

Supplementary Materials

Table S1. – *Drosophila* species and strains utilized.

Species	Strain	Obtained From	Collection Site
<i>D. albomicans</i>	15112-1751.00	UCSD Stock Center	Okinawa, Japan
<i>D. albomicans</i>	15112-1751.01	UCSD Stock Center	Alisha, Taiwan
<i>D. albomicans</i>	15112-1751.02	UCSD Stock Center	Penghu Islands
<i>D. albomicans</i>	15112-1751.03	UCSD Stock Center	Nankang, Taiwan
<i>D. albomicans</i>	15112-1751.05	UCSD Stock Center	Ishigaki Island, Japan
<i>D. albomicans</i>	15112-1751.07	UCSD Stock Center	Chiang Dao, Thailand
<i>D. albomicans</i>	15112-1751.08	UCSD Stock Center	Chakkarat, Thailand
<i>D. albomicans</i>	FKC20	EHIME Stock Center	Fukui, Japan
<i>D. albomicans</i>	KM070	Qi Zhou	Kunming, China
<i>D. albomicans</i>	KM126	Qi Zhou	Kunming, China
<i>D. albomicans</i>	KM134	Qi Zhou	Kunming, China
<i>D. albomicans</i>	KM165	Qi Zhou	Kunming, China
<i>D. albomicans</i>	KM55	Qi Zhou	Kunming, China
<i>D. albomicans</i>	SHL	EHIME Stock Center	Shillong, India
<i>D. albomicans</i>	NOU98	Masayoshi Watada	Noumea, New Caledonia
<i>D. immigrans</i>	15111-1731.13	UCSD Stock Center	Ehime, Japan
<i>D. kepulauanana</i>	15112-1761.01	UCSD Stock Center	Sarawak, Malaysia
<i>D. kepulauanana</i>	15112-1761.02	UCSD Stock Center	Brunei, Borneo
<i>D. kepulauanana</i>	15112-1761.03	UCSD Stock Center	Ulu Temburong, Borneo
<i>D. kohkoa</i>	15112-1771.00	UCSD Stock Center	Chakkarat, Thailand
<i>D. kohkoa</i>	15112-1771.01	UCSD Stock Center	Sarawak, Malaysia
<i>D. nasuta</i>	15112-1781.00	UCSD Stock Center	Mysore, India
<i>D. nasuta</i>	15112-1781.01	UCSD Stock Center	Seychelle Isles, France
<i>D. nasuta</i>	15112-1781.02	UCSD Stock Center	Seychelle Isles, France
<i>D. nasuta</i>	15112-1781.06	UCSD Stock Center	Mombasa Kenya
<i>D. nasuta</i>	15112-1781.07	UCSD Stock Center	Mombasa Kenya
<i>D. nasuta</i>	15112-1781.08	UCSD Stock Center	Antananarivo, Madagascar
<i>D. nasuta</i>	15112-1781.09	UCSD Stock Center	Antananarivo, Madagascar
<i>D. nasuta</i>	15112-1781.11	UCSD Stock Center	Sri Lanka
<i>D. nasuta</i>	15112-1781.12	UCSD Stock Center	Kandy, Sri Lanka
<i>D. nasuta</i>	15112-1781.13	UCSD Stock Center	Yaounde, Cameroon
<i>D. nasuta</i>	E-19502_MBA31	EHIME Stock Center	Mombasa Kenya
<i>D. nasuta</i>	E-19503_G86	EHIME Stock Center	Mauritius
<i>D. nasuta</i>	E-19504_NHO4	EHIME Stock Center	Nagarahole, India
<i>D. nasuta</i>	E-19505_SEZ11	EHIME Stock Center	Seychelle Isles, France
<i>D. niveifrons</i>	LAE276	Masayoshi Watada	Lae, Papua New Guinea
<i>D. niveifrons</i>	LAE221	Masayoshi Watada	Lae, Papua New Guinea
<i>Taxon F</i>	B208	Masayoshi Watada	Kunching, Sarawak, Malaysia
<i>D. pallidifrons</i>	PN175_E-19901	Masayoshi Watada	Ponape Micronesia
<i>D. pulaua</i>	O-30	Masayoshi Watada	Lae, Papua New Guinea
<i>D. pulaua</i>	15112.1801.00	UCSD Stock Center	Sarawak, Malaysia
<i>D. s. albostrigata</i>	15112-1771.04	UCSD Stock Center	Rizal, Phillipines
<i>D. s. albostrigata</i>	cambodia_1	Doris Bachtrog	Cambodia
<i>D. s. albostrigata</i>	cambodia_3	Doris Bachtrog	Cambodia
<i>D. s. albostrigata</i>	15112-1811.00	UCSD Stock Center	Kuala Lumpur, Malaysia
<i>D. s. albostrigata</i>	15112-1811.01	UCSD Stock Center	Akreiy Ksatr Commune
<i>D. s. albostrigata</i>	15112-1811.02	UCSD Stock Center	Chakkarat, Thailand
<i>D. s. albostrigata</i>	15112-1811.03	UCSD Stock Center	Sarawak, Malaysia

