

Table S1. Collection and voucher information of samples from putative species in the *Melanoplus scudderi* complex

Samples*	Species	Collection records	SRA accession #
arkansas1	<i>M. arkansas</i>	Mt. Magazine, Arkansas (35.177 N, 93.595 W)	SAMN12867852
arkansas2			SAMN12867853
arkansas3			SAMN12867854
arkansas4			SAMN12867855
arkansas5			SAMN12867856
chattahoochee1	<i>M. chattahoochee</i>	Black Creek, Mississippi (32.572 N, 84.408 W)	SAMN12867857
chattahoochee2			SAMN12867858
chattahoochee3			SAMN12867859
coreyi1	<i>M. coreyi</i>	Ft. Bragg, North Carolina (35.021 N, 79.549 W)	SAMN12867860
coreyi2			SAMN12867861
coreyi3			SAMN12867862
coreyi4			SAMN12867863
coreyi5			SAMN12867864
davisi1	<i>M. davisi</i>	Nokuse Plantation, Florida (30.543 N, 85.930 W)	SAMN12867865
davisi2			SAMN12867866
davisi3			SAMN12867867
davisi4			SAMN12867868
davisi5			SAMN12867869
folkerti1	<i>M. folkerti</i>	Splinter Hill Bog, Alabama (31.025 N, 87.685 W)	SAMN12867870
folkerti2			SAMN12867871
folkerti3			SAMN12867872
folkerti4			SAMN12867873
folkerti5			SAMN12867874
irwini1	<i>M. irwini</i>	Angelina National Forest, Texas (31.042 N, 94.188 W)	SAMN12867875
irwini2			SAMN12867876
irwini3			SAMN12867877
irwini4			SAMN12867878
irwini5			SAMN12867879
latus1	<i>M. ouachita</i>	Petit Jean State Park, Arkansas (35.115 N, 92.940 W)	SAMN12867880
latus2			SAMN12867881
latus3			SAMN12867882
latus4			SAMN12867883
latus5			SAMN12867884
mississippius1	<i>M. mississippi</i>	Village Creek State Park, Arkansas (35.156 N, 90.719 W)	SAMN12867885
mississippius2			SAMN12867886
mississippius3			SAMN12867887
mississippius4			SAMN12867888
mississippius5			SAMN12867889
muscogee1	<i>M. muscogee</i>	Black Creek, Mississippi (32.585 N, 84.506 W)	SAMN12867890
muscogee2			SAMN12867891
muscogee3			SAMN12867892
muscogee4			SAMN12867893
muscogee5			SAMN12867894

ottei1	<i>M. ottei</i>	Longhorn Caverns State Park, Texas (30.688 N, 98.352 W)	SAMN12867895
ottei2			SAMN12867896
ottei3			SAMN12867897
ottei4			SAMN12867898
ottei5			SAMN12867899
ouchita1	<i>M. baronei</i>	Perry Co., Arkansas (34.857 N, 92.767 W)	SAMN12867900
ouchita2			SAMN12867901
ouchita3			SAMN12867902
ouchita4			SAMN12867903
ouchita5			SAMN12867904
ozarkus1	<i>M. ozarkensis</i>	Baker Prairie, Arkansas (36.241 N, 93.134 W)	SAMN12867905
ozarkus2			SAMN12867906
ozarkus3			SAMN12867907
ozarkus4			SAMN12867908
ozarkus5			SAMN12867909
quercicola1	<i>M. quercicola</i>	Apalachicola BRP, Florida (30.494 N, 84.972 W)	SAMN12867910
quercicola2			SAMN12867911
quercicola3			SAMN12867912
quercicola4			SAMN12867913
quercicola5			SAMN12867914
relictus1	<i>M. relictus</i>	Apalachicola BRP, Florida (30.494 N, 84.972 W)	SAMN12867915
relictus2			SAMN12867916
relictus3			SAMN12867917
rex1	<i>M. optimus</i>	Ellis Co., Texas (32.314 N, 96.654 W)	SAMN12867918
rex2			SAMN12867919
rex3			SAMN12867920
rex4			SAMN12867921
rex5			SAMN12867922
scudderi1	<i>M. scudderi</i>	Osborn Prairie, Mississippi (33.506 N, 88.734 W)	SAMN12867923
scudderi2			SAMN12867924
scudderi3			SAMN12867925
scudderi4			SAMN12867926
scudderi5			SAMN12867927
seltzeri1	<i>M. seltzeri</i>	Middle fork Barrens, Arkansas (34.638 N, 92.842 W)	SAMN12867928
seltzeri2			SAMN12867929
seltzeri3			SAMN12867930
seltzeri4			SAMN12867931
seltzeri5			SAMN12867932
taurus1	<i>M. taurus</i>	Huntsville, Alabama (30.713 N, 95.525 W)	SAMN12867933
taurus2			SAMN12867934
taurus3			SAMN12867935
taurus4			SAMN12867936
taurus5			SAMN12867937
texarkana1	<i>M. texarkana</i>	Miller Co. Sandhills, Arkansas (33.190 N, 94.028 W)	SAMN12867938
texarkana2			SAMN12867939
texarkana3			SAMN12867940

texarkana4			SAMN12867941
texarkana5			SAMN12867942
texensis1	<i>M. texensis</i>	Lick Creek Park, Texas	SAMN12867943
texensis2		(30.564 N, 96.212 W)	SAMN12867944
texensis3			SAMN12867945
texensis4			SAMN12867946
texensis5			SAMN12867947

*All vouchered specimens, pinned and preserved in alcohol, are stored in the Mississippi Entomological Museum.

Table S2. Summary of pyRAD filtering results

Samples	Raw reads	Good reads	# clusters	m.depth	Heteroz.	# loci
arkansas1	2180978	1962028	65530	19.848	0.01032286	32919
arkansas2	1877446	1688624	60301	18.597	0.01063264	31418
arkansas3	1842931	1640520	58897	18.762	0.01059089	30990
arkansas4	7364748	6526277	123489	34.975	0.01086957	47742
arkansas5	2026015	1814602	67464	17.325	0.01063807	32965
chattahoochee1	1957052	1772926	62682	18.772	0.00946262	25732
chattahoochee2	2550364	2264726	65011	23.832	0.00940511	27757
chattahoochee3	2020174	1760771	61418	19.091	0.00952189	26523
coreyi1	2361932	2109736	64372	22.13	0.0101752	35486
coreyi2	2077141	1850621	64024	19.34	0.01185345	34365
coreyi3	2302691	2051201	67921	20.407	0.01013203	36345
coreyi4	3224785	2836928	82199	22.534	0.01163693	41032
coreyi5	2943182	2624003	78542	22.47	0.01003385	39549
davisi1	1300768	1187068	47128	16.084	0.00967823	19583
davisi2	1657871	1487246	52838	18.153	0.00994478	21683
davisi3	2002177	1752016	59861	19.033	0.00996965	24790
davisi4	2775118	2498576	72255	22.863	0.00975161	27481
davisi5	2729388	2402518	70948	21.788	0.00987738	27405
folkertsi1	1479141	1341556	51970	17.448	0.00960005	28743
folkertsi2	1910050	1714726	59398	19.509	0.00960447	32338
folkertsi3	2857169	2545786	70732	24.83	0.00972525	37456
folkertsi4	1630272	1471000	57709	16.717	0.00980329	30240
folkertsi5	2243303	1969331	64993	20.469	0.00947515	35299
irwini1	2543163	2254367	72096	21.167	0.0109618	35755
irwini2	1808785	1627330	60068	18.421	0.01111133	30815
irwini3	2948475	2633008	80563	21.977	0.01131966	38576
irwini4	1803520	1619324	63079	17.177	0.01097237	32072
irwini5	2253662	1986942	69591	18.994	0.01150718	34774
latus1	2215212	1986058	68777	19.122	0.01096115	34181
latus2	2236364	2011138	66228	20.592	0.01066336	34319
latus3	2415722	2187416	68384	21.552	0.0105649	35414
latus4	2335629	2031888	69347	19.288	0.0111562	35444
latus5	2193983	1923655	67787	18.704	0.01089119	34920

