**Supplementary material 1. Reference-based genome assembly for *Myotis austroriparius*.**

Whole-genome sequencing data from an Illumina paired-end read (2 x 150 bp) dataset of *Myotis austroriparius*, with 17 X coverage, was quality-filtered, and trimmed using Trimmomatic v0.27 (Bolger et al. 2014). Bases below the quality threshold of Q20 were removed from the ends of all sequences, and trimmed regions in sliding windows of 20 bp if the average quality dropped below Q20. We mapped both paired-end and single reads to the *Myotis lucifugus* de novo genome assembly (GCA\_000147115.1) using BWA-MEM v0.7.17 (Li & Dur 2009) and used Picard v2.5.0 (Institute 2019) to merge BAM files. Samtools (Li et al. 2009) was used to sort the merged BAM file, Picard to add read groups and remove duplicates, then the cleaned BAM was indexed via Samtools. We used Freebayes v1.1.0 (Garrison & Marth 2012) to call variant sites relative to *M. lucifugus*. In order to maintain more direct comparability to *M. lucifugus* features, such as gene annotations, we filtered variants to exclude indels, and kept only variant calls with site quality > 30, and read depth of 5-60, using GATK (Genome Analysis Tool Kit) v3.8.0 (McKenna et al. 2010). These calls were then left-aligned and trimmed, with MNPs (multiple nucleotide polymorphisms, e.g. ATTCG -> AATGA) broken into multiple individual SNP calls, again using GATK. These variants were then integrated into the *M. lucifugus* genome sequence to create a variant consensus sequence. This new consensus sequence was then used as the reference for the next round of read mapping and variant calling, and the whole process above was repeated for a total of seven iterations to produce the reference-based genome for *M. austroriparius* to reduce the likelihood of inclusion of lower-quality, inconsistent variant calls.

References

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