

Supplementary Table 1. Assembly statistics for initial genome assemblies performed with only short-read data. k=61 was selected as the optimal short-read assembly for further hybrid assembly with long-read data using DBG2OLC.

	k = 51	k = 61
# contigs (>= 0 bp)	62,285,342	53,534,813
# contigs (>= 500 bp)	750,475	966,258
# contigs (>= 1000 bp)	148,561	227,660
# contigs (>= 5000 bp)	850	1088
Total length (>= 0 bp)	5,689,084,525	5,960,525,287
Total length (>= 500 bp)	626,371,545	846,993,489
Total length (>= 1000 bp)	224,744,531	347,268,680
Total length (>= 5000 bp)	7,240,744	7,187,453
Largest contig (bp)	73,394	28,080
GC (%)	33.6	33.6
N50 (bp)	817	877

Supplementary Table 2. Sex ratios of *T. rathkei* broods reared in the lab. Wild-caught refers to a mated female captured in the wild and brought back to the lab to give birth. Only broods with at least 10 offspring are shown.

Cross type	Mother ID	Father ID	Brood number	Year born	Male	Female	Total Offspring	% Female
Wild-caught	8	unknown	1	2016	14	12	26	46.2
Wild-caught	10	unknown	1	2016	19	15	34	44.1
Wild-caught	11	unknown	1	2016	11	11	22	50.0
Wild-caught	13	unknown	1	2016	18	12	30	40.0
Wild-caught	14	unknown	1	2016	26	15	41	36.6
Wild-caught	14	unknown	2	2016	7	10	17	58.8
Wild-caught	17	unknown	1	2016	23	28	51	54.9
Wild-caught	17	unknown	2	2016	8	10	18	55.6
Wild-caught	18	unknown	1	2016	6	12	18	66.7
Wild-caught	19	unknown	1	2016	15	13	28	46.4
Wild-caught	20	unknown	1	2016	7	6	13	46.2
Wild-caught	21	unknown	1	2016	20	18	38	47.4
Wild-caught	21	unknown	2	2016	4	8	12	66.7
Wild-caught	22	unknown	1	2016	17	14	31	45.2
Wild-caught	25	unknown	1	2016	19	12	31	38.7
Wild-caught	26	unknown	1	2016	9	42	51	82.4
Wild-caught	26	unknown	2	2016	2	13	15	86.7
Wild-caught	28	unknown	1	2016	12	16	28	57.1
Wild-caught	28	unknown	2	2016	8	5	13	38.5
Wild-caught	29	unknown	1	2016	12	20	32	62.5
Wild-caught	29	unknown	2	2016	8	9	17	52.9
Wild-caught	30	unknown	1	2016	20	26	46	56.5
Wild-caught	32	unknown	1	2016	5	16	21	76.2
Wild-caught	34	unknown	1	2016	12	11	23	47.8
Wild-caught	35	unknown	1	2016	5	5	10	50.0
Wild-caught	39	unknown	1	2016	6	5	11	45.5
Wild-caught	42	unknown	1	2016	8	10	18	55.6
Wild-caught	45	unknown	1	2016	3	7	10	70.0
Wild-caught	46	unknown	1	2016	17	24	41	58.5
Wild-caught	47	unknown	1	2016	15	8	23	34.8