mississippius1	2543003	2300693	68949	22.927	0.00959481	37241
mississippius2	2092871	1890851	61440	21.015	0.00971108	33794
mississippius3	1597186	1404071	55245	16.535	0.01060188	30379
mississippius4	1715329	1520097	59417	16.997	0.01026571	32128
mississippius5	220186	196584	9977	9.624	0.01337138	4909
muscogee1	2192724	2002938	64958	21.063	0.01000913	35219
muscogee2	1576590	1435759	54926	17.56	0.01008228	30049
muscogee3	2304120	2058815	66546	20.525	0.01026988	35748
muscogee4	2365268	2105124	71631	19.104	0.01060951	36884
muscogee5	3173061	2803950	80957	22.563	0.01085604	40353
ottei1	1302318	1161278	49206	15.604	0.01057412	24656
ottei2	1957901	1764885	60277	19.95	0.00942892	29743
ottei3	286745	246104	12454	11.089	0.01290439	6125
ottei4	1044263	921499	43157	13.787	0.01019877	21940
ottei5	1288829	1137138	50627	14.438	0.01052701	25420
ouchita1	1466078	1315115	53115	16.445	0.01061418	27935
ouchita2	2061647	1845353	63165	19.681	0.01048919	33105
ouchita3	1661195	1485166	56784	17.773	0.01032217	30256
ouchita4	1823646	1594929	62170	16.679	0.0107347	32086
ouchita5	2116546	1846489	67215	18.075	0.01093256	34281
ozarkus1	2224284	2011651	66258	20.647	0.01004263	34870
ozarkus2	2245881	2016608	67609	19.585	0.01067987	34475
ozarkus3	1020363	912423	42103	13.53	0.01111062	22217
ozarkus4	1846357	1614355	62422	16.454	0.01107465	31753
ozarkus5	1937256	1718813	65189	16.93	0.01067959	32821
quercicola1	2107645	1914365	58870	21.276	0.00887547	24405
quercicola2	1100706	985444	40499	15.404	0.00901481	17819
quercicola3	1858039	1643297	56976	18.1	0.00957934	23847
quercicola4	2353103	2092342	64513	20.459	0.00930614	25447
quercicola5	2194855	1950705	63825	19.232	0.00958207	25261
relictus1	1565465	1400534	50966	18.369	0.00948144	28573
relictus2	1764955	1597295	58210	17.965	0.00943834	30318
relictus3	1722300	1547565	59668	16.9	0.00981675	31074
rex1	1248425	1113752	48543	14.981	0.01106487	25046
rex2	1950409	1751051	62868	18.465	0.0111062	31257
rex3	1349429	1184163	51799	14.695	0.01156243	26808
rex4	1831963	1621736	62695	17.305	0.01084114	31572
rex5	2521206	2249238	75444	19.863	0.01128068	36271
scudderi1	2231244	1977687	65126	20.297	0.01004768	35319
scudderi2	2513746	2254486	69334	21.954	0.01019156	36570
scudderi3	668278	608145	30894	12.318	0.01061745	16928
scudderi4	2061351	1811590	65029	18.434	0.01041667	34744
scudderi5	2078733	1829348	65398	18.285	0.01052028	35050
seltzeri1	1712559	1524477	58309	17.465	0.0106991	30278
seltzeri2	429889	387499	20768	10.969	0.01177747	10586
seltzeri3	1898101	1667946	62806	17.82	0.01085272	32590

seltzeri4	4134081	3662561	93213	26.274	0.01112821	42845
seltzeri5	1527343	1347213	55444	15.984	0.01128387	29366
taurus1	1971799	1779670	64657	18.339	0.01128565	30962
taurus2	521158	470855	25033	11.614	0.01195074	12340
taurus3	413505	373473	20564	10.986	0.01179519	10273
taurus4	1531407	1374393	58330	15.272	0.01143929	28041
taurus5	404521	357616	19098	10.637	0.01196721	9509
texarkana1	1797431	1615835	60107	18.101	0.01050776	31123
texarkana2	2826868	2506566	72160	23.561	0.01078029	36454
texarkana3	2738385	2415097	76670	20.741	0.01084312	37584
texarkana4	684113	613382	31802	11.735	0.01159315	16634
texarkana5	1999145	1785810	64788	18.266	0.01090344	33132
texensis1	177654	160880	8522	9.479	0.01264232	4018
texensis2	2441638	2205913	71823	20.401	0.01114934	33450
texensis3	1452206	1277789	54076	15.541	0.01147751	27423
texensis4	1511822	1311950	55110	15.342	0.01165301	27547
texensis5	2092731	1862379	67740	18.497	0.01122174	32823

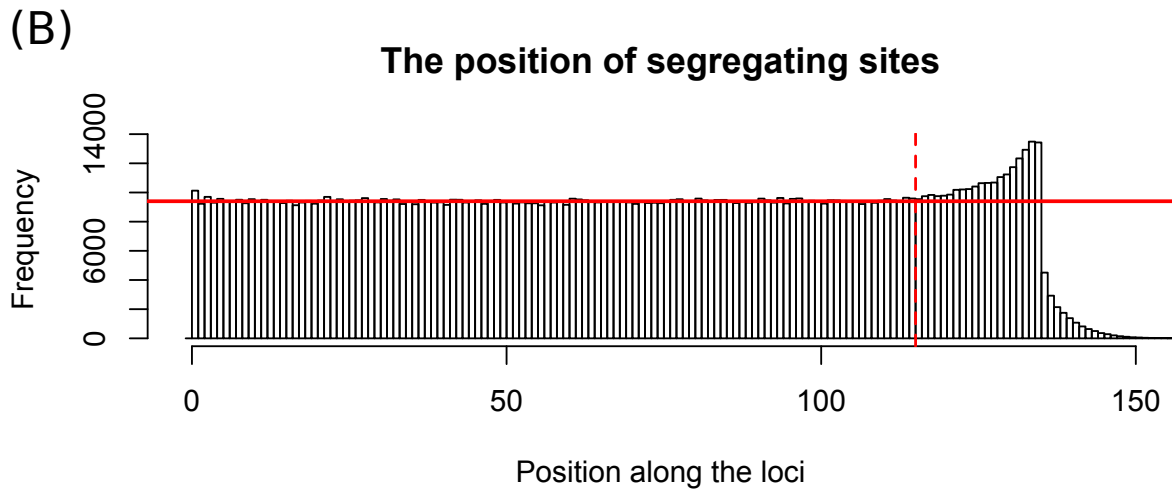
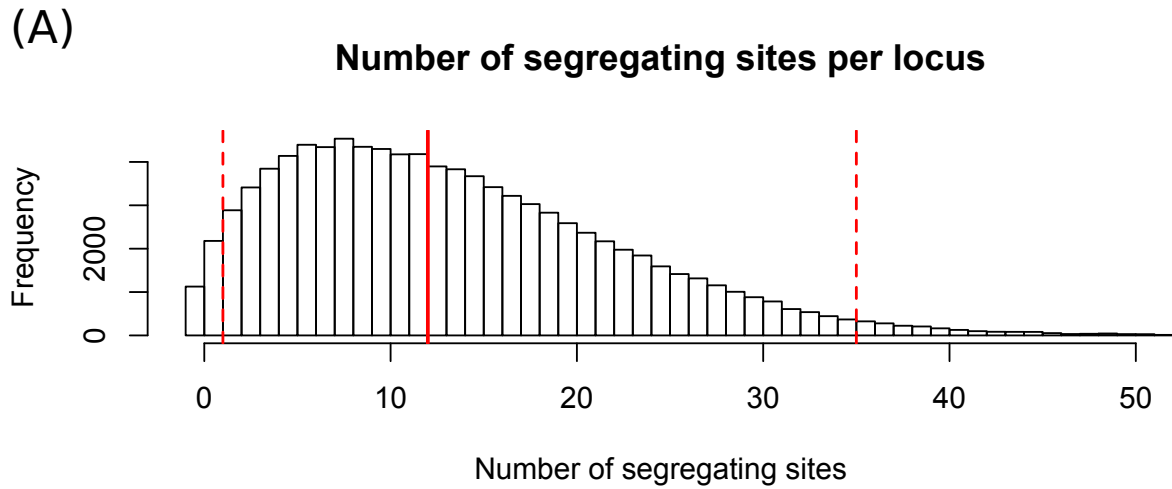


Fig. S1. The (A) distributions of site variation and (B) the position of the variable site of the retained loci after processing. The solid red line in (A) identifies the mean value and the dash lines the 95% range. The horizontal solid red line in (B) marks the average accumulated frequency of variable sites retained across all loci; the vertical dash red line marks the boundary of retained bases (i.e., < position 120).

