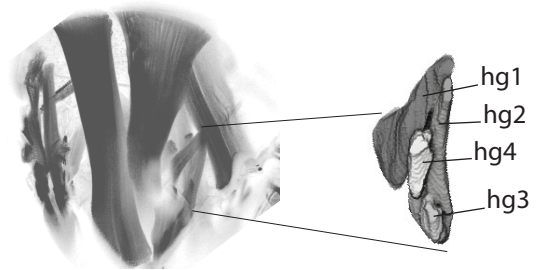
D. seguyi

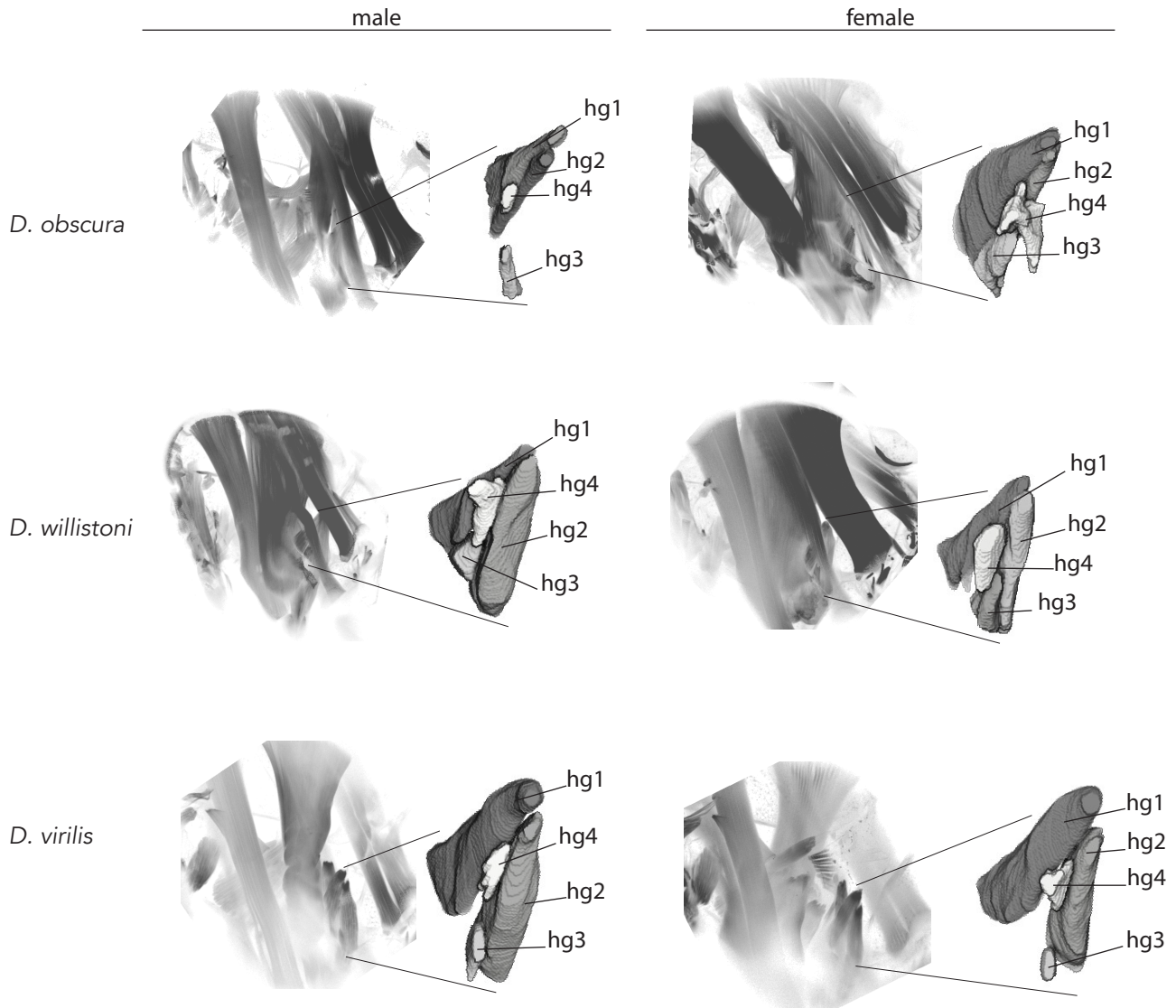


D. parabiptinata



D. ananassae





Supplemental Figure 4. Reconstructions of the hg1–4 muscles from males and females of the species used in this study. The relative position and shape of the muscles are generally conserved in males and females across all species. In all images, the anterior side of the thorax is to the left and the dorsal side of the thorax is to the top. Maximum projection image of the confocal slices that include the hg muscles is shown to the left of each panel. Reconstructions on the right were generated in Amira.

| Node | Hg1 | | Hg2 | | | Hg3 | | |
|------|-------------|-----------|-------------|----------------------|------------------------|------------|----------------------|------------------------|
| | Prob (mono) | Prob (di) | Prob (mono) | Prob (male enlarged) | Prob (female enlarged) | Prob(mono) | Prob (male enlarged) | Prob (female enlarged) |
| 1 | 0.702 | 0.298 | 0.966 | 0.017 | 0.017 | 0.956 | 0.022 | 0.022 |
| 2 | 0.719 | 0.281 | 0.978 | 0.011 | 0.012 | 0.970 | 0.015 | 0.015 |
| 3 | 0.785 | 0.215 | 0.990 | 0.004 | 0.006 | 0.989 | 0.005 | 0.005 |
| 4 | 0.843 | 0.157 | 0.991 | 0.002 | 0.007 | 0.998 | 0.001 | 0.001 |