Wild-caught	47	unknown	2	2017	33	30	63	47.6
Wild-caught	47	unknown	3	2017	27	20	47	42.6
Wild-caught	48	unknown	2	2017	23	25	48	52.1
Wild-caught	77	unknown	1	2016	6	8	14	57.1
Wild-caught	83	unknown	1	2016	6	4	10	40.0
Wild-caught	83	unknown	2	2017	15	20	35	57.1
Wild-caught	89	unknown	1	2016	12	7	19	36.8
Wild-caught	106	unknown	1	2016	8	10	18	55.6
Wild-caught	107	unknown	1	2016	5	5	10	50.0
Wild-caught	115	unknown	1	2016	8	4	12	33.3
Wild-caught	116	unknown	1	2016	7	9	16	56.3
Wild-caught	119	unknown	1	2017	24	26	50	52.0
Wild-caught	121	unknown	1	2016	11	8	19	42.1
Wild-caught	122	unknown	1	2016	6	12	18	66.7
Wild-caught	123	unknown	1	2016	7	5	12	41.7
Wild-caught	128	unknown	1	2016	3	8	11	72.7
Wild-caught	128	unknown	2	2017	24	17	41	41.5
Wild-caught	130	unknown	1	2016	11	2	13	15.4
Wild-caught	132	unknown	1	2016	9	12	21	57.1
Wild-caught	136	unknown	1	2016	5	6	11	54.5
Wild-caught	151	unknown	1	2017	23	25	48	52.1
Wild-caught	161	unknown	1	2017	15	15	30	50.0
Wild-caught	161	unknown	2	2017	17	24	41	58.5
Wild-caught	163	unknown	1	2016	23	14	37	37.8
Wild-caught	163	unknown	2	2017	28	47	75	62.7
Wild-caught	163	unknown	3	2017	4	18	22	81.8
Wild-caught	165	unknown	1	2017	21	24	45	53.3
Wild-caught	165	unknown	2	2017	28	16	44	36.4
Wild-caught	167	unknown	1	2016	22	21	43	48.8
Wild-caught	167	unknown	2	2017	7	5	12	41.7
Wild-caught	168	unknown	1	2017	4	9	13	69.2
Wild-caught	169	unknown	1	2016	24	20	44	45.5
Wild-caught	169	unknown	2, 3	2016	30	40	70	57.1

Wild-caught	171	unknown	1	2017	22	21	43	48.8
Wild-caught	171	unknown	2	2017	19	21	40	52.5
Wild-caught	171	unknown	3	2018	22	23	45	51.1
Wild-caught	174	unknown	1	2016	21	20	41	48.8
Wild-caught	174	unknown	2	2017	17	26	43	60.5
Wild-caught	175	unknown	1	2016	25	17	42	40.5
Wild-caught	175	unknown	2	2016	18	17	35	48.6
Wild-caught	175	unknown	3	2017	28	29	57	50.9
Wild-caught	177	unknown	1	2016	20	11	31	35.5
Wild-caught	177	unknown	2	2016	15	18	33	54.5
Wild-caught	178	unknown	1	2017	21	19	40	47.5
Wild-caught	178	unknown	2	2017	10	17	27	63.0
Wild-caught	178	unknown	3, 4	2018	19	22	41	53.7
Wild-caught	179	unknown	1	2017	19	18	37	48.6
Wild-caught	179	unknown	2	2017	15	21	36	58.3
Wild-caught	179	unknown	3	2018	10	10	20	50.0
Wild-caught	180	unknown	1, 2	2017	18	14	32	43.8
Wild-caught	182	unknown	1	2017	7	10	17	58.8
Wild-caught	182	unknown	2	2017	7	7	14	50.0
Wild-caught	182	unknown	3	2018	20	15	35	42.9
Wild-caught	184	unknown	1, 2	2017	27	36	63	57.1
Wild-caught	185	unknown	2	2017	8	10	18	55.6
Wild-caught	186	unknown	2	2017	8	10	18	55.6
Wild-caught	187	unknown	1, 2	2017	11	13	24	54.2
Wild-caught	191	unknown	1, 2	2017	39	33	72	45.8
Wild-caught	192	unknown	1	2017	19	21	40	52.5
Wild-caught	192	unknown	2	2017	6	11	17	64.7
Wild-caught	192	unknown	3	2018	10	6	16	37.5
Wild-caught	193	unknown	1	2017	8	2	10	20.0
Wild-caught	194	unknown	2	2017	4	15	19	78.9
Wild-caught	196	unknown	2	2017	17	21	38	55.3
Wild-caught	196	unknown	3	2018	14	15	29	51.7
Wild-caught	197	unknown	1	2017	19	25	44	56.8