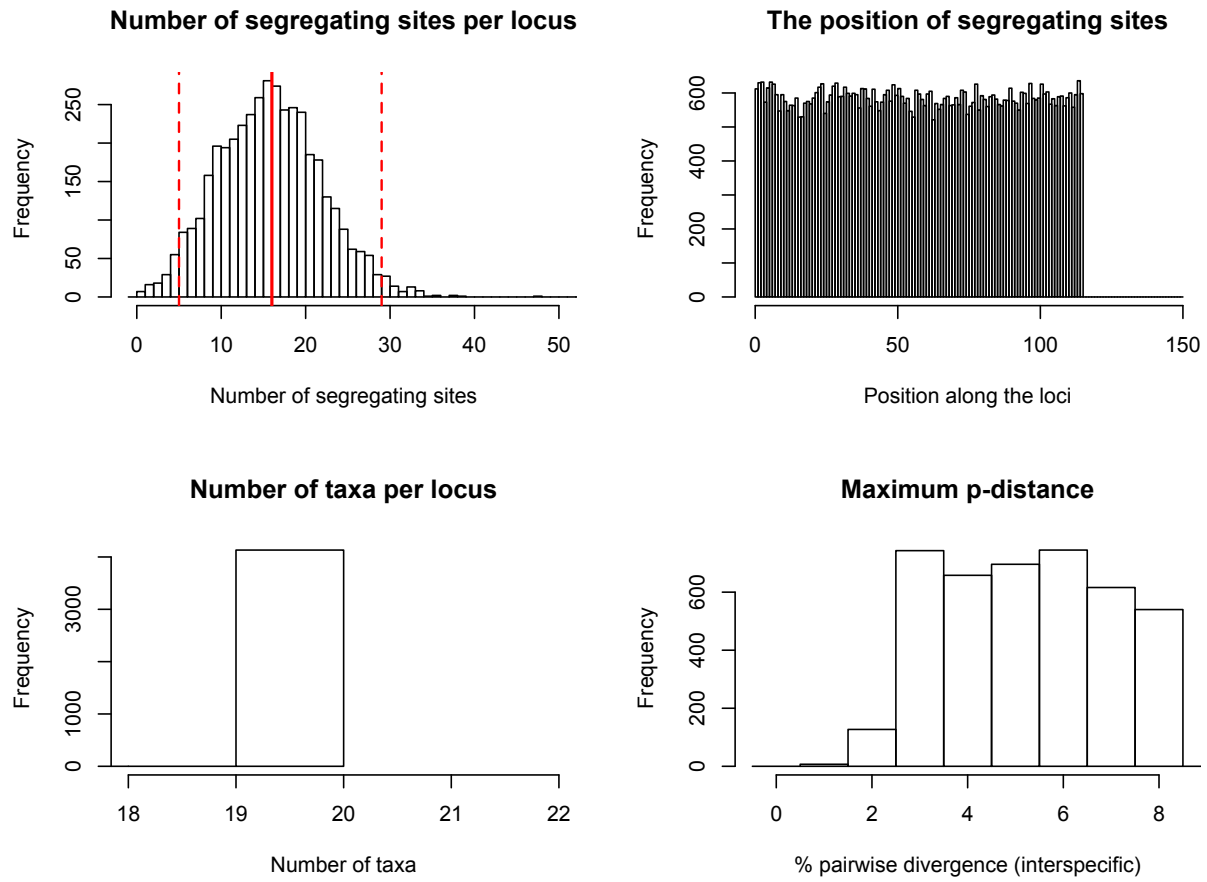


Fig. S2. Summaries of the genetic data of retained sequences after filtering the ddRADseq data.

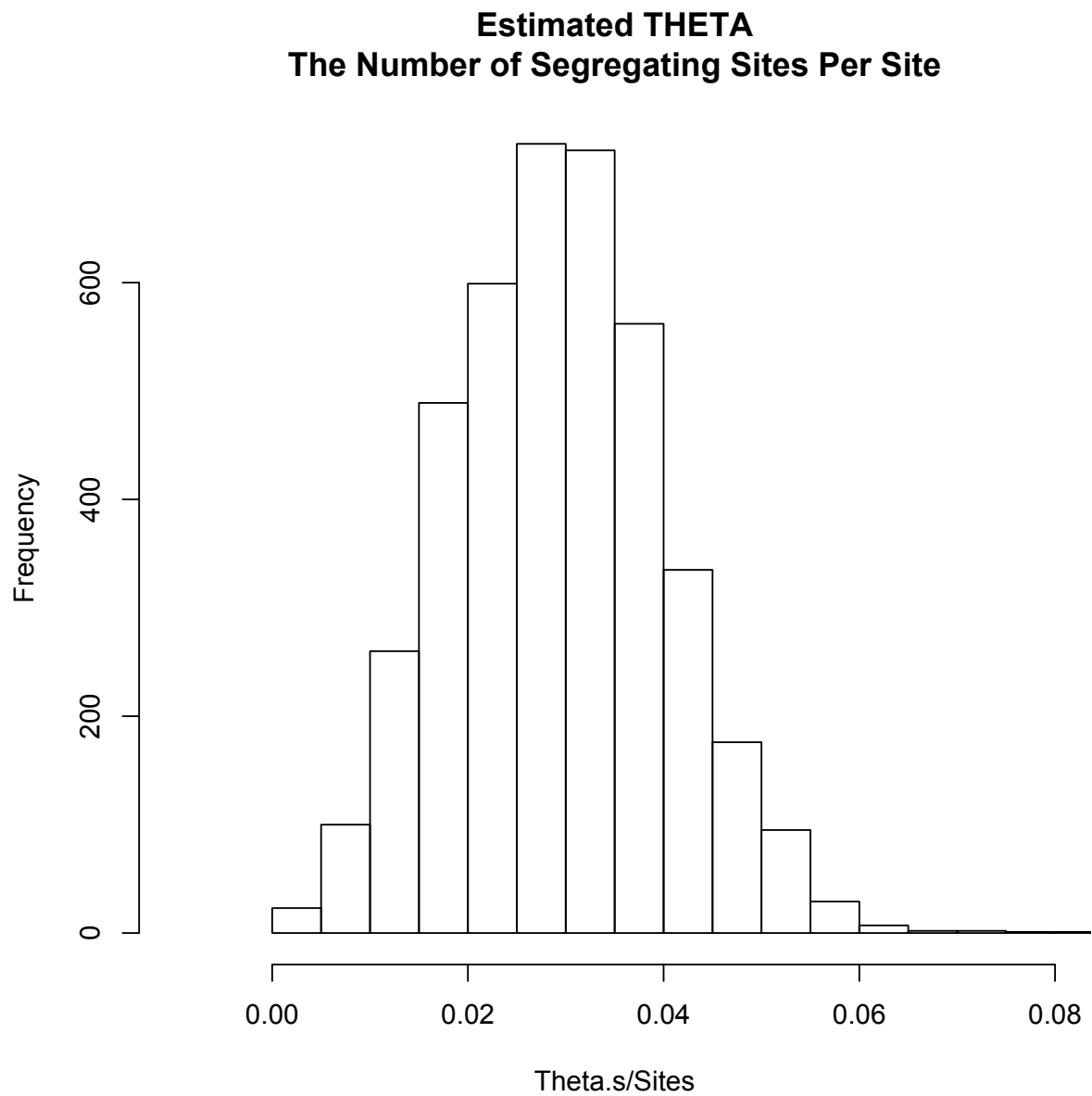


Fig. S3. The distribution of estimated theta values from the 4,132 retained variable loci used in phylogenetic and species delimitation analyses.

