

Supplement

| | AMA1 | AMA2 | AMA5 | AMA6 |
|-----------------------------------------|---------------|---------------|-------------|---------------|
| Population | California | California | Kansas | Kansas |
| Sex | male | female | male | female |
| Individuals | 35 | 32 | 25 | 35 |
| Raw read pairs ¹ | 467,707,106 | 426,450,124 | 322,106,487 | 371,673,480 |
| Per pool coverage (X) | 331.71 | 302.45 | 228.44 | 263.60 |
| Per individual coverage (mean X) | 9.48 | 9.45 | 9.14 | 7.53 |
| Mapped to <i>A. hypochondriacus</i> (%) | 90.54% | 92.55% | 92.12% | 92.06% |
| Filtered paired reads ² | 460,522,536 | 420,526,544 | 316,496,725 | 365,964,343 |
| Total k-mers ³ | 1,329,452,869 | 1,235,093,537 | 961,598,655 | 1,066,417,174 |
| k-mers/read pair (mean) | 2.89 | 2.94 | 3.04 | 2.91 |
| Sum male specific k-mers ⁴ | 17,259,830 | 0 | 13,168,285 | 52,698 |
| Sum female specific k-mers ⁴ | 83,397 | 324,143 | 12,994 | 249,012 |

Table S1 Data Summary ¹ Paired end 2x 150bp reads. ² Trimmed and quality filtered correctly paired reads. ³ 35-mers from filtered reads with $15 \leq \text{count} \leq 2000$. ⁴ unique k-mers *times* number of each k-mer after correction with Kansas population. Values > 0 in opposite pools result from filter steps. For instance, more than 2,000 or below 20 k-mers in opposite pool

| Scaffold | Scaffold size | Covered BPs | Covered (%) | Male specific BPs | Male specific (%) |
|----------|---------------|-------------|-------------|-------------------|-------------------|
| 20 | 1968246 | 428904 | 21.79 | 51615877 | 91.42 |
| 6 | 20110894 | 5314 | 0.03 | 888631 | 1.57 |
| 10 | 16397930 | 6057 | 0.04 | 641181 | 1.14 |
| 5 | 21298423 | 5123 | 0.02 | 577873 | 1.02 |
| 3 | 23382003 | 4432 | 0.02 | 343804 | 0.61 |
| 81 | 33215765 | 3631 | 0.01 | 306992 | 0.54 |
| 259 | 43907 | 2592 | 5.9 | 227170 | 0.4 |
| 9 | 16692146 | 2478 | 0.01 | 216295 | 0.38 |
| 2 | 25377341 | 1841 | 0.01 | 156778 | 0.28 |
| 22 | 1315334 | 1354 | 0.1 | 155948 | 0.28 |
| 4 | 21854484 | 1828 | 0.01 | 153487 | 0.27 |
| 1 | 31626665 | 2043 | 0.01 | 146878 | 0.26 |
| 11 | 16251303 | 2007 | 0.01 | 128730 | 0.23 |
| 12 | 14118028 | 1669 | 0.01 | 124206 | 0.22 |
| 80 | 34472010 | 1472 | 0 | 112312 | 0.2 |
| 8 | 18030403 | 1629 | 0.01 | 104343 | 0.18 |
| 16 | 8418444 | 1356 | 0.02 | 95906 | 0.17 |
| 7 | 19699934 | 1332 | 0.01 | 93398 | 0.17 |
| 73 | 47327 | 472 | 1 | 45973 | 0.08 |
| 19 | 2235478 | 612 | 0.03 | 42613 | 0.08 |
| 18 | 2575540 | 533 | 0.02 | 41511 | 0.07 |
| 92 | 286925 | 162 | 0.06 | 36415 | 0.06 |
| 17 | 6914372 | 521 | 0.01 | 35750 | 0.06 |
| 78 | 16516 | 320 | 1.94 | 32983 | 0.06 |
| 14 | 11695121 | 533 | 0 | 31634 | 0.06 |
| 84 | 2698915 | 269 | 0.01 | 18466 | 0.03 |
| 183 | 51914 | 239 | 0.46 | 14837 | 0.03 |
| 247 | 304540 | 234 | 0.08 | 14812 | 0.03 |
| 86 | 829192 | 115 | 0.01 | 7284 | 0.01 |
| 248 | 225155 | 125 | 0.06 | 6945 | 0.01 |
| 15 | 8446923 | 102 | 0 | 6220 | 0.01 |
| 27 | 286531 | 110 | 0.04 | 5959 | 0.01 |
| 25 | 438162 | 97 | 0.02 | 5419 | 0.01 |
| 121 | 98696 | 86 | 0.09 | 4535 | 0.01 |
| 90 | 308815 | 84 | 0.03 | 4200 | 0.01 |
| 113 | 124836 | 65 | 0.05 | 3586 | 0.01 |
| 31 | 199361 | 45 | 0.02 | 2385 | 0 |
| 213 | 33128 | 40 | 0.12 | 2107 | 0 |
| 226 | 27712 | 35 | 0.13 | 1854 | 0 |
| 56 | 82381 | 22 | 0.03 | 1109 | 0 |
| 130 | 86235 | 10 | 0.01 | 500 | 0 |
| 237 | 17867 | 4 | 0.02 | 200 | 0 |

