

## Description of Additional Supplementary Files

File Name: Supplementary Data 1

Description: Overview of 368 fire ant samples used, including species identities, colony identifiers, GPS coordinates, supergene variants, castes, sequencing amounts, NCBI identifiers, mapping rates to the *S. invicta* reference genome (samtools flagstat), RFLP results and genotype information.

File Name: Supplementary Data 2

Description: Overview of the 5,851 single-copy genes used in our analysis, including BUSCO identifiers, substitution models for coding and non-coding regions, average numbers of single nucleotide polymorphisms per individual among the 342 *S. invicta*, *S. macdonaghi* or *S. richteri* males, results of the maximum likelihood phylogenetic tree inference, genomic region, genomic coordinates, BUSCO profile completeness, BUSCO profile score and length.

File Name: Supplementary Data 3

Description: Pairwise Bray-Curtis dissimilarities between *Sb* supergene haplotypes, based on the genotypes of the 24,435 SNPs in the supergene region that were bi-allelic among the *Sb* samples.

File Name: Supplementary Data 4

Description: Statistics, data sources and Busco scores of fire ant assemblies used for the dating analysis.