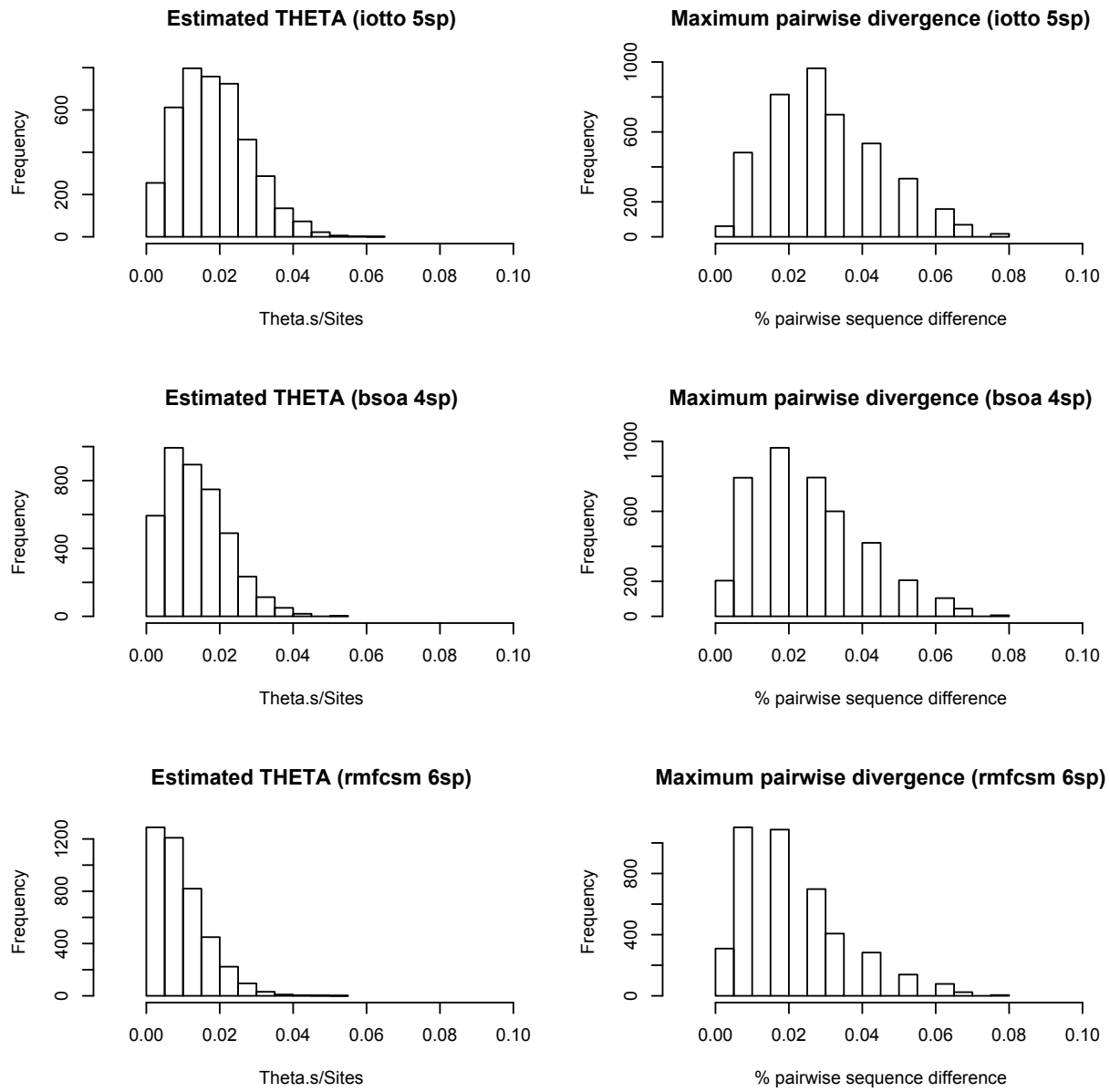


Fig. S4. The distributions of estimated theta values and maximum pairwise genetic differences from each of the three groups of lineages (see Fig. 1) for each of the separate species delimitation analyses, and served to inform the priors on parameters in the analyses.

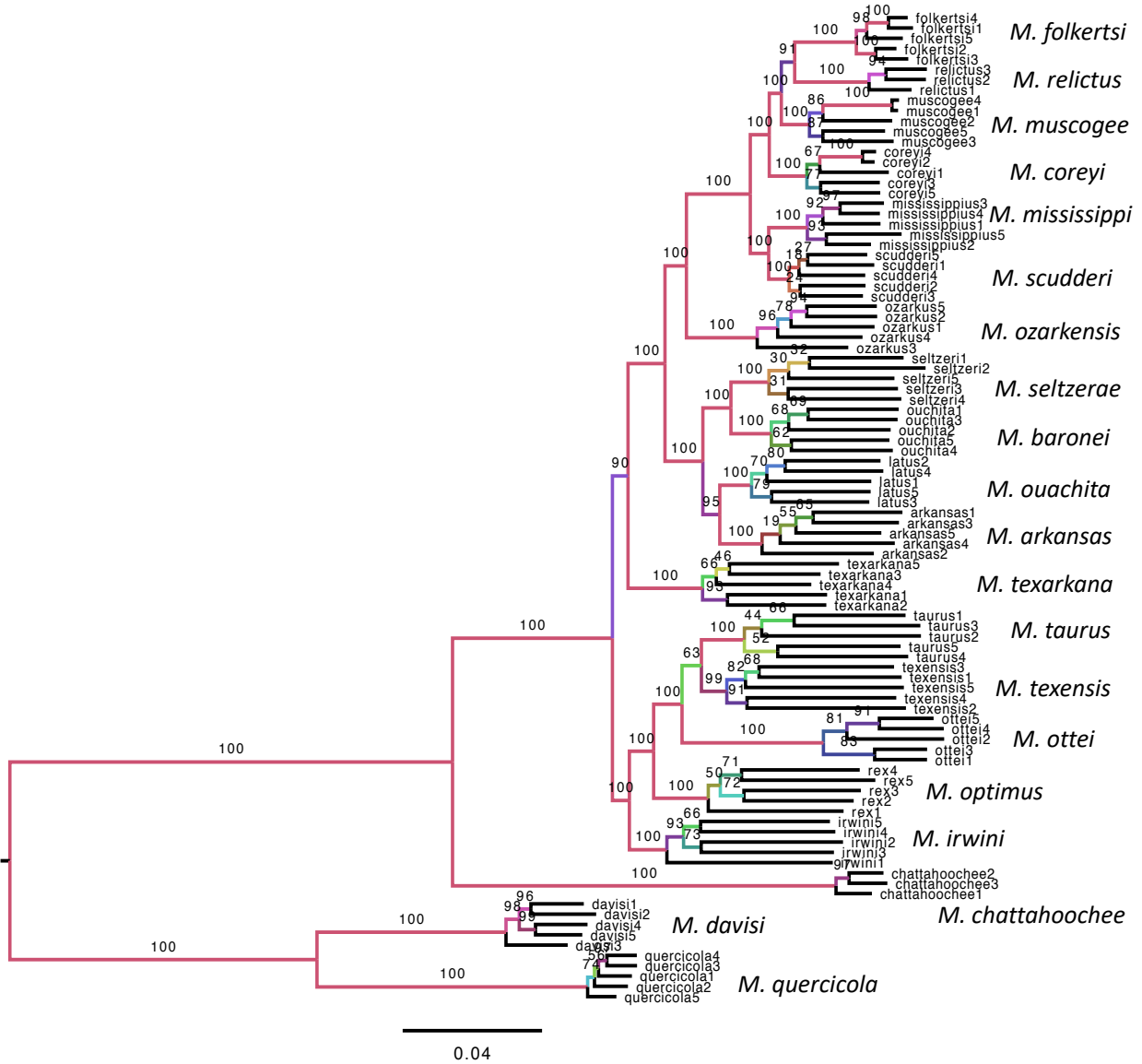


Fig. S5. A maximum likelihood phylogenetic estimate of the 96 sampled individuals based on a concatenated data set of SNPs; bootstrap support values are given on the branches. Species names were provided next to the sample names.