<i>D. s. albostrigata</i>	15112-1811.04	UCSD Stock Center	Siem Reap, Cambodia
<i>D. s. albostrigata</i>	15112-1811.05	UCSD Stock Center	Brunei, Borneo
<i>D. s. albostrigata</i>	15112-1811.07	UCSD Stock Center	Singapore
<i>D. s. albostrigata</i>	15112-1811.08	UCSD Stock Center	Kandy, Sri Lanka
<i>D. s. bilimbata</i>	15112-1821.00	UCSD Stock Center	Oahu, Hawai'i
<i>D. s. bilimbata</i>	15112-1821.02	UCSD Stock Center	Palmyra Islands
<i>D. s. bilimbata</i>	15112-1821.03	UCSD Stock Center	Savai'i, Samoa
<i>D. s. bilimbata</i>	15112-1821.04	UCSD Stock Center	Upolu, Samoa
<i>D. s. bilimbata</i>	15112-1821.05	UCSD Stock Center	Tongatapu, Tonga Islands
<i>D. s. bilimbata</i>	15112-1821.06	UCSD Stock Center	Viti Levu, Fiji
<i>D. s. bilimbata</i>	15112-1821.08	UCSD Stock Center	Guam, Mariana Islands
<i>D. s. bilimbata</i>	15112-1821.09	UCSD Stock Center	Upolu, Samoa
<i>D. s. bilimbata</i>	15112-1821.10	UCSD Stock Center	Guam
<i>D. s. neonasuta</i>	15114-1861.00	UCSD Stock Center	Mysore, India
<i>D. s. neonasuta</i>	E-20702_CJB53	EHIME Stock Center	Coinbatore, India
<i>D. s. sulfurigaster</i>	WAU-18	Masayoshi Watada	WAU, Papua New Guinea
<i>D. s. sulfurigaster</i>	15112-1831.00	UCSD Stock Center	Queensland, Australia
<i>D. s. sulfurigaster</i>	15112-1831.01	UCSD Stock Center	Kavieng, New Ireland
<i>D. s. sulfurigaster</i>	15112-1831.02	UCSD Stock Center	Wau, Papua New Guinea
<i>D. s. sulfurigaster</i>	15112-1831.04	UCSD Stock Center	Kavieng, New Ireland

Table S2. – The number of BUSCOs found in the *D. albomicans* genome assembly. Nearly all complete, single copy genes were found.

Busco Stats		
Complete	1046	98.1 %
Complete and Single Copy	1037	97.3 %
Complete and Duplicated	9	0.8 %
Fragmented	2	0.2 %
Missing	18	1.7 %
Total Searched	1066	100 %

Table S3. – Mean and median levels of average pairwise diversity (π) in the different species.

Species	median π (%)	mean π (%)
<i>D. albomicans</i>	0.61	0.60
<i>D. nasuta</i>	0.49	0.47
<i>D. kepulauan</i>	0.58	0.57
<i>D. s. albostrigata</i>	0.52	0.51
<i>D. s. neonasuta</i>	0.49	0.48
<i>D. s. bilimbata</i>	0.18	0.18
<i>D. s. sulfurigaster</i>	0.40	0.39
<i>D. pulaua</i>	0.31	0.33
<i>D. kohkoa</i>	0.44	0.44
<i>D. niveifrons</i>	0.29	0.30