Wild-caught	197	unknown	2	2017	9	11	20	55.0
Wild-caught	199	unknown	1	2017	9	7	16	43.8
Wild-caught	199	unknown	2	2017	7	6	13	46.2
Wild-caught	201	unknown	1, 2	2017	11	12	23	52.2
Wild-caught	201	unknown	3	2018	7	7	14	50.0
Wild-caught	202	unknown	1	2017	7	13	20	65.0
Wild-caught	202	unknown	2	2018	5	9	14	64.3
Wild-caught	203	unknown	1	2017	7	14	21	66.7
Wild-caught	204	unknown	1	2017	17	13	30	43.3
Wild-caught	204	unknown	2	2017	15	14	29	48.3
Wild-caught	205	unknown	1	2017	21	13	34	38.2
Wild-caught	205	unknown	2	2017	6	11	17	64.7
Wild-caught	207	unknown	1, 2	2017	37	32	69	46.4
Wild-caught	208	unknown	1	2017	8	9	17	52.9
Wild-caught	208	unknown	2	2017	10	14	24	58.3
Wild-caught	211	unknown	1, 2	2017	27	13	40	32.5
Lab cross	10-F1	18-M1	1	2019	24	24	48	50.0
Lab cross	13-F1	107-M1	1	2018	8	9	17	52.9
Lab cross	151-M1	178-F1	1	2019	21	35	56	62.5
Lab cross	163-F1	163-M1	1	2019	34	42	76	55.3
Lab cross	169-F1	179-M1	1	2019	16	18	34	52.9
Lab cross	171-F1	162-M1	1	2018	5	8	13	61.5
Lab cross	177-F1	177/162-M1	1	2019	5	13	18	72.2
Lab cross	177-F2	177/162-M2	1	2019	10	20	30	66.7
Lab cross	199-F1	199-M1	1	2018	7	9	16	56.3
Lab cross	199-F1	199-M1	2	2018	6	12	18	66.7
Lab cross	199-F1	199-M1	3	2019	3	11	14	78.6
Lab cross	205-F1	205-M1	1	2018	19	10	29	34.5
Lab cross	208-F1	208-M1	1	2018	20	24	44	54.5
Lab cross	211-F1	211-M1	1	2019	15	4	19	21.1
Lab cross	34-F1	174-M1	1	2019	17	20	37	54.1
Lab cross	47-F1	47-M1	1	2018	38	37	75	49.3
Lab cross	47-F1	47-M1	2	2019	19	14	33	42.4

Lab cross	48-M1	48-F1	1	2019	26	27	53	50.9
Lab cross	83-F1	203-M1	1	2019	5	5	10	50.0
Lab cross	9-M1	132-F1	1	2018	25	10	35	28.6

Supplementary Table 3. Top 100 regions of the *Trachelipus rathkei* assembly matching one or more *Wolbachia* genomes.

Contig	Matching <i>Wolbachia</i> genome	E-value	Bitscore	Length	Percent Identity
contig41205:46995-50826	NZ_QPIP01000040.1	0	5802	3898	92.894
contig21655:23176-27650	NZ_CP016430.1	0	4834	4092	85.973
contig82518:11250-14109	NZ_QPIP01000225.1	0	4420	2894	93.988
contig24014:19607-22010	NZ_QPIP01000012.1	0	3807	2393	95.153
contig82518:31954-35917	NZ_CAOH01000056.1	0	3714	4061	80.325
contig22648:52901-55829	NZ_QPIP01000225.1	0	3610	2955	87.174
contig60599:36848-52880	NZ_CP041215.1	0	3606	4456	77.536
contig111166:29090-31313	NZ_MNCG01000080.1	0	2438	2274	83.641
contig101838:5953-13532	NZ_CP041215.1	0	2355	2341	81.888
contig104492:6893-11374	NZ_CP041215.1	0	1894	2067	79.971
contig24014:16199-17706	NZ_MUJL01000059.1	0	1819	1384	88.584
contig140396:3608-5757	NZ_QPIP01000011.1	0	1807	1695	83.481
contig6767:28422-30540	NZ_QPIP01000011.1	0	1807	1695	83.481
contig62661:89289-93752	NZ_CP041215.1	0	1794	1975	79.747
contig54256:25557-27714	NZ_QPIP01000011.1	0	1784	1705	82.991
contig10767:48224-50433	NZ_NSDS01000079.1	0	1773	2019	79.693
contig83347:6124-8304	NZ_CP016430.1	0	1707	2000	79.1
contig8667:11430-15085	NZ_CP015510.2	0	1693	2461	75.457
contig82518:22137-23924	NZ_QPIP01000225.1	0	1681	1160	92.5
contig4956:73158-74195	NZ_CAOH01000048.1	0	1635	1039	94.706
contig54255:38034-40169	NZ_QPIP01000011.1	0	1634	1708	81.03
contig28080:13103-16290	NZ_CP015510.2	0	1625	2450	74.735
contig54256:19275-20850	NZ_LRUH01000003.1	0	1618	1434	85.146
contig41156:47828-49356	NZ_QPIP01000070.1	0	1611	1570	83.312
contig120126:10876-13379	NZ_KB223538.1	0	1608	1371	86.287
contig6767:22276-23850	NZ_LRUH01000003.1	0	1604	1441	84.733
contig4956:78999-82475	NZ_VCEG01000122.1	0	1592	1355	85.83
contig4865:65822-67934	NZ_QPIP01000120.1	0	1591	1221	88.37
contig4956:77436-78667	NZ_QPIP01000116.1	0	1537	1206	88.557
contig110376:12951-16136	NZ_CP015510.2	0	1481	2536	73.226
contig45554:30664-32437	NZ_QPIP01000040.1	0	1466	970	93.505
contig26619:11904-13635	NZ_KB223538.1	0	1457	1494	81.124
contig12055:11602-13784	NZ_QPIP01000093.1	0	1446	1213	86.562
contig55499:34817-37014	NZ_KB223538.1	0	1446	1236	86.084
contig120015:7288-11014	NZ_CP015510.2	0	1439	2357	73.568
contig56420:32935-36636	NZ_CP015510.2	0	1405	2481	72.229
contig57015:7061-9509	NZ_CAOH01000065.1	0	1397	1300	83.692