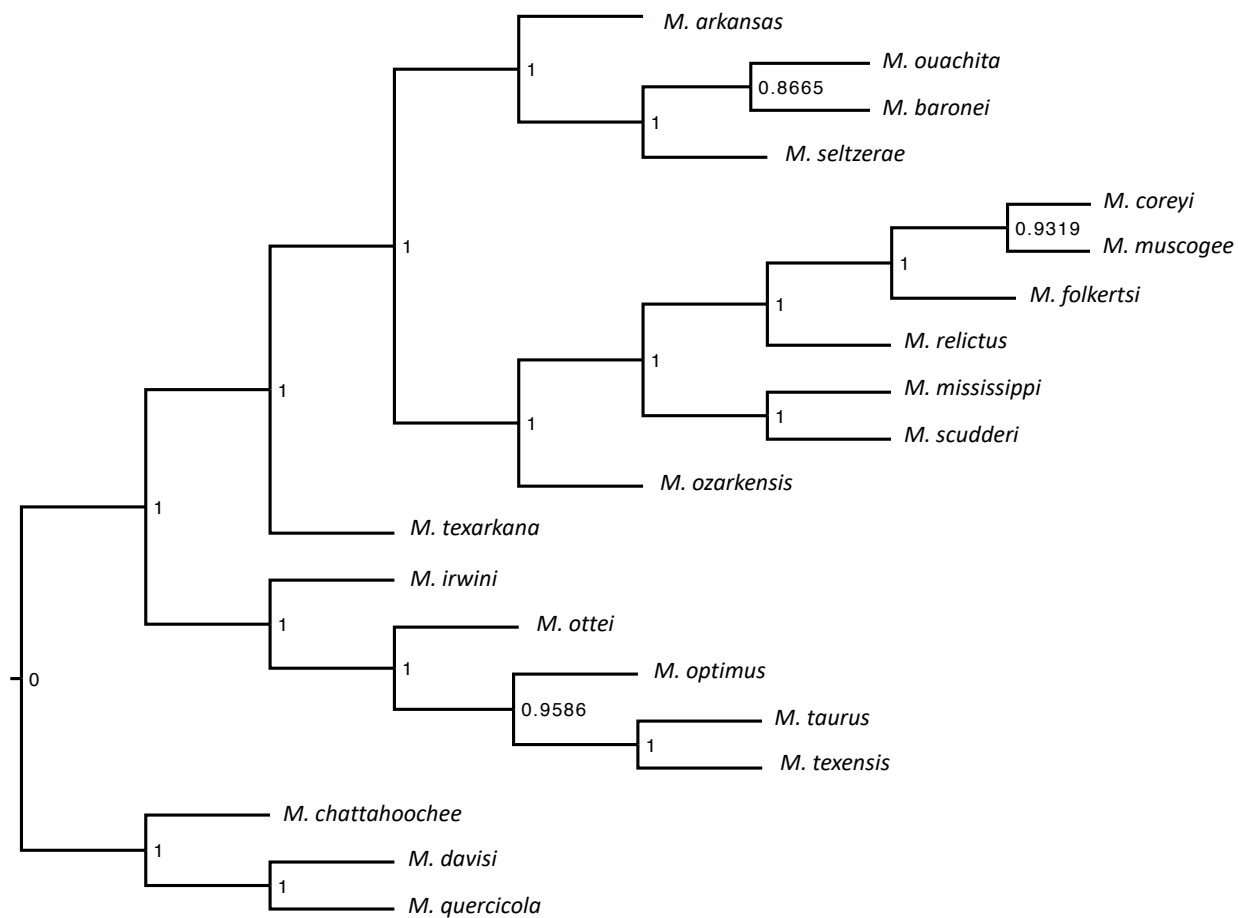


Fig. S6. The best species tree topology from bpp 4 analyses. Number on nodes denote posterior probabilities.

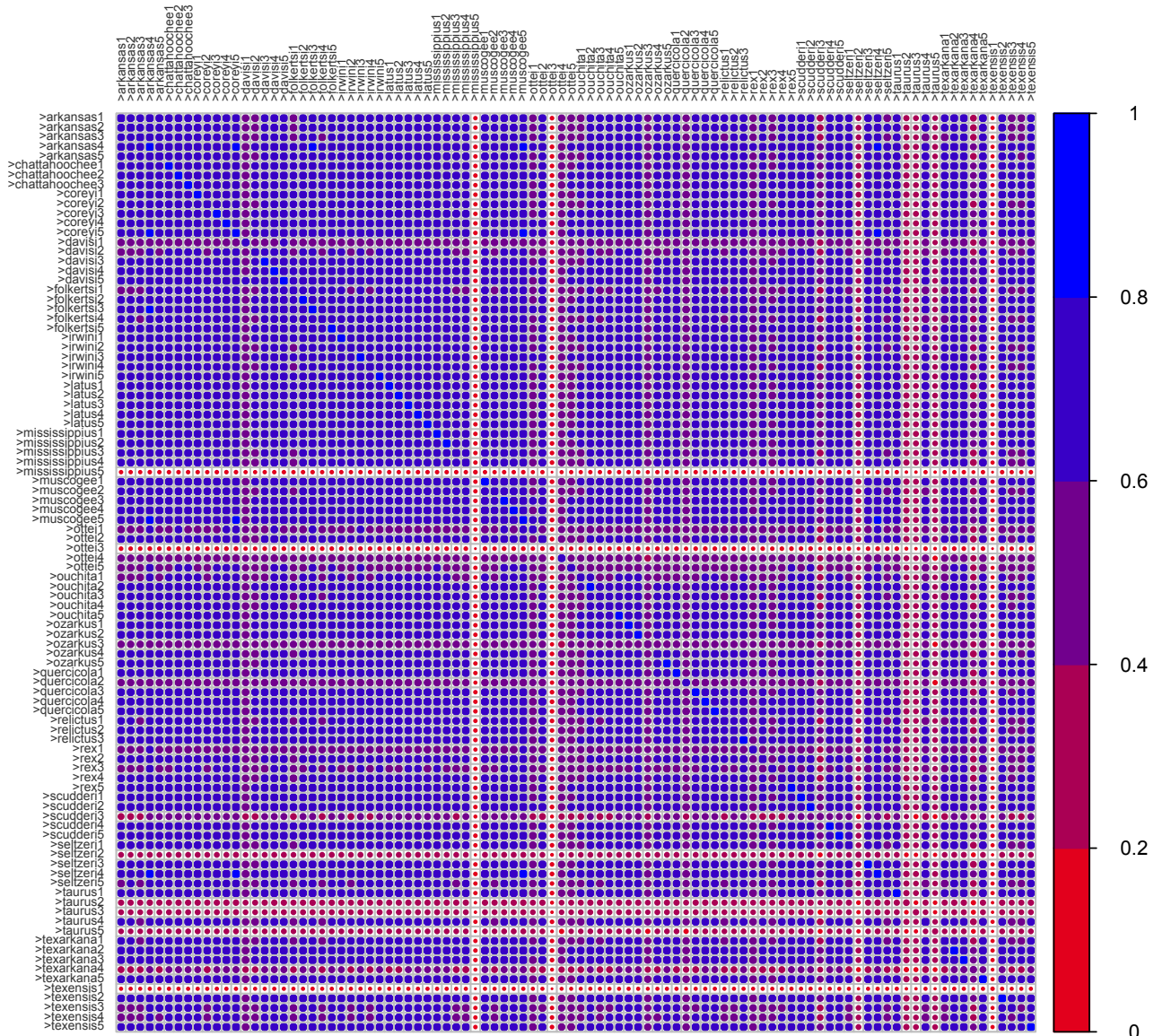


Fig. S7. A pairwise estimation of shared loci between samples from a 4132 loci data set. Colder color represents higher percentage of shared loci between pairs of samples.