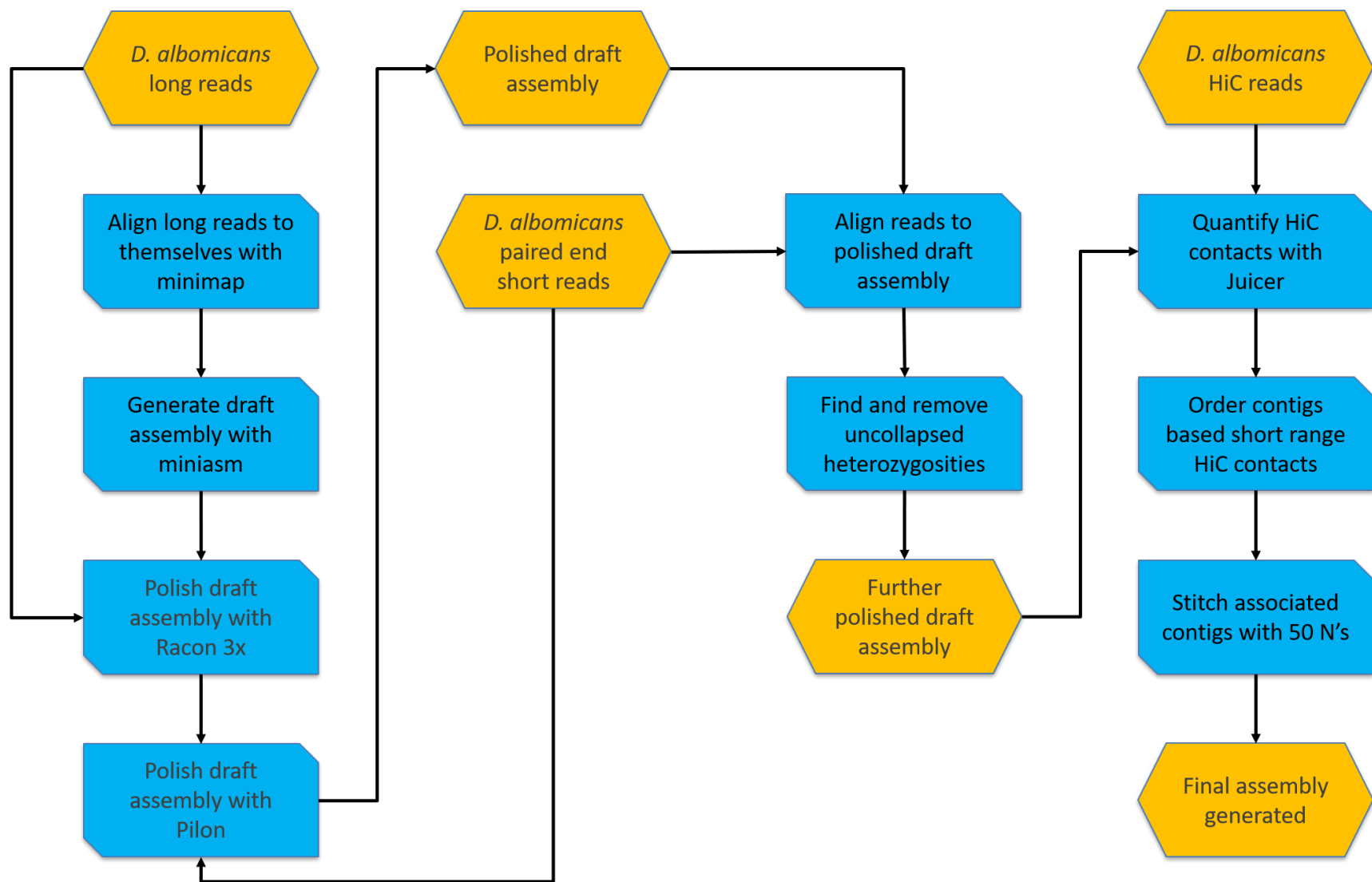


Figure S1. Assembly pipeline. The yellow hexagons represent data and the blue rectangles are the computational steps performed to obtain a high-quality genome assembly of *D. albomicans*.

