File S1

Supplementary Methods

*T. rathkei mitochondrial genome assembly*—Initial quality filtering was performed using Trimmomatic (*Bolger et al.* 2014). Adapter sequences were removed, allowing two mismatches in the seed, with a palindrome clip threshold of 40, and a simple clip threshold of 15. Leading and trailing bases with a quality score below 5 were trimmed, and a sliding window cleaning approach was used with a window size of 4 and a minimum average quality score of 5. Reads shorter than 36 bases after trimming were discarded.

An initial assembly of all sequence reads was performed using Minia 1.6906 (*Chikh* and *Rizk* 2012; *Salikhov* and *Sacomoto* 2013) with a kmer size of 53 and requiring kmers to appear at least 10 times in the dataset to be included in the assembly. The average sequencing depth of each contig was then estimated by mapping the filtered reads to this initial assembly with bwa mem (*Li* and *Durbin* 2009; *Li* 2013) and the samtools depth command (*Li et al.* 2009). Finally, all contigs were compared against the *Armadillidium vulgare* mitochondrial genome (*Marcadé et al.* 2007) using BLAST (*Altschul et al.* 1990) to identify putative mitochondrial contigs. This search identified a single contig with multiple high-confidence hits in the *A. vulgare* mitochondrial genome (*E* < 10^{-100}); this contig was about 13kb in length and had ~1600x and 680x coverage in the male and female samples, respectively, and therefore was inferred to represent the *T. rathkei* mitochondrial genome.
To obtain a more complete mitochondrial genome assembly, we used an iterative mapping approach, similar to those adopted by others (Hahn et al. 2013). First, all reads from each sequencing sample mapping to the putative mitochondrial contig identified earlier with a mapping score of at least 60 were identified, along with their mates, even if the mates themselves did not necessarily map. The rationale behind this procedure is that reads mapping to the ends of the contig may have mates that lie beyond the contig’s boundaries. Subsequently, these reads were assembled, again using Minia with the same parameters as earlier. Reads mapping to the new assembly were then identified, and this cycle was repeated for ten iterations.

To construct a final optimized assembly, the reads that mapped to the assembly generated by the tenth iteration were identified. We then assembled the male and female mitochondrial reads separately, in case sequence polymorphisms might hinder the assembly process. In addition, for each sample, we performed assemblies using the full set of putative mitochondrial reads, as well as a reduced set of 25,000 putative mitochondrial reads (~350x coverage), because at extremely high sequencing depth, sequencing errors might also reduce assembly quality. We performed all these assemblies across a range of different parameter values: kmer sizes of 43, 47, 51, 53, 57, 61, 63, 67, and 73; and minimum kmer depths of 3, 5, 8, 10, 15, 20, 25, 30, 50, 80, 100, 120, 150 for the reduced datasets, and minimum kmer depths of 5, 10, 20, 30, 50, 60, 80, 100, 130, 160, 200, 250, and 300 for the full datasets. Finally, we selected the assembly with the single longest contig as our final reference mitochondrial genome for *T. rathkei*. 

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