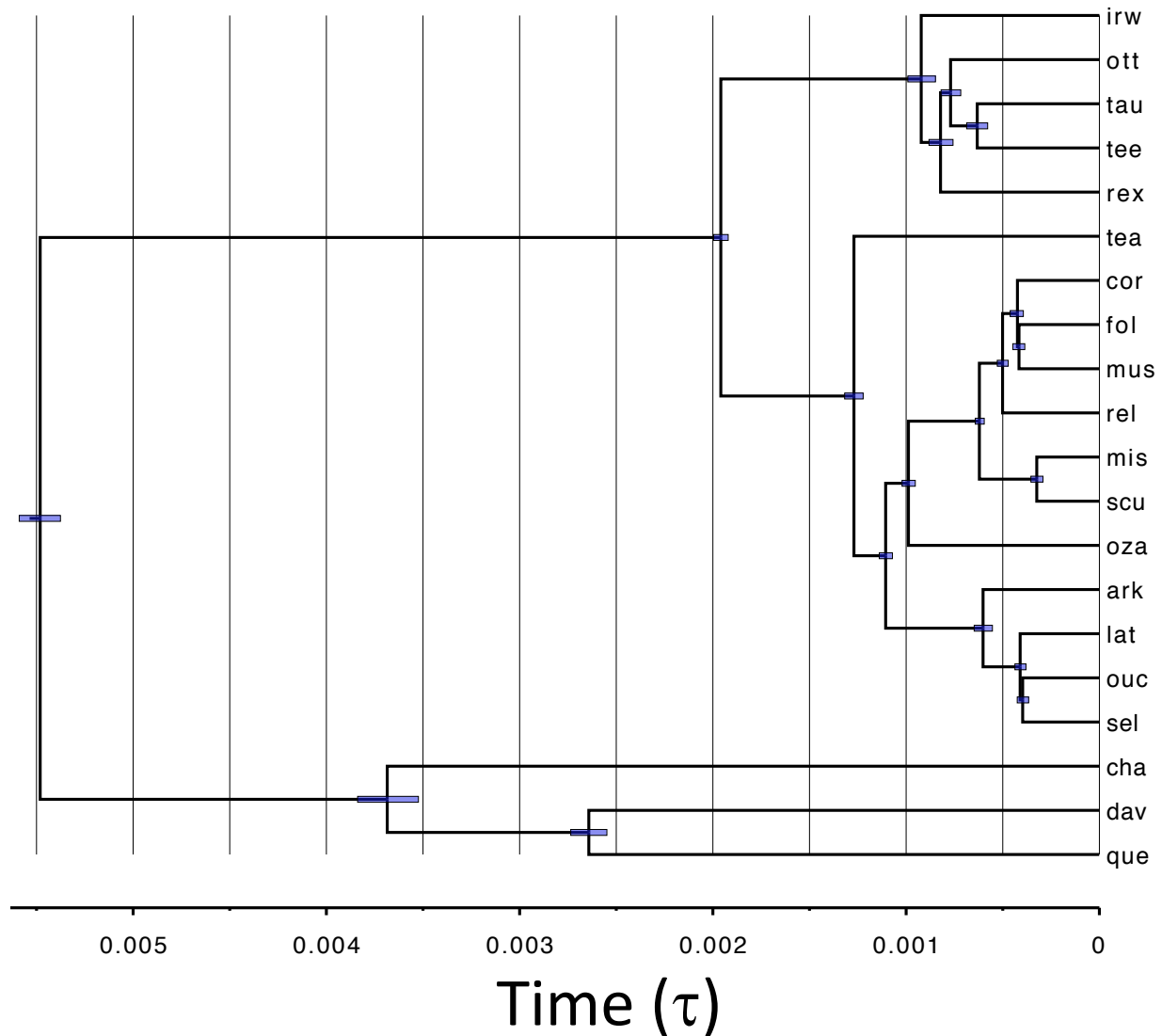


Fig. S8. The estimated species tree and divergence times using a total of 4,132 variable loci that do not have any missing taxon in the individual locus with a combination of SVDQuartets and bpp4 analyses. Bars on nodes indicate the posterior distribution of the estimated divergence times.

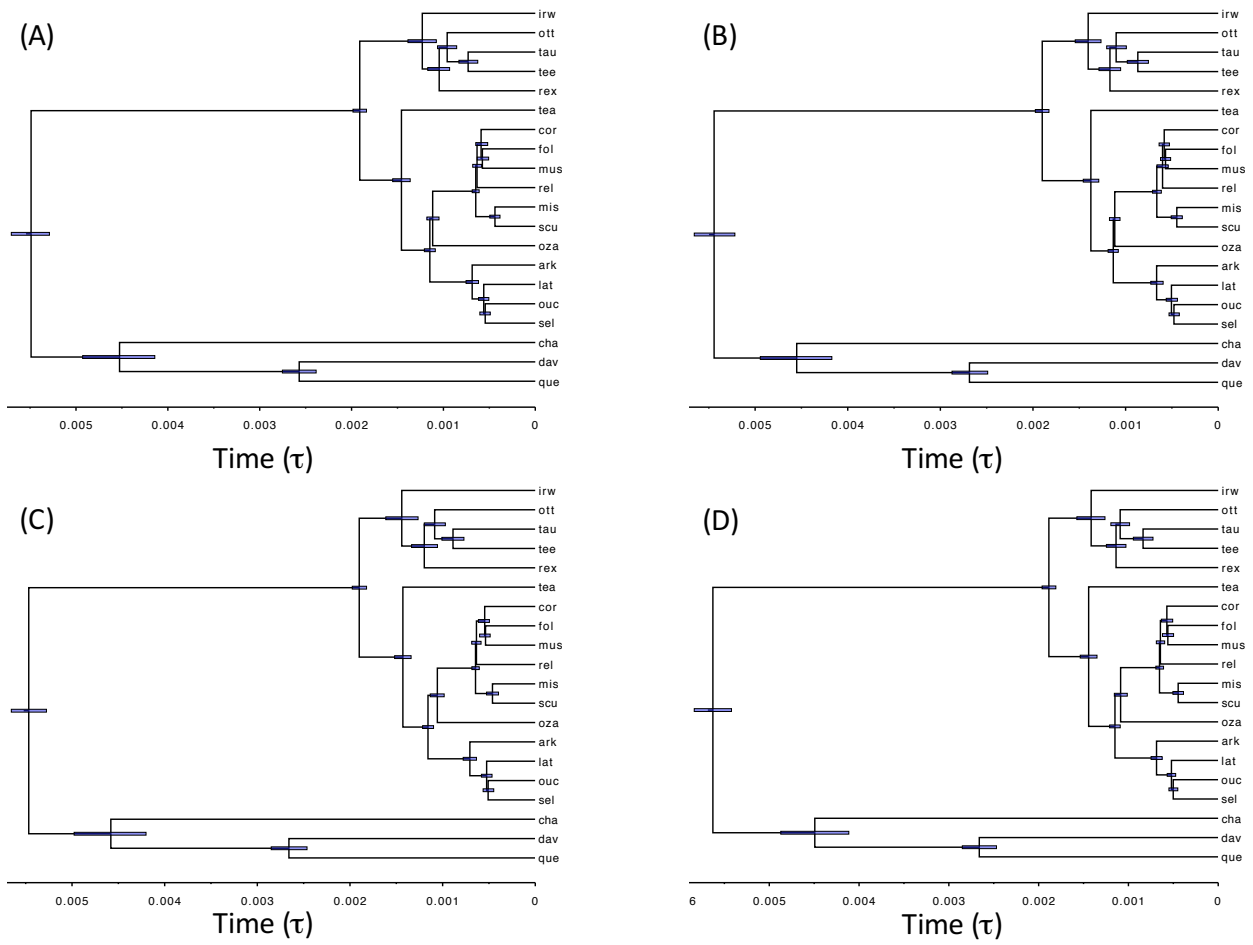


Fig. S9. The estimated species tree and divergence times using four different sets of 1000 variable loci. Bars on nodes indicate the 95% probability of the estimated divergence times.