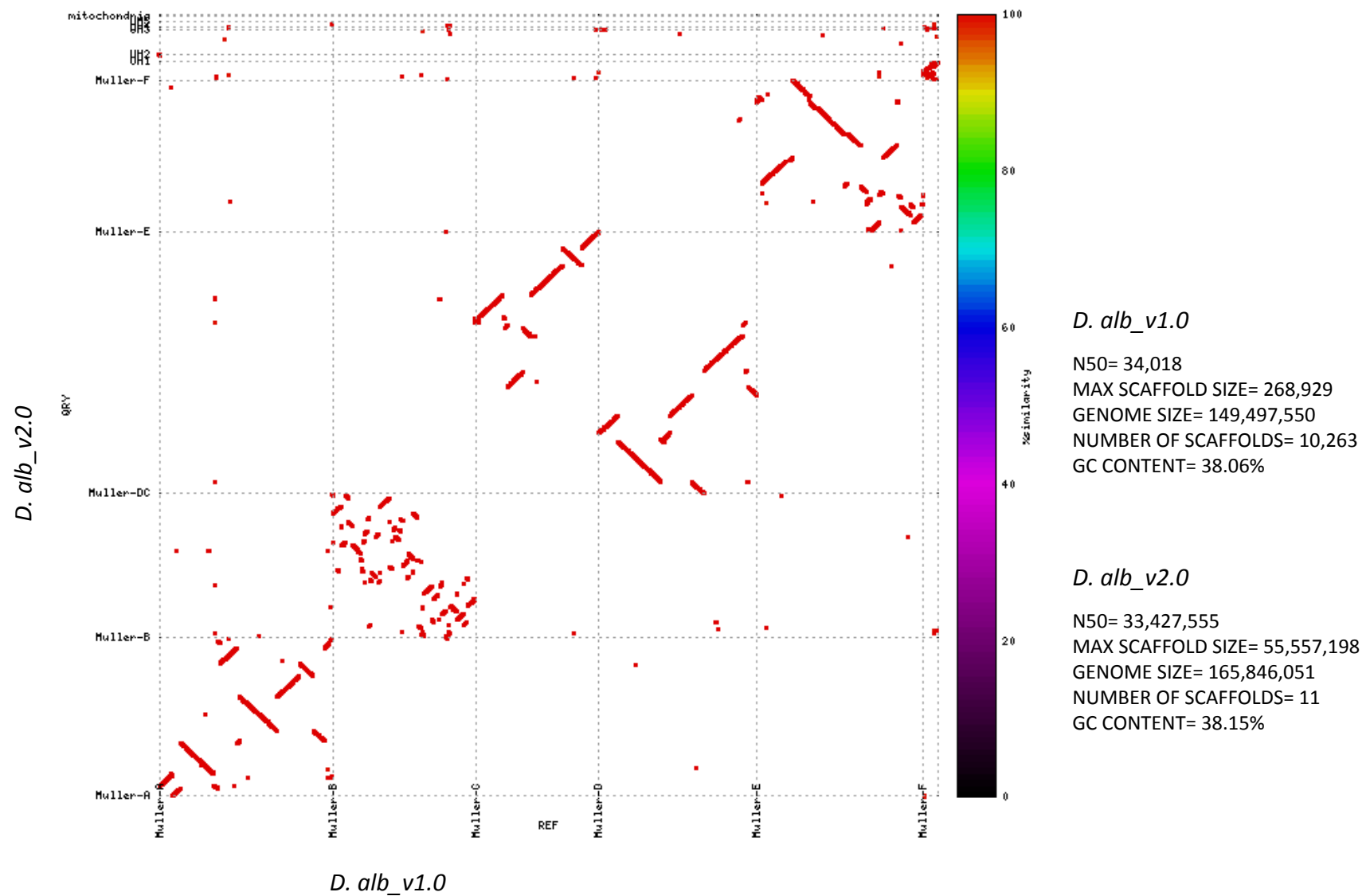


Figure S2. Comparison to previous assembly of *D. albomicans* (*D. alb_v1.0*) to current assembly (*D. alb_v2.0*). *D. alb_v1.0* was based on an Illumina assembly, and scaffolding of contigs using the *D. virilis* genome as a reference.