| 5 | 0.811 | 0.189 | 0.999 | 0.000 | 0.001 | 0.999 | 0.001 | 0.000 |
| 6 | 0.563 | 0.437 | 0.999 | 0.000 | 0.000 | 0.992 | 0.007 | 0.001 |
| 7 | 0.161 | 0.839 | 0.979 | 0.017 | 0.004 | 0.999 | 0.001 | 0.000 |
| 8 | 0.018 | 0.982 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 9 | 0.013 | 0.987 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 10 | 0.086 | 0.914 | 1.000 | 0.000 | 0.000 | 0.980 | 0.000 | 0.019 |
| 11 | 0.001 | 0.999 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 12 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.990 | 0.010 | 0.000 |
| 13 | 0.141 | 0.859 | 0.897 | 0.085 | 0.018 | 0.998 | 0.002 | 0.000 |
| 14 | 0.081 | 0.919 | 0.435 | 0.473 | 0.092 | 0.993 | 0.006 | 0.001 |
| 15 | 0.029 | 0.971 | 0.169 | 0.516 | 0.315 | 0.885 | 0.109 | 0.006 |
| 16 | 0.579 | 0.421 | 1.000 | 0.000 | 0.000 | 0.960 | 0.038 | 0.003 |
| 17 | 0.990 | 0.010 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 18 | 0.973 | 0.027 | 0.871 | 0.006 | 0.124 | 0.999 | 0.000 | 0.000 |

Supplemental Table 1. Results from the ancestral state reconstruction for hg1–hg3 with the probability of each node being monomorphic or dimorphic. Results for hg4 are not shown, as no dimorphisms were present in the dataset (all nodes are inferred to be monomorphic).