Fig. S10. Outlines of the lateral view of the male genitalia shapes.

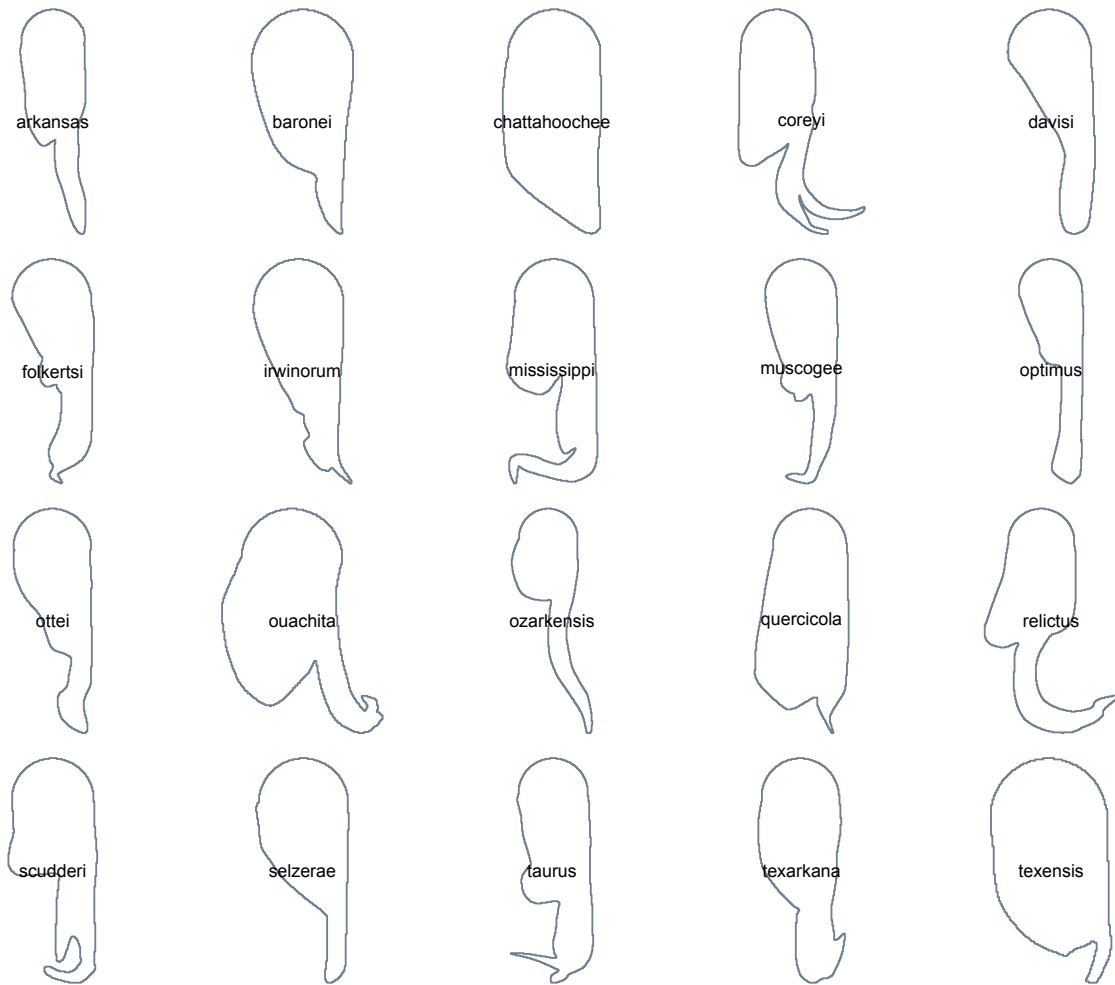


Fig. S11. Outlines of the ventral view of the male genitalia shapes.

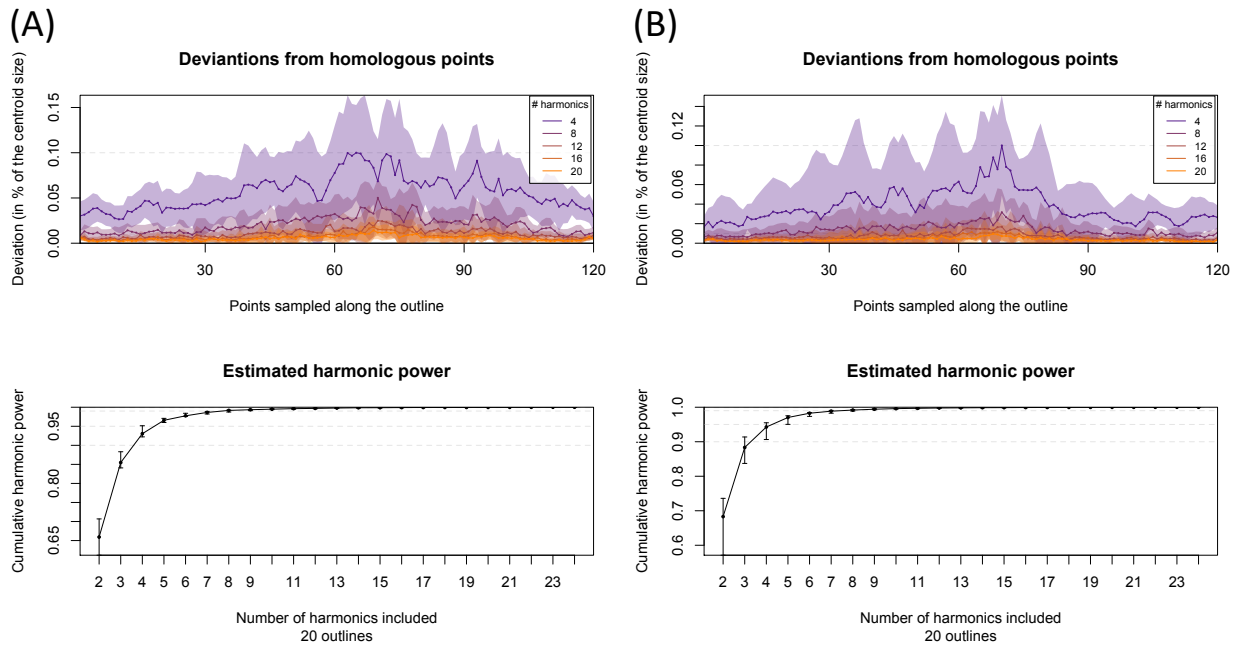


Fig. S12. Results from elliptical Fourier analyses using different number of harmonics and the shape data for the (A) lateral and (B) ventral view of the male genitalia.