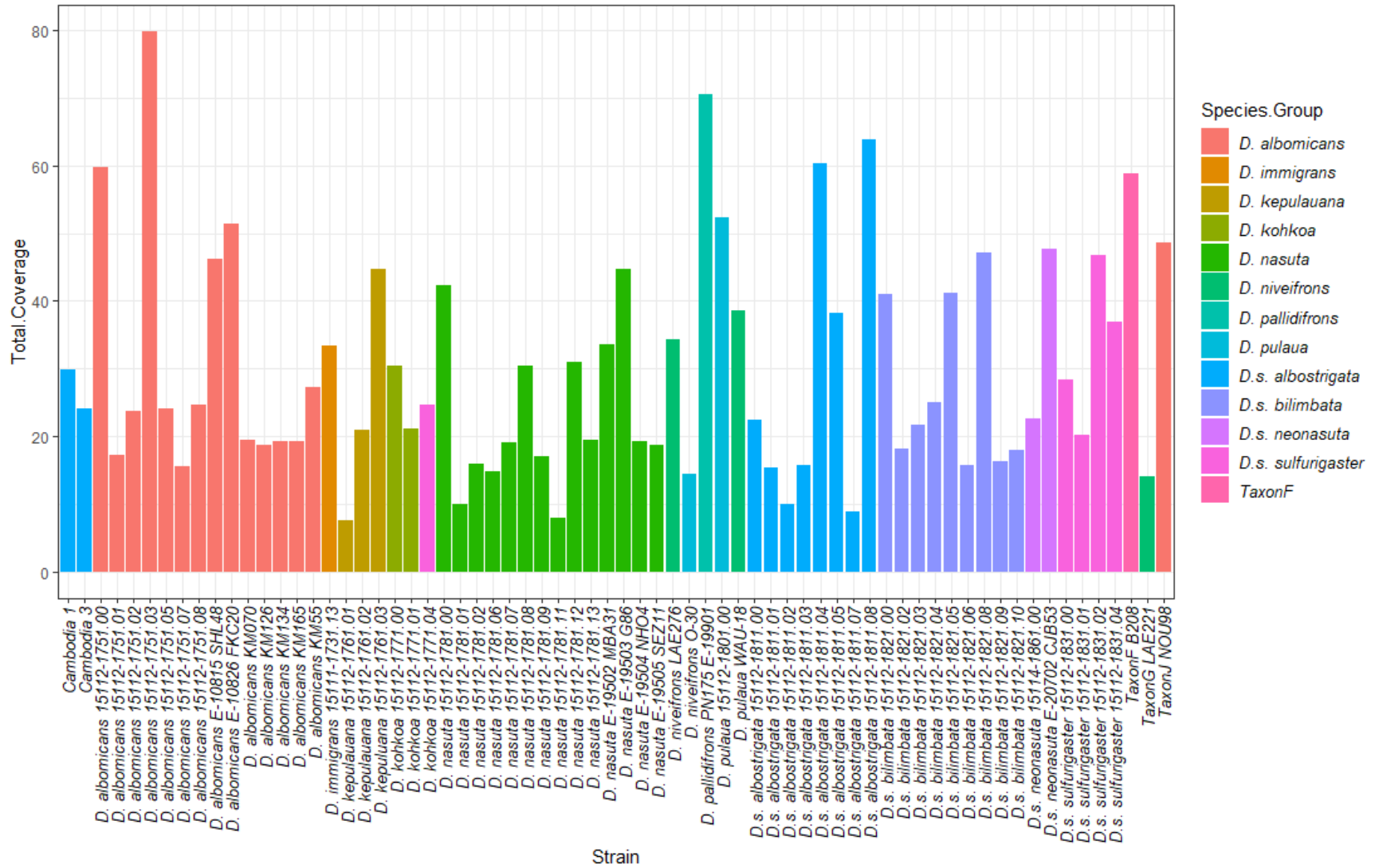


Figure S3. Sequencing read depth for each line investigated. Coverage ranges from 7.5x to 79.7x among strains.

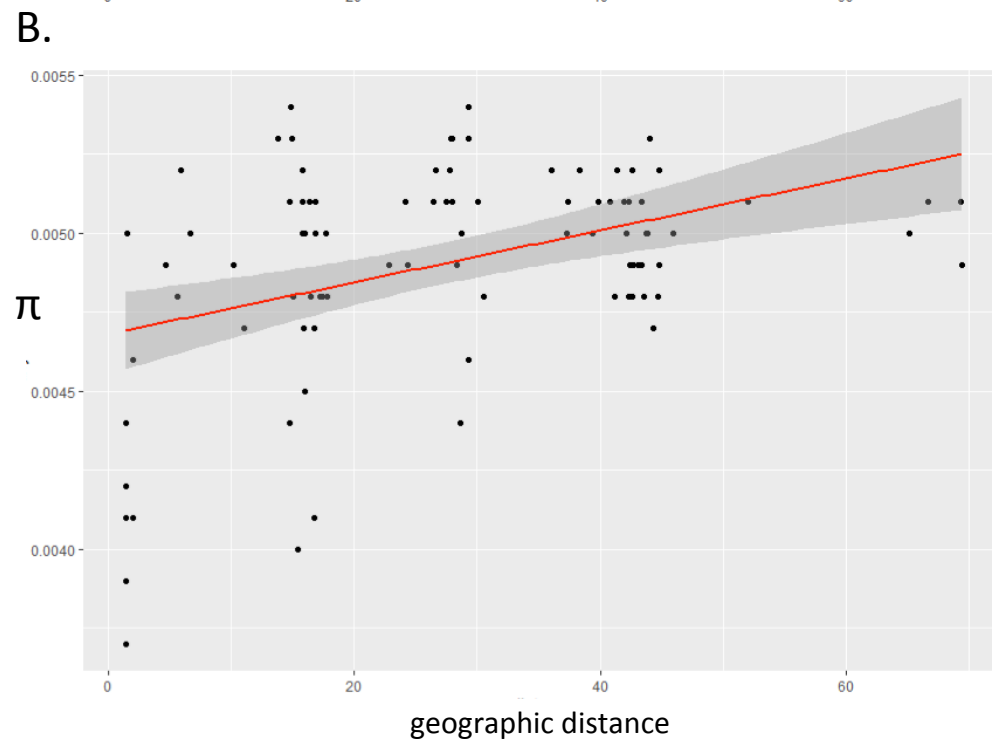
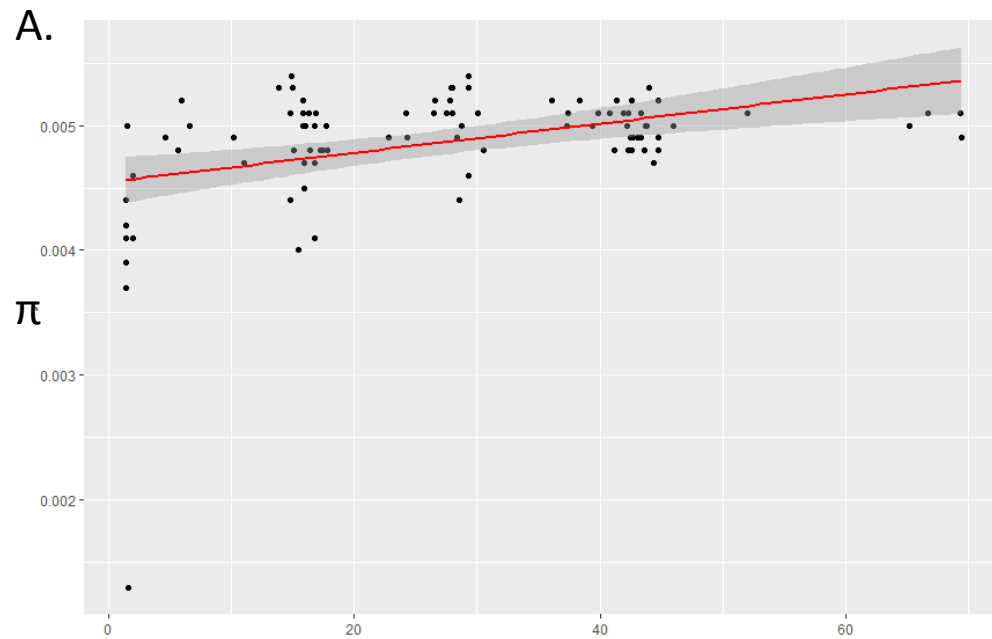