Table S2 Mapping of extracted male specific reads to *A. palmeri* draft genome v1.1. Covered describes the size of the scaffold covered by male specific reads. "Male specific" indicates the amount and proportion of male all specific reads that mapped to a specific scaffold

| Scaffold | Scaffold size | Covered BPs | Covered (%) | Female specific BPs | Female specific (%) |
|----------|---------------|-------------|-------------|---------------------|---------------------|
| 20 | 1968246 | 308 | 0.02 | 18829 | 63.86 |
| 4 | 21854484 | 125 | 0 | 7138 | 24.21 |
| 3 | 23382003 | 55 | 0 | 2854 | 9.68 |
| 1 | 31626665 | 8 | 0 | 408 | 1.38 |
| 6 | 20110894 | 5 | 0 | 255 | 0.86 |

Table S3 Mapping of extracted female specific reads to *A. palmeri* draft genome v1.1. Covered describes the size of the scaffold covered by female specific reads. "Female specific" indicates the amount and proportion of female all specific reads that mapped to a specific scaffold

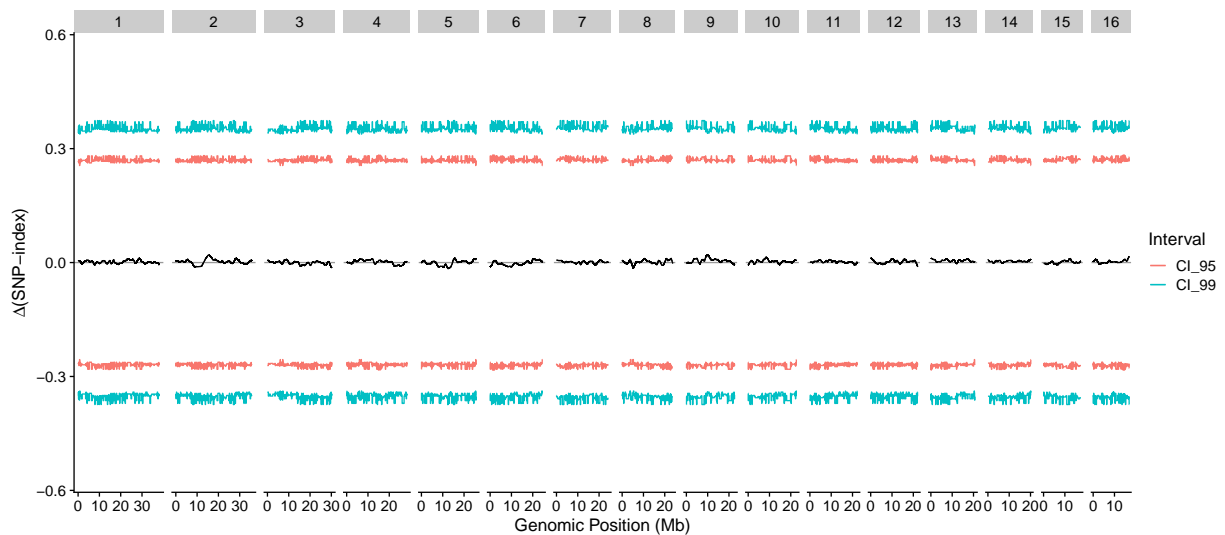


Figure S1 Differences in allele frequencies between male and female pools of the Kansas population along the genome, relative to the *A. hypochondiracus* reference. Red and blue lines represent 95% and 99% confidence intervals for frequency outliers.