contig97794:44314-47449	NZ_CP015510.2	0	1366	2461	72.694
contig43090:31693-34077	NZ_CP015510.2	0	1361	1863	76.436
contig59781:1759-2780	NZ_CAOH01000048.1	0	1343	1043	90.604
contig57015:21936-26306	NZ_CAOH01000065.1	0	1337	1259	83.4
contig36754:35171-36510	NZ_KB223538.1	0	1330	1402	80.243
contig581:4071-5440	NZ_NSDS01000070.1	0	1269	1152	84.549
contig42215:18348-19410	NZ_NSDS01000070.1	0	1257	1080	85.741
contig131151:4311-5431	NZ_NSDS01000070.1	0	1247	1139	84.197
contig6767:8936-12307	NZ_NSDS01000070.1	0	1242	1154	83.882
contig47840:6928-9012	NZ_NSDS01000070.1	0	1236	1145	83.843
contig108259:12794-14948	NZ_NSDS01000070.1	0	1228	1138	83.743
contig110377:9706-11307	NZ_RWIK01000003.1	0	1224	1581	77.293
contig97486:21655-25569	NZ_CP041215.1	0	1200	1590	76.541
contig66402:15885-18640	NZ_LK055284.1	0	1199	1815	74.49
contig10737:33945-36112	NZ_NSDS01000070.1	0	1194	1146	83.333
contig107168:4876-8422	NZ_LK055284.1	0	1191	1822	74.424
contig56531:7008-8374	NZ_LSY01000126.1	0	1179	810	91.728
contig25813:9937-11304	NZ_LSY01000126.1	0	1177	811	91.615
contig8667:23939-25563	NZ_RWIK01000003.1	0	1177	1651	75.954
contig56531:9367-10734	NZ_LSY01000126.1	0	1172	811	91.492
contig17820:18438-21324	NZ_CTEH01000015.1	0	1169	829	91.435
contig79792:23503-24741	NZ_LSY01000126.1	0	1166	812	91.379
contig79792:26192-27559	NZ_LSY01000126.1	0	1163	811	91.245
contig28080:22749-24395	NZ_RWIK01000003.1	0	1162	1561	77.13
contig98868:31083-33755	NZ_CTEH01000006.1	0	1154	2240	71.607
contig56531:4623-6003	NZ_LSY01000126.1	0	1150	824	90.413
contig72521:1-909	NZ_CP041215.1	0	1095	932	85.73
contig109514:3774-5787	NZ_NSDS01000070.1	0	1070	996	83.735
contig136622:7675-8872	NZ_CAOH01000065.1	0	1049	963	84.112
contig32215:18066-20042	NZ_CTEH01000006.1	0	1040	2005	71.521
contig98868:20772-22347	NZ_RWIK01000003.1	0	1028	1637	74.221
contig2827:52033-53374	NZ_CTEH01000015.1	0	1027	1351	76.166
contig42215:16743-18227	NC_021084.1	0	1022	946	83.932
contig58604:31642-34836	NZ_CTEH01000006.1	0	1022	2450	69.918
contig84414:19934-21197	NZ_CP041215.1	0	1020	922	84.382
contig97794:50638-52378	NZ_RWIK01000003.1	0	1017	1380	76.304
contig131151:5506-7007	NZ_KB223533.1	0	1003	935	83.316
contig103716:10622-12081	NZ_NSDS01000070.1	0	998	1026	81.189
contig136941:1093-1804	NZ_QPIP01000070.1	0	980	714	91.036
contig4956:87666-89184	NZ_CAOH01000037.1	0	952	750	88