Figure S4. – Mean pairwise diversity versus geographic distance for *D. nasuta*. **A.** Shown is mean π in 50-kb sliding windows versus geographic distance for all pairwise *D. nasuta* strain comparisons. Geographic distance is the Euclidean distance between GPS coordinates. Genetic distance correlates with geographic distance (Adjusted R-squared: 0.1393; p-value: 0.0001587). **B.** Same as A, but outliers removed (Adjusted R-squared: 0.1525; p-value: 8.374e-05).

Proportion Fixed SNPs in Nasuta Subgroup												
<i>niveifrons</i>	0.492	0.602	0.523	0.359	0.631	0.673	0.54	0.653	0.537	0.325	0.803	
<i>taxonF</i>	0.523	0.696	0.576	0.365	0.724	0.812	0.599	0.769	0.568	0.014		0.753
<i>pallidifrons</i>	0.125	0.2	0.162	0.11	0.225	0.268	0.189	0.239	0.122		0.018	0.306
<i>kohkoa</i>	0.201	0.332	0.258	0.162	0.384	0.456	0.309	0.416		0.069	0.458	0.478
<i>pulaua</i>	0.362	0.514	0.419	0.108	0.327	0.323	0.13		0.294	0.175	0.672	0.577
<i>s.sulfurigaster</i>	0.268	0.385	0.318	0.075	0.207	0.176		0.064	0.206	0.129	0.498	0.472
<i>s.bilimbata</i>	0.394	0.551	0.453	0.142	0.397		0.061	0.126	0.262	0.163	0.597	0.53
<i>s.neonasuta</i>	0.33	0.474	0.388	0		0.158	0.105	0.171	0.207	0.126	0.521	0.493
<i>s.albostrigata</i>	0.134	0.21	0.174		0	0.054	0.035	0.054	0.078	0.055	0.243	0.28
<i>kepulauana</i>	0.111	0.234		0.072	0.171	0.216	0.173	0.238	0.137	0.088	0.407	0.406
<i>nasuta</i>	0.135		0.135	0.108	0.264	0.324	0.262	0.366	0.228	0.143	0.588	0.521
<i>albomicans</i>		0.089	0.066	0.063	0.168	0.215	0.171	0.241	0.135	0.085	0.425	0.422
<i>albomicans</i>		<i>nasuta</i>	<i>kepulauana</i>	<i>s.albostrigata</i>	<i>s.neonasuta</i>	<i>s.bilimbata</i>	<i>s.sulfurigaster</i>	<i>pulaua</i>	<i>kohkoa</i>	<i>pallidifrons</i>	<i>taxonF</i>	<i>niveifrons</i>

Proportion shared SNPs in Nasuta Subgroup												
<i>niveifrons</i>	0.004	0.002	0.003	0.005	0.002	0.002	0.002	0.002	0.003	0.006	0	
<i>taxonF</i>	0.001	0.001	0.001	0.001	0	0.001	0.001	0.001	0.001	0.01		0
<i>pallidifrons</i>	0.02	0.009	0.015	0.027	0.01	0.008	0.014	0.008	0.021		0.013	0.009
<i>kohkoa</i>	0.016	0.008	0.012	0.016	0.007	0.005	0.009	0.005		0.04	0.002	0.005
<i>pulaua</i>	0.008	0.004	0.006	0.036	0.026	0.035	0.126		0.015	0.016	0.001	0.003
<i>s.sulfurigaster</i>	0.014	0.007	0.011	0.069	0.041	0.056		0.166	0.024	0.027	0.001	0.005
<i>s.bilimbata</i>	0.008	0.004	0.006	0.025	0.016		0.207	0.107	0.018	0.02	0.001	0.005
<i>s.neonasuta</i>	0.01	0.005	0.008	0.193		0.053	0.076	0.054	0.027	0.029	0.001	0.005
<i>s.albostrigata</i>	0.025	0.011	0.018		0.269	0.063	0.092	0.057	0.037	0.049	0.001	0.009
<i>kepulauana</i>	0.069	0.03		0.057	0.035	0.024	0.032	0.019	0.036	0.04	0.001	0.007
<i>nasuta</i>	0.056		0.054	0.038	0.024	0.017	0.023	0.014	0.024	0.026	0.001	0.004
<i>albomicans</i>		0.079	0.087	0.06	0.041	0.027	0.036	0.021	0.037	0.039	0.002	0.007
<i>albomicans</i>		<i>nasuta</i>	<i>kepulauana</i>	<i>s.albostrigata</i>	<i>s.neonasuta</i>	<i>s.bilimbata</i>	<i>s.sulfurigaster</i>	<i>pulaua</i>	<i>kohkoa</i>	<i>pallidifrons</i>	<i>taxonF</i>	<i>niveifrons</i>