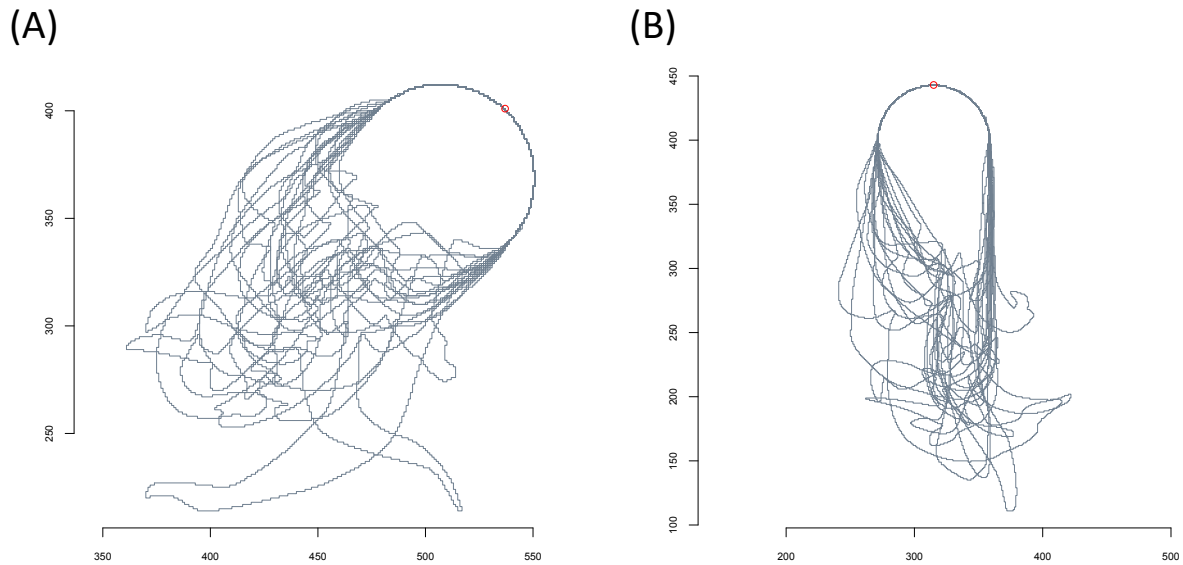


Fig. S13. A overlay of all the outlines of the (A) lateral and (B) ventral male genitalia shape variation across individuals. A red circle indicates a common origin point.

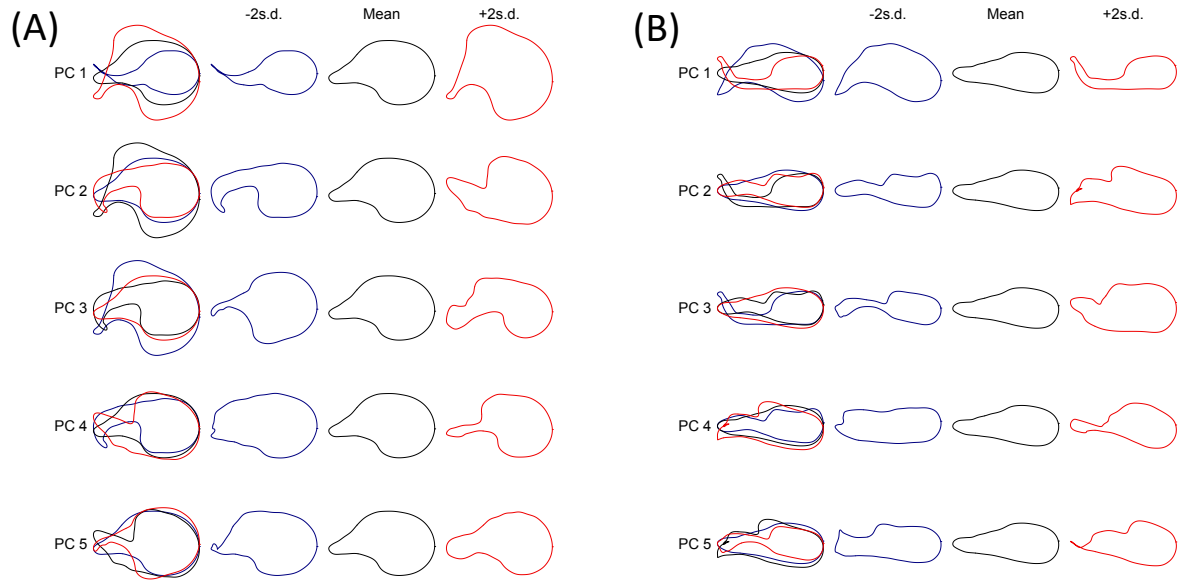


Fig. S14. The first five PCs from shape analyses based on the (A) lateral and (B) ventral views of the male genitalia shape. Blue and red outlines indicate the overlap of shape variation (2 standard deviation) relative to the mean shape (black outline) for each PC in the first column.

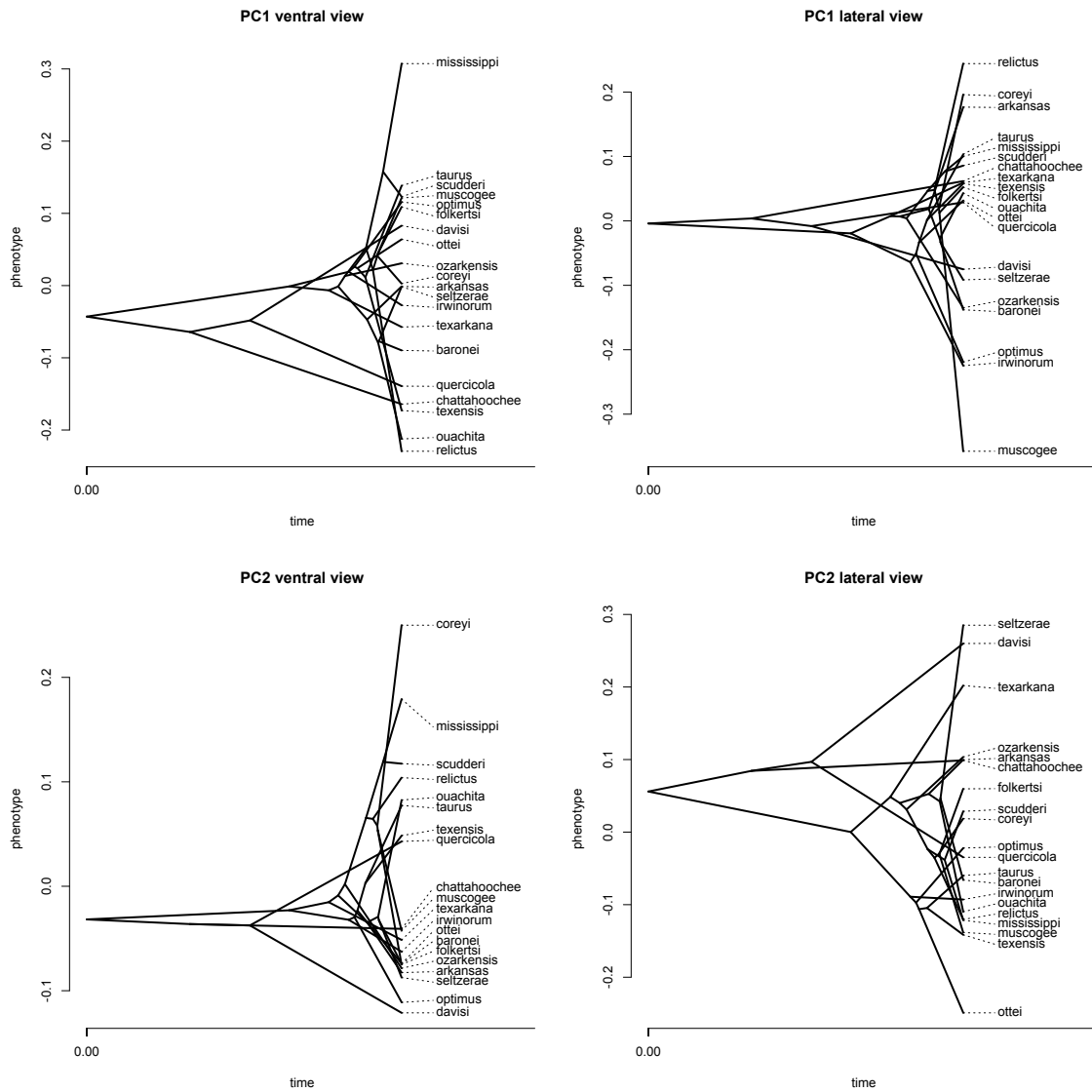


Fig. S15. Phenograms based on individual PCs and the reconstructed species tree (Fig. 1) were generated using the *phenogram* function in *phytools*.