contig113691:13388-16226	NZ_CP041215.1	0	940	1375	75.345
contig62357:26051-29274	NC_010981.1	0	930	2607	68.7
contig22648:60781-63093	NZ_CP041215.1	0	915	1236	75.971
contig90834:10305-13093	NZ_CTEH01000006.1	0	900	1594	72.271
contig5091:23161-24553	NZ_QPIP01000017.1	0	898	1403	74.626
contig24485:37434-40025	NZ_CP041215.1	0	889	1709	72.147
contig98868:22871-23971	NZ_RWIK01000003.1	0	884	992	80.444
contig28080:19443-20340	NZ_RWIK01000003.1	0	864	882	81.859
contig20306:1-723	NZ_CP011148.1	0	850	729	85.597
NODE_4409_length_2226_cov_13.672390:1-980	NZ_CAOH01000065.1	0	847	745	85.235
contig8667:22312-23414	NZ_RWIK01000003.1	0	837	972	79.835
contig64403:13606-14996	NZ_KB223533.1	0	830	667	87.706
contig51480:5191-10342	NZ_CP041215.1	0	825	1196	75.084
contig110377:12182-13291	NZ_RWIK01000003.1	0	820	984	78.455
contig67097:19963-22152	NZ_CP041215.1	0	815	1132	75.883
NODE_1677_length_3365_cov_23.488464:190-1484	NZ_CAOH01000066.1	0	792	963	78.712
contig42215:13837-14533	NZ_RWIK01000007.1	0	775	668	85.629
contig21865:12389-13705	NZ_KB223533.1	0	769	679	85.272
contig55499:23126-24227	NZ_CP034335.1	0	750	735	82.313
contig4956:83945-85499	NZ_CAOH01000037.1	0	736	727	82.256
contig122193:35489-36065	NZ_NSDS01000070.1	0	725	578	87.889
contig82518:38729-39602	NZ_LSYY01000140.1	0	720	885	78.418

Supplementary Table 4. Initial set of candidate primers for Y chromosome markers. None of the primer pairs from this initial set produced the expected patterns of male-specific amplification.

ID	Forward primer	Reverse primer	Expected product size
TrYJa19_3	GGGACACTGTTGTGAGAGCA	CGCCAACAACCAGATGCG	160
TrYJa19_21	CTCTCACGTTTCGGAACCACT	TCACTTTGGTTTTGCATGTCGA	118
TrYJa19_9	TTCCTTTCCACCCCTATTTGCA	GCAAAGTGCTTACATGATTGCC	100
TrYJa19_19	CGTGTCATTTTCTGTCAGCGA	CCCAAGGCAATGAAGCTGA	114
TrYJa19_4	ACATCAGCAATCGCATCGT	TCGAGGTTGCGGATTCTGT	120
TrYJa19_5	AGGCCCTATTAGGAAGACCCA	AAGGCCTGCTACCATGCG	104
TrYJa19_22	AGCCTTGTGTGACCTTTTCGA	CCTCACAGGCGGGACAAA	145
TrYJa19_16	TGGTGTCATTTTTAACGGCAGA	GCAGGCAGTTTGTGTTGCGA	140
TrYJa19_18	GAGCAACAAGAATTCCTCGTTG	TCCGAGAACAAGTAAATCGTGC	100
TrYJa19_20	CGCAGCCGCTGAAGGTAT	CACTTCCCATCAAAGTGTCCA	176
TrYJa19_1	TGCAACTCATGGATTGTAAGCA	GGACACTGTCCCGTTCAAAAC	101
TrYJa19_7	TTTGGCGTGGTTATGGCA	GTGTCCCAGTTATCAGGAACCT	108
TrYJa19_10	TGGTGGCATTCTTTTCTCTGC	ACCACTAAGGGTTACCGCT	237
TrYJa19_6	CCCAGGATTCTTCGAGCCC	TGCAGAGTGATCCCCGGT	234
TrYJa19_17	GAAGGAGTCAGGCGCGTT	GCTTCTTGCGTTAGACGAAAT	236
TrYJa19_8	CATCTTCTGGATGCGGCT	TGGCATCGTGTGTTCCGA	228