Figure S5. Patterns of genome-wide differentiation in the *nasuta* group. Proportion of fixed (top) and shared (bottom) SNPs in the *nasuta* group between the X chromosomes (red) and autosomes (blue). Darker shading indicates larger values. Flies are down-sampled to two individuals in *D. nasuta* and *D. albomicans*.

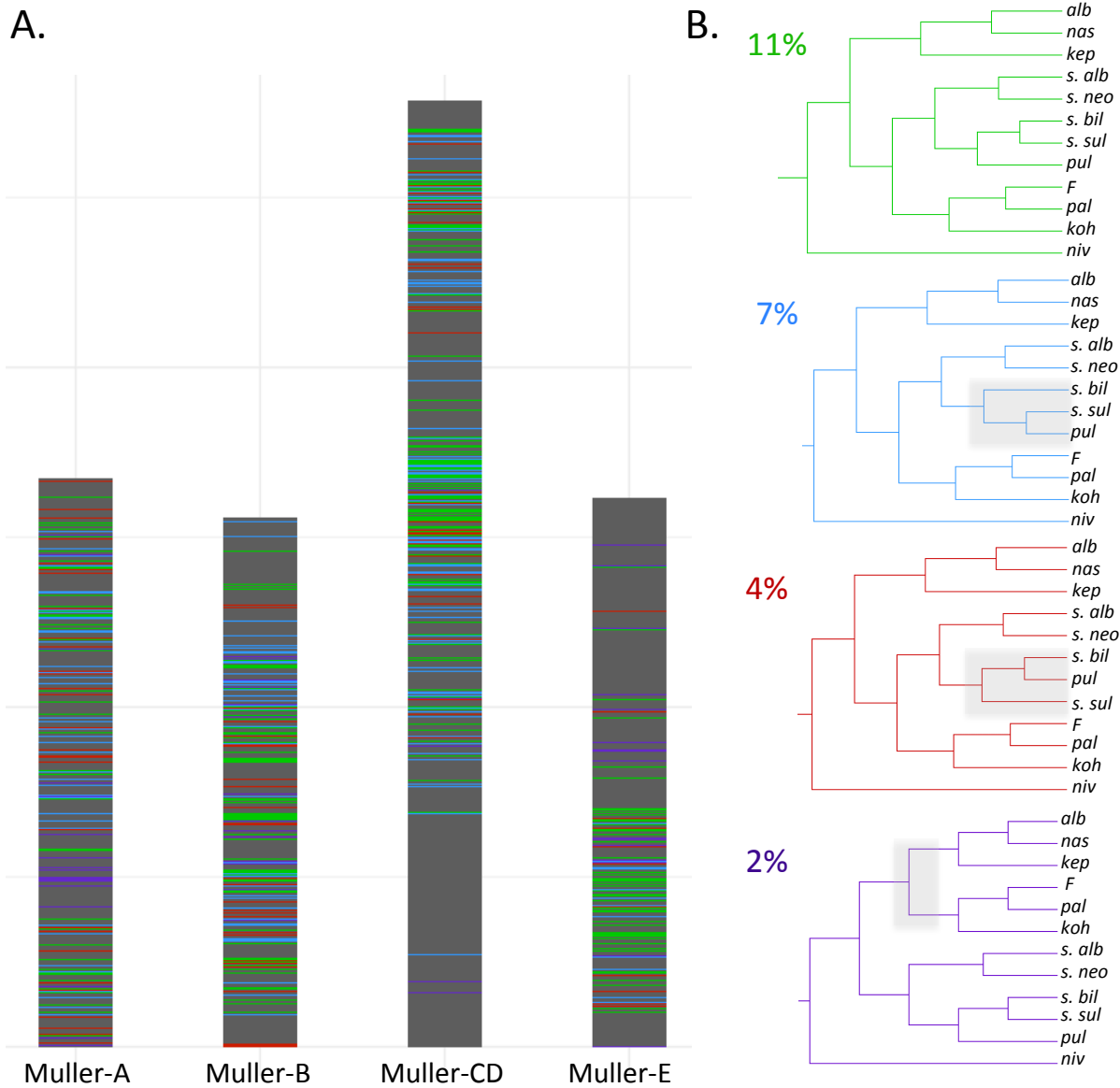


Figure S7. – Local evolutionary history in the *D. nasuta* group varies across the genome. A. Tree topology across the genome. For each 50-kb window, we color-code the topology recovered from that region (colors correspond to topologies in B). Note that while tree 1 (green) dominates on the autosomes, tree 2 (blue) dominates on the X. Coordinates are in terms of *D. albomicans* genome. Grey regions show alternative topologies. **B.** Common topologies. The four most common trees are shown. The value in the top left corner is the percentage of all 50-kb windows that recovers that topology.