| | ADH | amyrel | Ddc | Gphd | Sod | Xdh |
|------------------------------------|-------------|----------|----------|-------------|----------|----------|
| <i>Chymomyza amoena</i> | AF059887 | AY736544 | AY124490 | L36961 | X61687 | AF093217 |
| <i>Chymomyza procnemis</i> | AB026521 | - | - | L41252 | AF021821 | - |
| <i>Drosophila ananassae</i> | XM001961486 | AF024691 | - | XM001962674 | - | - |
| <i>Drosophila biarmipes</i> | DQ363229 | AF462597 | DQ363223 | AY098467 | - | DQ363235 |
| <i>Drosophila elegans</i> | DQ363230 | AF136930 | DQ363224 | AB032146 | - | DQ363236 |
| <i>Drosophila erecta</i> | XM001968861 | AF039562 | - | XM001968825 | AF127156 | - |
| <i>Drosophila eugracilis</i> | AY279326 | AF250055 | DQ363225 | AY098472 | - | AY279341 |
| <i>Drosophila ficusphila</i> | DQ363232 | AF462600 | DQ363226 | AB032149 | - | DQ363237 |
| <i>Drosophila flavohirta</i> | - | AY733051 | - | - | - | - |
| <i>Drosophila fuyamai</i> | - | - | - | AY098471 | - | - |
| <i>Drosophila gunungcola</i> | AM181670 | - | - | AB032145 | - | - |
| <i>Drosophila levii</i> | - | AF491635 | - | - | - | - |
| <i>Drosophila lucipennis</i> | AF459751 | AF251138 | - | AY098470 | - | - |
| <i>Drosophila lutescens</i> | AY279327 | AF491637 | - | - | - | AY279343 |
| <i>Drosophila mauritiana</i> | X63953 | U96157 | - | - | AF127158 | - |
| <i>Drosophila melanogaster</i> | X78384 | NM057914 | X04661 | J04567 | X17332 | Y00308 |
| <i>Drosophila mimetica</i> | AY279328 | AY733058 | - | AY098465 | - | AY279342 |
| <i>Drosophila nikananu</i> | AF459767 | AF251136 | - | - | - | - |
| <i>Drosophila obscura</i> | U90955 | AF306713 | - | U47881 | U47892 | - |
| <i>Drosophila orena</i> | Z00032 | U96158 | - | DQ167752 | AF127155 | AY279340 |
| <i>Drosophila parabipectinata</i> | AB194414 | AY736489 | - | AJ844754 | AJ844865 | - |
| <i>Drosophila paralutea</i> | AF459747 | - | - | - | - | - |
| <i>Drosophila prostipennis</i> | AF459748 | - | - | AB027282 | - | - |
| <i>Drosophila pseudotakahashii</i> | - | AY736499 | - | - | - | - |
| <i>Drosophila santomea</i> | AY804554 | AY736503 | - | - | AY804512 | AY804550 |
| <i>Drosophila sechellia</i> | XM002035803 | AF039558 | - | - | AF127157 | - |
| <i>Drosophila seguyi</i> | AF459765 | - | - | - | - | - |

| | | | | | | |
|-------------------------------|-------------|-------------|----------|-------------|----------|----------|
| <i>Drosophila simulans</i> | XM002079432 | U96160 | AY197770 | L41647 | X15685 | AY279336 |
| <i>Drosophila subelegans</i> | - | AY736507 | - | - | - | - |
| <i>Drosophila takahashii</i> | AF459749 | U96161 | - | - | - | - |
| <i>Drosophila teissieri</i> | X54118 | AF039557 | - | U47809 | AF127160 | AY279337 |
| <i>Drosophila virilis</i> | XM002057547 | XM002048980 | AF293749 | XM002051332 | X13831 | AF093215 |
| <i>Drosophila willistoni</i> | XM002066420 | AF039560 | AF293750 | XM002065330 | L13281 | AF093206 |
| <i>Drosophila yakuba</i> | XM002089270 | AF039561 | - | XM002089126 | AF127159 | AY279338 |
| <i>Leucophenga orientalis</i> | AB033638 | - | - | - | - | - |

Supplemental Table 2. GenBank accession numbers of species used in the phylogenetic analysis. All data was used for the Maximum Likelihood estimation of the phylogeny, and the bolded individuals were the only ones retained on the phylogeny for ancestral state reconstruction.