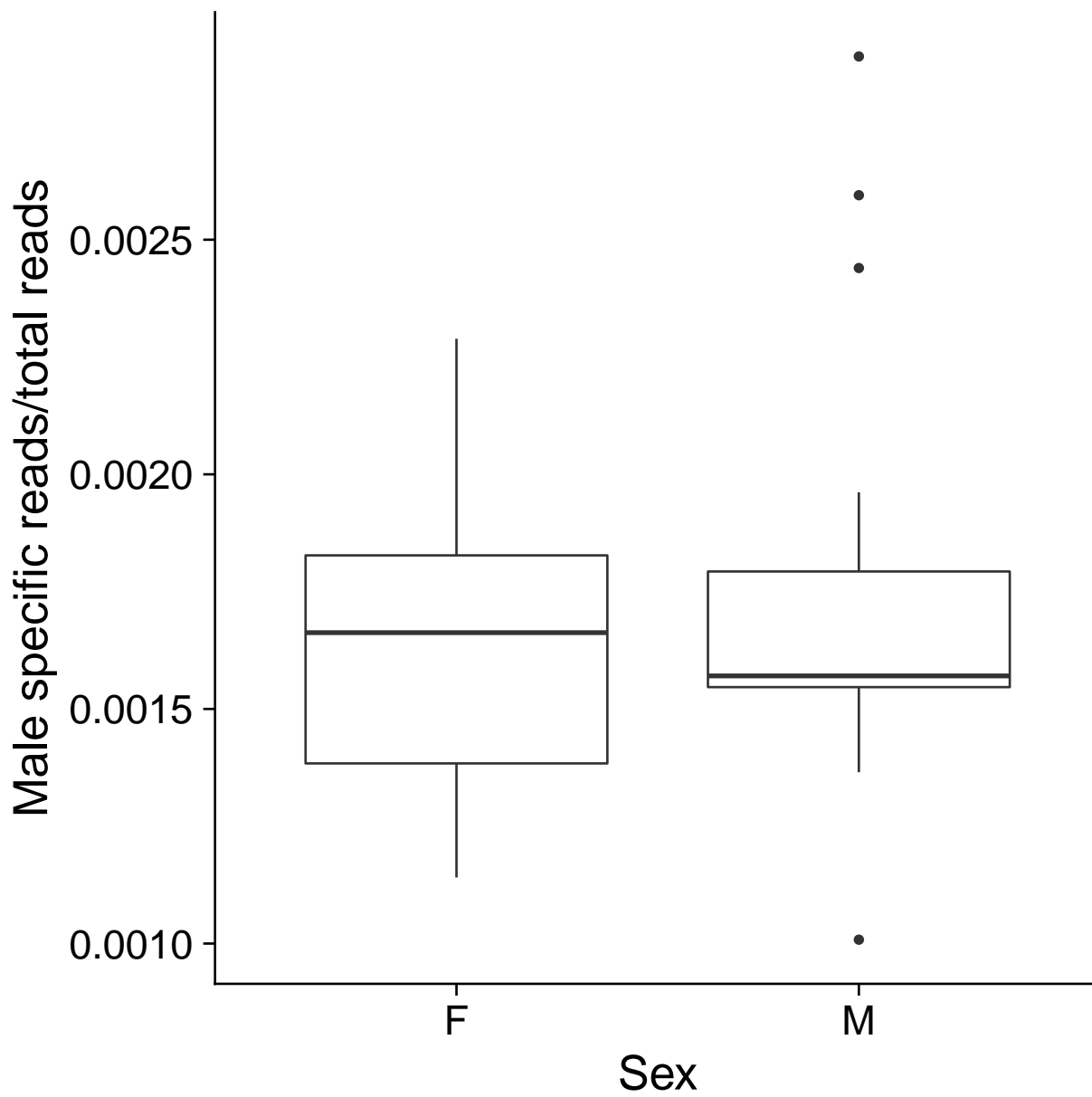


Figure S2 *A. tuberculatus* reads extracted Ratio of *A. palmeri* male specific k-mer containing reads in *A. tuberculatus* samples. No significant difference between female (n=19) and male (n=25)

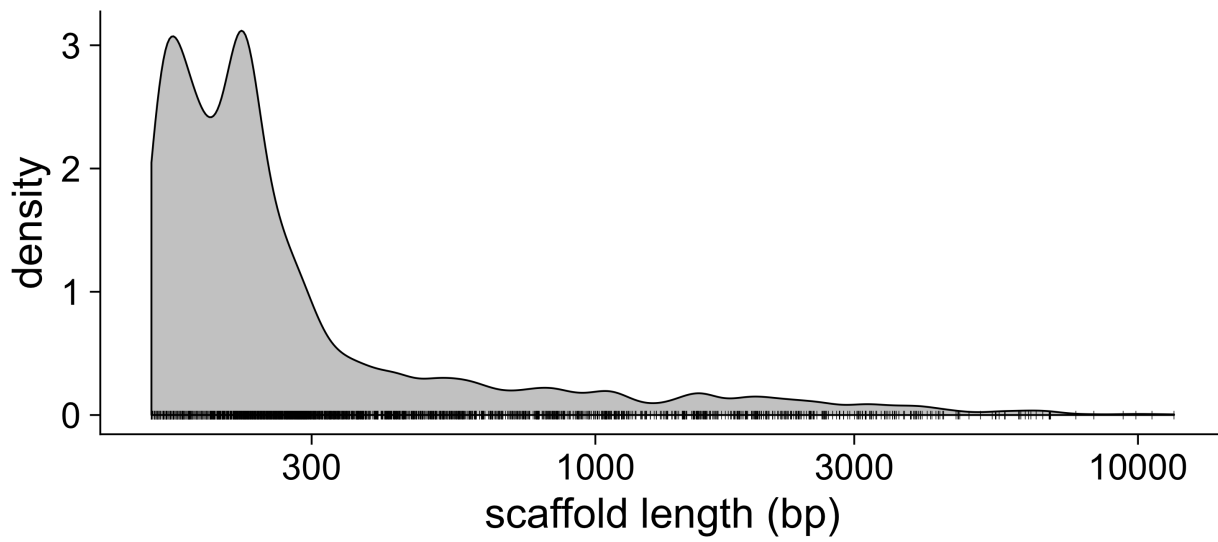


Figure S3 Scaffold size distribution Size distribution of scaffolds assembled from sex specific reads. Ticks on the x-axis show value distribution