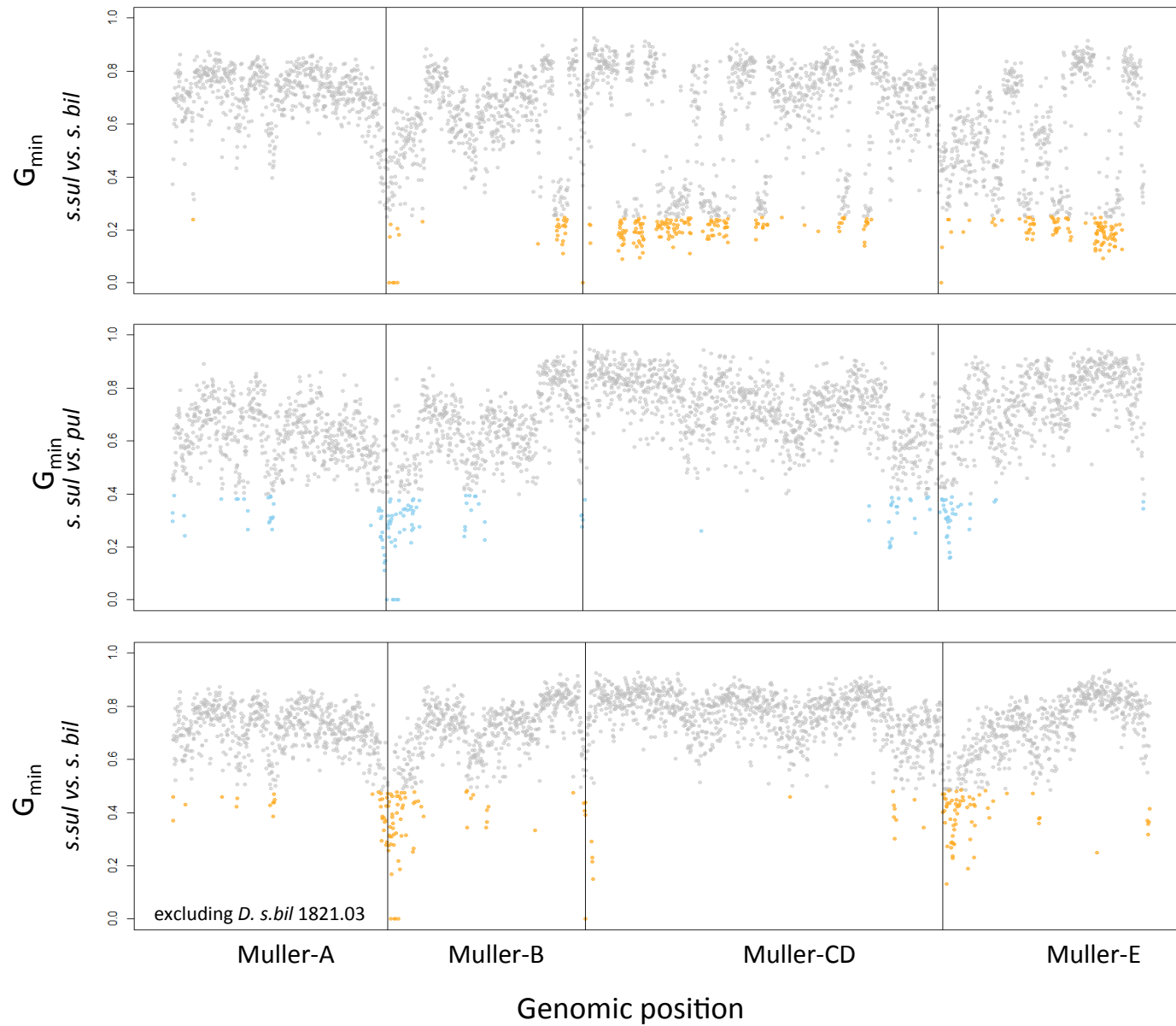


Figure S8. – G_{\min} across the genome. Shown is the G_{\min} statistics across the genome (in 50-kb windows) between *D. s. bilimbata* and *D. s. sulfurigaster* (top); *D. s. sulfurigaster* and *D. pulaua* (middle) and between *D. s. bilimbata* (but excluding strain *D. s. bil* 1821.03) and *D. s. sulfurigaster* (bottom). Significant windows are color-coded.